THAILAND
HALAL
ASSEMBLY 2021

Preface

Thailand Halal Assembly 2021, International Halal Science and Technology Conference (IHSATEC) 2021; The 14th Halal Science Industry and Business (HASIB) Conference is organized by the Halal Science Center Chulalongkorn University (HSC-CU) in collaboration with the Central Islamic Council of Thailand (CICOT), the Halal Standard Institute of Thailand (HSIT), Research Synergy Foundation (RSF) as well as the other organizations. Owing to the ongoing global pandemic of COVID-19, this IHSATEC 2021; 14th HASIB conference during 14-15 December 2021 was the second fully virtual conference with the government's preventive measures, to pay a tribute under the main theme **"A VIRTUAL WAY FOR ACTUAL HALAL WORLD."**

This proceedings book contains 24 peer-reviewed papers on halal topics given by local and foreign researchers during IHSATEC 2021; 14th HASIB conference. This book expands on previous discussions about the halal sectors by offering findings from scientific research studies that use quantitative and qualitative methods, as well as findings from social research studies. Papers published in this book cover many areas, including Halal science and innovation, health and physical science, cosmetic science, blockchain and internet of things (IoT), digital marketing and data driven marketing, and mobile commerce, are among the topics covered in this book. This book is an invitation to a deeper understanding and discussion of the global halal movement.

On behalf of the committee, my deepest thanks go to our dedicated staff committees for their devotion, enthusiasm, and tremendous effort in making the conference fruitful and memorable. A special thanks to all of the academic staff at HSC-CU, CICOT, RSF, as well as the authors and all reviewers for their precious response and feedback. Our appreciation also goes to Research Synergy Press for assisting us with the publication of these academic publications. Your participation in this conference is much appreciated.

Associate Prof. Dr. Winai Dahlan
Chairman of the Organizing Committee
Thailand Halal Assembly 2021
Editor: Proceedings of The IHSATEC 2021; 14th HASIB Book

Focus and Scope

The International Halal Science and Technology Conference (IHSATEC) is an international conference devoted to various spectrums of Halal Science and Technology (Industry and Business) for oral and poster presentation in the Academic Presentation Session of Thailand Halal Assembly 2021. The general objective of the conference is to provide a platform and stimulate discussions on issues surrounding Food Science, Nutrition, Food Safety, Management, Natural Products, etc. Topic included in the proceeding are: Food Science and Nutrition, Food Safety and Management, Natural Products and Bioactive Compounds, Biotechnology and Molecular Biology, Blockchain and Internet of Things (IoT), Health and Physical Science, Nanoscience and Technology, Environment and Green Technology, Post COVID-19 Management, Digital Marketing and Data Driven Marketing, E-Business and Mobile Commerce, Artificial Intelligence (AI), Islamic Finance, and Cosmetic Science.

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Exploration of The Selective Binding Property of The MIP-grafted Paper for Cochineal Dye

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Abstract

A molecularly imprinted polymer (MIP) is a synthetic polymer that provides specific cavities for its analyte. In this study, the MIP specific to carminic acid, an insect-derived pigment, has been synthesized using methacrylic acid (MAA) and 4-vinyl pyridine (4Vpy) as monomers and ethylene glycol dimethacrylate (EDGMA) as a cross-linker. The imprinted surface particles were characterized by Scanning Electron Microscope (SEM). The rough surface of the synthesized MIP represented the specific binding site for carminic acid. The paper-based MIP polymerization was performed by pre-treatment the cellulose paper with aminopropyltriethoxysilane (APTES) before polymerization with the MIP solution. The novel membrane-grafted MIP exhibits good performance for selective recognition with the target carminic acid, which can be demonstrated by the imprinted factor of 1.94 as compared to those of nonimprinted polymer. According to the Scatchard analysis, it was estimated that there are two types of binding strategy, including high and low affinity, which corresponded to the Ka of 1.24x103 mM and 0.10 x103 mM, respectively. It was thus preliminary concluded that the membrane-grafted MIP fabricated in this study has the potential to be implemented in many applications such as extraction and pre-concentration.

Keywords: *Molecularly imprinted polymer, carminic acid, halal, Cochineal red color, E120*

INTRODUCTION

Molecular Imprinting Technology (MIT) is a technique creating artificial recognition receptors with a predetermined selectivity and specificity for its analyte, which can be used as ideal materials in various application fields. The polymer created by this technology is known as molecularly imprinted polymer or MIP. This polymer is a robust molecular recognition element that is used mimically for natural recognition elements, such as antibodies and biological receptors (Vasapollo et al., 2011). The advantages of MIP compared with antibodies include a reduced molecular mass, enhanced stability, and cost-effective production methods. In addition, more efficient selection and screening procedures are offered in comparison with the screening

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procedure for the selection of aptamer, an alternative affinity receptor based on nucleic acids (Ruigrok et al., 2011).

Carminic acid is a natural red pigment derived from the Cochineal insect (E120). This dye is used as a colorant in various industries, including food, confectionery, drink and beverages, cosmetics, and the pharmaceutical industry. This dye is also used in the textile industry due to its relatively high chemical and biological stability. However, because this dye is extracted from insects, there are allergic cases of patients who have symptoms due to the consumption of food containing insect proteins (Takeo et al., 2018). In addition, for Muslim consumers, the use of this dye has some critical issues, including whether the use of dyes derived from insects is permitted. In addition, another issue has also risen because of the way they treat insects with strong conditions. To this point, the insects are boiled in hot water or heated at high temperatures for color extraction. This process is believed that the insect is treated improperly. Therefore, the color obtained from this process is an unlawful color for application in Halal products.

Because there are issues with using carminic acid in Halal industries, the sensitive detection method for insect-derived carminic acid is important for traceability and safety management. Previous studies for determination of this color are included using spectrophotometry (Samari et al., 2010, Ordoudi et al., 2018), high-performance liquid chromatography (HPLC) (Carvalho and Collins, 1997, Lancaster and Lawrence, 1996, Nishizaki et al., 2018, Ordoudi et al., 2018), FT-IR (Ordoudi et al., 2018) and electrochemistry (Yilmaz et al., 2014). Although these techniques are known as a sensitive procedure for determination in dilute samples, they require specific materials for selective binding or separation before analysis when detected in the sample with high complexity. Therefore, appropriate sample pre-treatment or sample manipulation is required before analysis.

Combined with the specific recognition of MIP, the synthesis of polymers with specific recognition for carminic acid is promising for selective extraction of carminic acid (Bibi et al., 2012). This polymer has the ability to apply for solid-phase extraction of carminic acid from dried body insects to obtain a highly effective yield similar to those previous reports (Bhawani et al., 2018, Karuehanon et al., 2018, Stevenson, 1999). In addition, in food and cosmetic products where the Cochineals color is added and appears at a very low content because of the FDA regulation (Harp and Barrows, 2015), this material could improve the selective absorption of the target carminic acid. Therefore, the better performance of the extraction could be obtained. This led to improvement of the sensitivity of the detection (Chianella et al., 2003, Andersson et al., 1997, Pichon and Combès, 2016, Mei et al., 2011).

Although the technique for detection of Cochineals color is necessary, there are few studies focusing on the development of the detection method for this dye. This limitation is not only for on-site detection but also for in-house techniques using high instrumentation. With regarding this necessarily, the development of the facile absorption paper with the specific binding sites for

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carminic acid created by MIP polymerized on the cellulose-based membrane was fabricated. The binding performance of the developed material will be demonstrated in the text. This material shows the potential to be implemented for solid-phase extraction to gain higher recovery. Moreover, this material provided further opportunity to implement in a pre-concentration step which was combined prior to the analysis. In addition, this material also provided the potential for the paper-based colorimetric sensor. Therefore, the developed material has promised further development in various fields of study. The preliminary study provided in this study will thus have high benefits.

LITERATURE REVIEW

Scatchard Model

Scatchard analysis is a common model used to evaluate the binding behavior of MIP in the rebinding experiment. Typically, non-covalent binding between a template and the functional monomer gives two straight lines indicating heterogeneous affinity of the high and low-affinity binding site (Zhi et al., 2018, Royani and Abdullah, 2014).

The Scatchard equation is as follows;

$$\frac{B}{[CA]_0} = (B_{max} - B)K_a$$

Where [CA]0 is the initial concentration of carminic acid. B represented bound carminic acid to the MIP. Ka is the association constant, Bmax is the maximum number of binding sites. Ka and Bmax values could be obtained from the slope and interception, respectively.

Imprinted factor (IF)

It can be calculated from the following equation:

$$IF = \frac{Q_{MIP}}{Q_{NIP}}$$

Where QMIP and QNIP are the adsorption capacity of the template of carminic acid@-MIPs and carminic acid@-NIPs, respectively.

RESEARCH METHOD

Preparation of MIP of carminic acid

The standard grades of 4-vinyl pyridine (4Vpy) and methacrylic acid (MAA) were purchased from Sigma Aldrich (St. Louis, MO). The ethylene glycol dimethacrylate (EDGMA) was obtained from Merck & Co., Inc. 2,2-Azobis (2-isobutyronitrile) (AIBN) was from Fluka (Steinem, Germany).

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Dimethylsulfoxide (DMSO) was purchased was prepared from reagent grade chemicals from Sigma Aldrich (St. Louis, MO). Aminopropyltriethoxysilane (APTES) was obtained from the Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University. Deionized water was obtained using a Milli-Q system (Thermo Fisher Scientific). Whatman® filter paper, Grade 1004, was purchased from GE Healthcare UK Limited, UK. The MultiskanTM microplate reader (Thermo Fisher Scientific) was used for spectrophotometric studies. The immobilised membrane was employed from FT-IR Tensor ll (Bruker, Germany). A scanning electron microscope (SEM Quanta, FEI 250) was employed for the surface morphology study.

Preparation of MIP of carminic acid

The MIP of the carminic acid was firstly prepared by bulk synthesis according to previous studies (Bibi et al., 2012). The co-monomer, 4-vinyl pyridine (4Vpy) and methacrylic acid (MAA), were utilized in the presence of ethylene glycol dimethacrylate (EDGMA). For the polymer preparation, 0.081 mmol of carminic acid was mixed with the co-monomers (0.93 mmol each) for 1 hour at room temperature. Subsequently, EGDMA (4.66 mmol) was added to the reaction mixture to proceed with the reaction for additional 4 hours at 24°C. Finally, 10 mg of AIBN (an initiator) and 1.27 ml of dimethylsulfoxide (DMSO), a porogen, were added to the above solution mixture. The mixture was then purged by nitrogen gas for 10 minutes at room temperature. After purging, the tube was carefully sealed and was incubated at 60 °C for 16 hours to allow the polymerization. The solid polymer was crushed and ground. The template was removed by repeated washing with a mixture of 10 percent of acetic acid in methanol several times. The obtained polymer was then dried at 80 °C for 24 hours. The resultant polymer was further analyzed by FT-IR and SEM to demonstrate the physiological property of the synthesized polymer. The binding capacity of the polymer with the target carminic acid was also observed by HPLC. Furthermore, nonimprinted (NIP) was prepared in the same manner but without the addition of the template.

Physiological characterization of polymer

1. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR analysis of the synthesized MIP and NIP obtained from the bulk polymerization method was carried out using FT-IR Tensor ll. The transmission region was studied in wavenumbers ranging from 400 cm−1 to 4000 cm−1. A number of 32 scans were operated. The data was analyzed by OPUS software.

2. Scanning Electron Microscope (SEM)

The morphology of the synthesized MIP and NIP was studied using SEM. The ground MIP and NIP were subjected to critical point drying using liquid CO2 substitution before analysis. Dehydrated samples were adhered to the stubs with clear nail polish, coated with gold-palladium, and analyzed under SEM. The MIP grafted membrane was analyzed in the same manner.

Preparation of MIP grafted on cellulose membrane

1. Activation of cellulose membrane by coupling agent

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Surface modification of the cellulose membrane was prepared according to the previous reported with slight modification (Howarter and Youngblood, 2006). Briefly, the cellulose membrane was cut into 0.5 x 1 cm and dried at 110 °C in the oven for 2 hours. Dried cellulose membrane was then submersed in 5% of aminopropyltriethoxysilane (APTES) in ethanol for 15 minutes at room temperature. Washing was performed carefully by rinsing with ethanol several times. The modified membrane paper was dried in the oven at 110°C overnight. The modified paper was kept in the desiccator before modification and polymerization.

2. Membrane grafting with MIP

The solution mixture MIP (2.0 mL) was dropped onto the silanized membrane, which was placed on a cleaned glass slide. The cover slit was placed onto the paper soaked with the polymerization solution to form a thin film on the cellulose membrane. Once the air was removed from the MIP-treated membrane, the polymerization was allowed to perform at 60°C for 4 hours. The grafted cellulose membrane was rinsed several times to remove carminic acid with 10% of acetic acid in methanol, as mentioned previously. The cellulose membrane grafted with NIP was prepared in the same manner using the solution mixture of NIP in which the target carminic acid was not included.

3. Rebinding analysis

The rebinding analysis was performed by incubation of carminic acid with the developed MIP and NIP. The reduction of the carminic acid after the binding was determined with ultra-high performance liquid chromatography (UHPLC). In this study, the carminic acid at the concentration ranged from 0 – 2.0 mM in 50% methanol. The reduction of the carminic acid concentration was calculated from the calibration curve of carminic acid prepared in the same manner. The rebinding parameter was calculated from the equation 1, and 2 explained in the above session.

FINDINGS AND DISCUSSION

Physiological characteristic of the synthesized carminic acid@-MIP

Recently, the MIP prepared by polymerization technique was interested in being used in solid-phase extraction to improve its recovery and yield of the target analyte. The bulk polymer synthesized with the co-monomer prepared in this study was solid. The resulting solid polymer was ground in a mortar and sieved to obtain the defined cut of particle size of 25 – 100 µm. Considering the solid MIP with FT-IR, the spectra are depicted in Figure 1. According to the ATR-FTIR spectra of carminic acid@-MIP (pink), the bands are dominant, including the band at 2960 cm-1 of the –CH stretch, the band at 1640 cm-1, which is the combination of –OH bend and intermolecular hydrogen bond between –COOH groups, the band at 1150 cm-1 which referred the stretch of the –C-O-C in hexose ring of carminic acid. These characteristics are in accordance with a previous report (Silverstein and Bassler, 1962). In addition, the bands typically for carminic acid are obviously visible at 1712 cm-1, which referred to the –C=O stretch, and at 1620 cm-1 –CC stretch in aromatics, 1245 cm-1 – assigned to catechol functions (Guillermin et al., 2019), except

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for the bands centered at 1570 cm-1 (–CC stretch in aromatics) and 1040 cm-1 (–CC stretch in glucose unit). These bands were not observed in carminic acid@-NIP (black). Therefore, it was thus confirmed that there were some interactions between the target template carminic acid and the polymerization solvent, including monomers, linkers, and initiators.

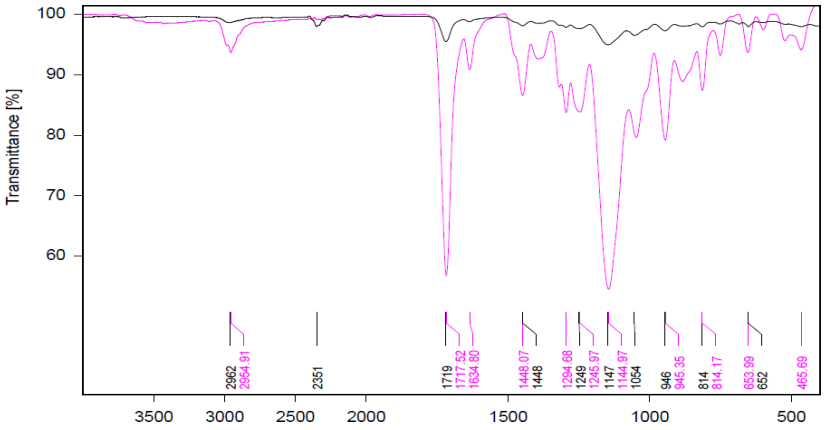

Figure 1. FTIR spectrum of NIP (black) and MIP (pink)

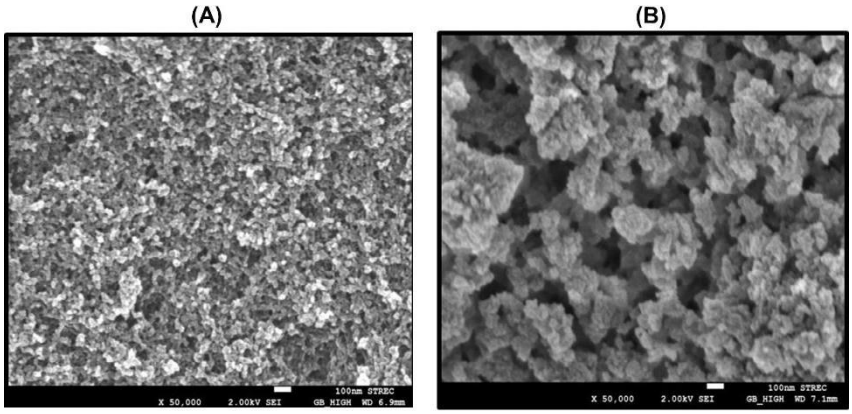

Figure 2. SEM micrographs of polymer (A) nonimprinted and (B) imprinted with carminic acid as the molecule template (the magnify of 50,000x).

The scanning electron micrograph shows the morphology, and the structure of the carminic acid imprinted MIP and its nonimprinted analog. Figure 2 (A) corresponds to the NIP, which was synthesized exactly by the same method but excluding the template. The SEM image shows an appreciable difference in the surface morphology. The NIP had a more uniform and smooth shape than that of the MIP, in which the irregular and rough morphology was distinguished under SEM. It could be described by the fact that the regular morphology attributed to that there is no specific binding for the target carminic acid in NIP. On the contrary, the cavity in the MIP was probably caused by the structure of the template carminic acid.

Preparation of MIP grafted on cellulose membrane

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MIP-grafted cellulose membranes were obtained by in situ polymerizations of a MIP layer on the surfaces of the cellulose membrane. For covalent grafting on a cellulose membrane, the membrane was silanized with ATPES. Figure 3 showed the FTIR spectrum of the bare cellulose membrane (black) and C=C modified cellulose membrane (pink), which can be indicated from the stretching peak at 1711 cm-1. PVDF membrane was also used for silanization, but the membrane was degraded after the silanization procedure. Therefore, it was not suggested to be used as a scaffold (data not shown). The thin film formed on the cellulose membrane could be clearly observed for the MIP-grafted cellulose membrane. Then, removal of the template was performed by soaking it with 10% acetic acid in methanol. The rebinding properties of MIP- grafted cellulose membranes were analyzed.

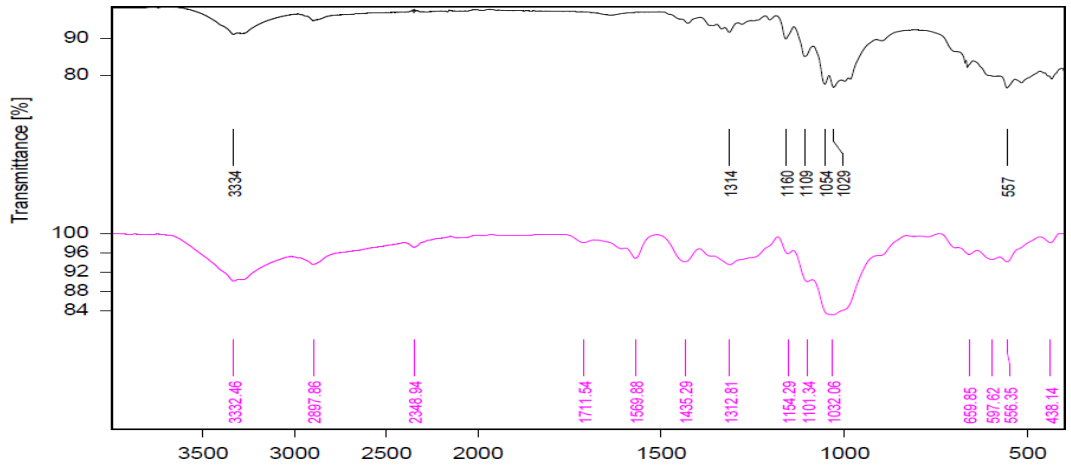

Figure 3. the FTIR spectrum of the bare cellulose membrane (black) and the cellulose membrane after silanization (pink)

Rebinding analysis

Because the MIP was made with the proposed to selectively bind a target, the absorption of the carminic acid at the binding sites was thus analyzed. The molecular recognition of the target at the binding sites or binding cavities is mostly attributed to the non-covalent interaction such as hydrogen bond, ion pairing, and dispersive forces. In addition, steric exclusion of the other interfering compounds from the cavities was also expected (Dorkó et al., 2015). This property is very promising for MIP as similar to the specific binding property of antibody, a biological recognition element. The binding interaction could be explained by the dissociation constant (Kd) and association constant (Ka) of a similar magnitude to antibodies when binding with the proteins such as melting (Vaidya et al., 2001) and trypsin (Hoshino et al., 2008). Besides, MIP has more potential for recognition and binding to the small molecule. It could be explained in the same manner. Therefore, in this study, the binding property of the synthesized solid polymer was characterized by the dissociation constant to explain the specific binding of the binding cavities of the MIP to be potentially used as a sorbent in the solid-phase extraction.

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The rebinding property of MIP towards carminic acid template was performed by the Binding isotherm and the Scatchard analysis as a demonstration in Figures 4 and 5, respectively. The calibration curve of carminic acid in 50% methanol was achieved with the linear equation of y = 5x106 x with the R2 = 0.999. The linear range was obtained at the carminic acid concentration between 0 – 2.0 mM. In Figure 4, the saturated curve of NIP was observed when carminic acid concentration was exceeded 2.5 mM. On the contrary, for MIP, the saturated curve was observed when increasing carminic acid to as higher as 4.5 mM. This is due to the increasing contribution of the specific and non-specific binding site of MIP (Alenazi et al., 2016). Furthermore, the imprinted factor (IF), a parameter that usually used to characterize the molecular recognition abilities of the imprinted polymer, was calculated as 1.94. This value indicated a satisfactory imprinting factor (1.5 – 3) (Zahedi et al., 2016).

The association constant (Ka) and the maximal biding site (Bmax) of MIP can be archived from the Scatchard plot, as was shown in Figure 4. Two straight lines of the high and low-affinity binding site of the synthesized polymer were obtained. The Ka and Bmax calculated from the slope and intercept were summarized in Table 1. The two straight lines fitting the Scatchard equation indicated the affinity of the binding site in the synthesized MIP are heterogeneous, and thus the two association constants have corresponded to the high and low affinity (Hervy and Bicout, 2019).

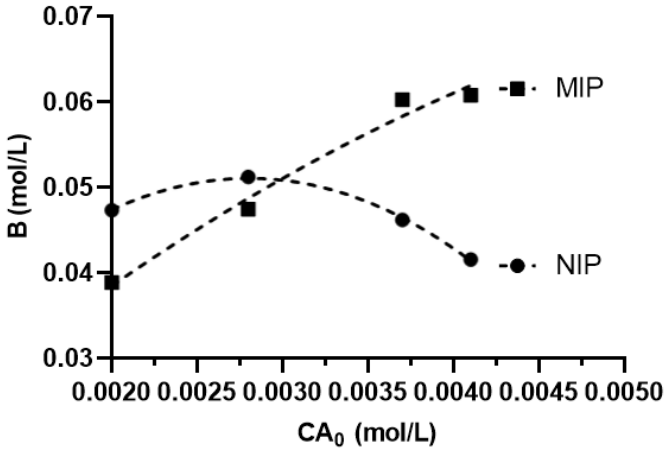

Figure 4. Binding isotherm study for the synthesized MIP and NIP.

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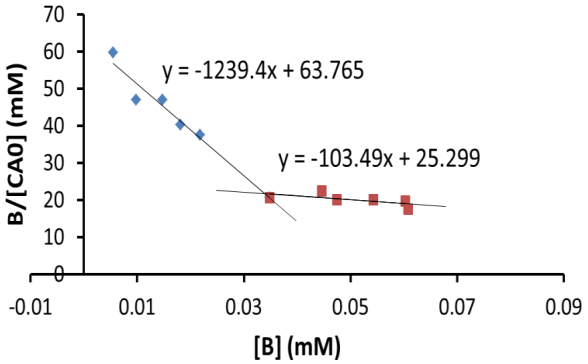

Figure 5. The Scatchard plot of the synthesized MIP. The two straight lines exhibit high and low-affinity binding.

Table 1. Ka and Bmax of high and low-affinity binding of MIP towards the carminic acid template

Polymer	Ka (103 mM)	Bmax (mmol/g of polymer)
MIP (high affinity)	1.24	0.051
MIP (low affinity)	0.10	0.244

CONCLUSION AND FUTURE RESEARCH

In this study, the selective molecularly imprinted polymer to carminic acid was obtained from the co-monomer polymerization technique. The imprinted factor of 1.95 indicated the higher specific binding compared to those that of nonimprinted polymer. The novelty of the immobilized MIP on the modified cellulose membrane was demonstrated using cellulose membrane as a substrate for forming polymerization. The membrane treated with silane derivative provided the functional moiety to react with the MIP. The obtained MIP grafted membrane could thus exhibit superior advantages because of its specificity, mobility, and facility. Nonetheless, it shows the potential to be further applied not limited to the application for the on-site extraction but on-site pre-concentration and sensor.

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REFERENCES

Alenazi, N. A., Manthorpe, J. M. & Lai, E. P. C. 2016. Selectivity Enhancement in Molecularly Imprinted Polymers for Binding of Bisphenol A. Sensors (Basel, Switzerland), 16, 1697.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Andersson, L., Paprica, A. & Arvidsson, T. 1997. A highly selective solid-phase extraction sorbent for pre-concentration of sameridine made by molecular imprinting. J Chromatographia, 46, 57-62.
Bhawani, S. A., Sen, T. S. & Ibrahim, M. N. M. 2018. Synthesis of molecular imprinting polymers for extraction of gallic acid from urine. Chemistry Central Journal, 12, 19.
Bibi, N. S., Galvis, L., Grasselli, M. & Fernández-Lahore, M. 2012. Synthesis and sorption performance of highly specific imprinted particles for the direct recovery of carminic acid. J Process Biochemistry, 47, 1327-1334.
Carvalho, P. & Collins, C. 1997. HPLC determination of carminic acid in foodstuffs and beverages using diode array and fluorescence detection. J Chromatographia, 45, 63-66.
Chianella, I., Piletsky, S., Tothill, I., Chen, B. & Turner, A. 2003. MIP-based solid phase extraction cartridges combined with MIP-based sensors for the detection of microcystin-LR. J Biosensors Bioelectronics, 18, 119-127.
Dorkó, Z., Szakolczai, A., Verbić, T. & Horvai, G. 2015. Binding capacity of molecularly imprinted polymers and their nonimprinted analogs. J Sep Sci, 38, 4240-7.
Guillermin, D., Debroise, T., Trigueiro, P., De Viguerie, L., Rigaud, B., Morlet-Savary, F., Balme, S., Janot, J.-M., Tielens, F. & Michot, L. 2019. New pigments based on carminic acid and smectites: A molecular investigation. J Dyes Pigments, 160, 971-982.
Harp, B. & Barrows, J. 2015. US regulation of color additives in foods. Colour Additives for Foods and Beverages. Elsevier.
Hervy, J. & Bicout, D. 2019. Determining the binding parameters from co-sedimentation assays. Physical Biology.
Hoshino, Y., Kodama, T., Okahata, Y. & Shea, K. J. 2008. Peptide imprinted polymer nanoparticles: a plastic antibody. J Journal of the American Chemical Society, 130, 15242-15243.
Howarter, J. A. & Youngblood, J. P. 2006. Optimization of silica silanization by 3-aminopropyltriethoxysilane. J Langmuir, 22, 11142-11147.
Karuehanon, W., Wongthep, T. & Wanggnorn, C. 2018. Synthesis of Molecularly Imprinted Polymers for Extraction of Quercetin from Mulberry Leaves.
Lancaster, F. E. & Lawrence, J. F. 1996. High-performance liquid chromatographic separation of carminic acid, α-and β-bixin, and α-and β-norbixin, and the determination of carminic acid in foods. J Journal of Chromatography A, 732, 394-398.
Mei, S., Wu, D., Jiang, M., Lu, B., Lim, J.-M., Zhou, Y.-K. & Lee, Y.-I. 2011. Determination of trace bisphenol A in complex samples using selective molecularly imprinted solid-phase extraction coupled with capillary electrophoresis. J Microchemical Journal, 98, 150-155.
Nishizaki, Y., Sato-Masumoto, N., Yokota, A., Mikawa, T., Nakashima, K., Yamazaki, T., Kuroe, M., Numata, M., Ihara, T., Ito, Y., Sugimoto, N. & Sato, K. 2018. HPLC/PDA determination of carminic acid and 4-aminocarminic acid using relative molar sensitivities with respect to caffeine. Food Additives & Contaminants: Part A, 35, 838-847.
Ordoudi, S. A., Staikidou, C., Kyriakoudi, A. & Tsimidou, M. Z. 2018. A stepwise approach for the detection of carminic acid in saffron with regard to religious food certification. J Food chemistry, 267, 410-419.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Pichon, V. & Combès, A. 2016. Selective tools for the solid-phase extraction of Ochratoxin A from various complex samples: immunosorbents, oligosorbents, and molecularly imprinted polymers. J Analytical bioanalytical chemistry, 408, 6983-6999.

Royani, I. & Abdullah, M. 2014. An atrazine molecularly imprinted polymer synthesized using a cooling-heating method with repeated washing: Its physico-chemical characteristics and enhanced cavities.

Ruigrok, V. J., Levisson, M., Eppink, M. H., Smidt, H. & Van Der Oost, J. 2011. Alternative affinity tools: more attractive than antibodies? Biochem Journal, 436, 1-13.

Samari, F., Hemmateenejad, B. & Shamsipur, M. 2010. Spectrophotometric determination of carminic acid in human plasma and fruit juices by second order calibration of the absorbance spectra-pH data matrices coupled with standard addition method. Anal Chim Acta, 667, 49-56.

Silverstein, R. M. & Bassler, G. C. 1962. Spectrometric identification of organic compounds. J Journal of Chemical Education, 39, 546.

Stevenson, D. 1999. Molecular imprinted polymers for solid-phase extraction. TrAC Trends in Analytical Chemistry, 18, 154-158.

Takeo, N., Nakamura, M., Nakayama, S., Okamoto, O., Sugimoto, N., Sugiura, S., Sato, N., Harada, S., Yamaguchi, M., Mitsui, N., Kubota, Y., Suzuki, K., Terada, M., Nagai, A., Sowa-Osako, J., Hatano, Y., Akiyama, H., Yagami, A., Fujiwara, S. & Matsunaga, K. 2018. Cochineal dye-induced immediate allergy: Review of Japanese cases and proposed new diagnostic chart. Allergology International, 67.

Vaidya, A., Lele, B., Kulkarni, M. & Mashelkar, R. 2001. Creating a macromolecular receptor by affinity imprinting. J Journal of applied polymer science, 81, 1075-1083.

Vasapollo, G., Sole, R. D., Mergola, L., Lazzoi, M. R., Scardino, A., Scorrano, S. & Mele, G. 2011. Molecularly imprinted polymers: present and future prospective. J International journal of molecular sciences, 12, 5908-5945.

Yilmaz, U. T., Ergun, F. & Yilmaz, H. 2014. Determination of the food dye carmine in milk and candy products by differential pulse polarography. Journal of Food and Drug Analysis, 22, 329-335.

Zahedi, P., Ziaee, M., Abdouss, M., Farazin, A. & Mizaikoff, B. 2016. Biomacromolecule template-based molecularly imprinted polymers with an emphasis on their synthesis strategies: a review. J Polymers for advanced technologies, 27, 1124-1142.

Zhi, K., Wang, L., Zhang, Y., Zhang, X., Zhang, L., Liu, L., Yao, J. & Xiang, W. 2018. Preparation and evaluation of molecularly imprinted polymer for selective recognition and adsorption of gossypol. 31, e2627

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Response Surface Methodology based Optimization of Microbial Amylase Production using Banana Peels as Carbon Source

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Abstract

Amylase is an enzyme that catalyzes the hydrolysis of polysaccharides such as starch into small units include disaccharides and monosaccharides such as glucose. It is found diversely in different sources, including animals, plants, vegetables, fruits as well as microbes. Amylases of microbial origin are favorable due to many advantages. Besides, microbial enzymes production is more economical compared to other sources. Optimization of enzyme production is quite challenging, especially when it is conducted conventionally due to the many parameters involved. Hence, applying Response Surface Methodology facilitates the design of the experiment and optimizes the production effectively. In this study, three independent variables, namely (A) Temperature, (B) pH, and (C) Banana peels concentration, were selected for the optimization of the amylase production. The result of the study indicated that the run-6 has the highest activity of amylase at 4.10 U/mL, with the optimum temperature at 60°C, pH 6 and 25% (w/v) of banana peels concentration. Further optimization of the amylase production, including recombinant gene expression, different expression hosts, and purification of the crude amylase, are highly recommended.

Keywords: *Amylase, Response Surface Methodology, Banana Peels.*

INTRODUCTION

Amylases were one of the first enzymes to be produced commercially by microorganisms. The use of microorganisms in amylase production is economical as microbes are easy to be manipulated to obtain enzymes of desired characteristics (Nagarajan, Deborah Paripuranam & Umamaheshwari, 2010). Genus Bacillus is known as a good producer of amylase for various applications. Bacillus is widely used for the production of amylase, and these bacteria need a rich source of nutritional medium to grow. Different fruit and vegetable peels are usually considered as waste providing a rich source of starch and nutrients for bacteria (Paul & Sumathy, 2013).

Amylases are amylolytic enzymes that have the ability to degrade starch or glycogen into valuable products, thus representing an important biocatalyst in carbohydrate metabolism (Shobhana et al., 2013). Amylases are identified by the differing in the glycoside bond they attack and are classified into three major classes; α, β, and γ amylase. However, only α-amylase is the most useful

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amylase type in industrial applications, and it is also one of the major classes of amylase that have been identified in microorganisms other than glucoamylase (Paul & Sumathy, 2013). Amylases are used in industry due to advantages such as cost-effectiveness, consistency, less time and space required for production, and ease of process modification and optimization. An increase in the numbers of competitive industries and the application of technology in amylase production leads to improved properties of the product, such as raw starch degrading amylases (Paul & Sumathy, 2013). During the saccharification or liquefaction of starch, amylase is used for wrap sizing of textile fibers, clarification of haze formed in beer or fruits juices, and for pre-treatment of animal feed to improve the digestibility (Shaishta Kokab et al., 2003). However, the cost of production is expensive, and a search for the most cost-effective fermentation strategy does not guarantee obtaining expected results.

Most products produced are being made using enzymes at over 500 industries (Johannes & Zhao, 2006; Kumar & Singh, 2013). Amylase is commonly used in industry due to its increased demand in present-day biotechnology and accounts for about 30% of the world's enzyme production (Swetha Sivaramakrishnan et al., 2006). Besides, Adrio and Demain (2014) reported that amylase has a wide range of applications, including paper industries, in the production of textiles and detergents, in chemical and food and beverages, biofuels, animal feed, sugar syrup, bakery, and pharmaceutical. In industrial sectors, amylase production usually meets process demands, improves the process of fermentation, and has successfully replaced the chemical hydrolysis of starch in starch-processing industries (Vengadaramana, 2013).

Banana wastes are highly rich in carbohydrates and other basic nutrients that support microbial growth (Schiebar & Saldana, 2009). Hence, many suggested that banana peels could be utilized as a promising carbon source for the production of amylase (Paul & Sumathy, 2013). Amylases are one of the main well-known enzymes used in industry. It is an amylolytic enzyme that degrades starch or glycogen. Thus, amylase represents a group of catalytic proteins providing great importance in carbohydrate metabolism (Shobhana, Pooja, Komal & Sayali, 2013). Industrially, amylases are important in many applications, including foods, brewing, baking, textile, analytical chemistry, detergent, and pharmaceutical industries (Krishna et al., 2012). The demand for amylase production increases due to its advantageous characteristics thus has attracted various industries in developing enzymes with better properties.

As a waste, banana peel is commonly disposed of once being used or consumed. Approximately more than 10kg of banana is used in each local stall per day (personal communication), leaving massive of wastes being disposed of due to its non-functional value. In the process of these products, the banana peel accumulates in bulk leading to a serious problem to the environment (Krishna, Srivastava, Ramaswamy, Suprasanna & D'Souza, 2012). Hence, emphasizing the use of this waste as a cheap carbon source plays a significant potential in the application of agro-industrial wastes. Several efforts have been accomplished to reduce the negative impacts brought upon these wastes, at the same time utilizing them for the production of enzymes.

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The high production cost of enzymes has been one of the major barriers to their commercial production in many countries; alternatively, utilizing cheap carbon sources may reduce the cost of the production. In addition, banana peels are considered as one of the massive wastes being disposed of daily due to their non-functional value. Thus, banana fruits that are widely available in Malaysia can be used as a cheap carbon source due to the fact that banana peels are rich in starch composition, which makes them suitable as a medium for bacterial growth.

The RSM is a collection of mathematical and statistical techniques designed to analyze the effects of different independent variables in which a response of interest is influenced. Thus, the response can be optimized by simplifying the multi-parameters of variables simultaneously (Lee, Chung & Hung, 2004). The RSM emphasis designing, formulating, modeling, developing, and analyzing scientific data and products (Bradley, 2007). The optimization of enzyme production can be done by using the Response Surface Methodology (RSM) in order to seek the optimum conditions for a multivariable system (Shakti may Kar, Tapan Kumar Datta & Ramesh Chandra Ray, 2010). The RSM provides a mathematical model and statistical techniques designed to analyze the experimental data on the different effects of independent variables, as well as to provide an interaction prediction between the response and the variables. (Granato & Calado, 2014).

RESEARCH METHOD

Qualitative Enzyme Determination
The thermophilic bacteria culture sample was inoculated and streaked onto the plates containing Nutrient Agar (NA) added with 2% of starch. These plates were then placed in a ziplock bag of 60°C incubators for 24 h. On the next day, an iodine solution was flooded onto the bacterial plates and left for two h at room temperature.

Determination of Quantitative Enzyme
The quantitative enzyme assay was conducted according to the method described by Bernfeld (1955). An amount of 0.2 mL crude enzyme was added to a reaction mixture comprising 0.8 mL of 2 percent (v/v) soluble starch as a substrate (pH 7). The mixture was incubated in a waterbath at 60°C. After that, 2mL of DNS reagent was added to each test tube holding the combination, which was then boiled for 5 minutes in a boiling waterbath to stop the reaction before being allowed to cool at room temperature, then measured the absorbance at 540 nm.

Optimization of pH of the Enzyme
The optimal pH for thermophilic bacterial growth was identified by assaying amylase at various pH ranges from pH 4 to pH7. The test was conducted by aseptically inoculating a thermophilic bacterial sample into 100mL of sterile NB in a 250mL conical flask. Each of the conical flasks was adjusted to the appropriate pH and incubated at 60°C for 24 hours. After 24 h of incubation, the crude enzyme was harvested by centrifugation at 10,000 rpm for 10 min. The crude enzyme was then used to perform a quantitative enzyme assay.

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Effect of Banana Peels and Starch as Carbon Source in the Production Media

In order to determine the ability of banana peels waste served as a natural carbon source to effective produce enzyme amylase in comparison to commercial carbon source, in this case 2% (w/v) of starch was used which served as a control experiment. 20g of banana peels and 2% of starch were added separately in conical flask containing NB, adjusted to pH 6. After inoculation of thermophilic bacteria, the flasks were incubated at 60°C for 24 h. After 24 h, the contents were collected and proceed with the quantitative enzyme assay.

Effect of Different Banana Peels Concentrations on Enzyme Production

To determine the optimal concentration of banana peel waste for bacterial growth, different peel concentrations ranging from 5-35 percent (w/v) were examined. Banana peels were weighed per 100mL of NB in grams (5-35 g). Each conical flask containing the mixture was adjusted to pH 6 (the optimal pH determined in step 3), labeled, and autoclaved for 15 minutes at 121°C. Each conical flask containing varying banana peel concentrations received 500uL of inoculum. The bacterial culture was kept at 60°C for 24 hours. The samples were collected the next day for the quantitative enzyme test.

Optimization of Amylase Production Using RSM

In this study, for the optimization of amylase enzyme production, three independent variables were selected, namely, (A) Temperature, (B) pH, and (C) Banana peel concentration, which was determined and designed using a Response Surface Methodology (RSM) software; Design Expert 10. Each independent variable was designed using Central Composite Design (CCD) at differently designed levels, namely; Low level (-1) and High level (+1), with a total set of 2 blocks and 20-set run experiments adjusted to Face Centered Central Composite Design (FCCCD) with the axial (star) points set at 1.0 coded units from CCD choice. Once the selected range of factors was chosen and entered as low/high level of variables, the effects of different independent variables were analyzed, thus predicting the interest optimum variable responses (Lokeswari, 2010).

The optimal point in optimization of the amylase enzyme was predicted using a second-order model (quadratic model) equation which can be expressed in equation (eq.3). y is a dependent variable or also known as the optimum value of amylase enzyme (IU/mL/min). $\beta 0$ is an intercept, $\beta 1$, $\beta 2$, and $\beta 3$ are linear, quadratic, and interaction coefficients, while xABC are factors (independent variables) and lastly ε is the error. Statistical significance can be determined from the evaluation of ANOVA, including the variance and coefficient of determination value (R2).

FINDINGS AND DISCUSSION

Qualitative Enzyme Determination

The qualitative enzyme determination was successfully conducted. The formation of a clear zone around the bacterial colonies indicated the positive amylase production by the isolate, as indicated

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in Figure 1. The formation of the clearing zone has been explained due to the ability of the amylase enzyme to hydrolyze the substrate (2% w/w starch) into smaller molecules such as maltose or glucose. The appearance of blue color on the NA palate, on the other hand, was due to the reaction of the iodine solution when it was flooded on the surface of the NA plate, with the starch present in the NA. Therefore, the result of the experiment has confirmed the production of the thermostable amylase by the isolated bacteria.

Figure 1. The qualitative determination of thermostable amylase on NA plate supplemented with 2% of commercial starch.

pH Optimization of the Enzyme

The optimum pH for the production of thermostable amylase by the isolates was found at pH 6.00. Figure 2 shows that at this pH, the maximum amylase enzyme activity (1.4 U/ml) was recorded. Further raising or decreasing the pH resulted in reduced enzyme activity.

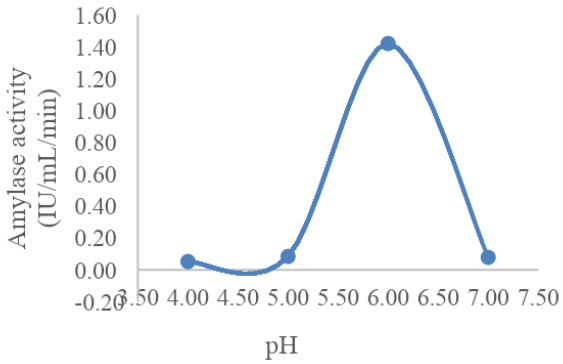

Figure 2. Optimum pH for thermostable amylase production by the isolate 3.

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Effect of Carbon Source from Banana Peels and Commercial in the Production Media

According to Happi et al. (2011) an average of 7.0-15.0 % of starch was reported in the banana peels, while Bezerra, Cruz Rodrigues, Amante, and Silva (2013) reported an average of 10.0 % of starch in the banana peels waste. In the present study, it is estimated about 100g of banana peels is equivalent to ≈10g of starch. Hence, 2g of the identical number of starches in 2% (w/v) of commercial starch and 20g banana peels were used in a comparison between commercial starch and banana peels starch (Table 1).

Table 1. Comparison between commercial starch and banana peels starch

Carbon source	Amount used in the experiment (g)	Starch content (g)	Amylase activity (IU/mL/min)
Commercial starch	2.00	2.00	1.49
Banana peel waste	20.00	2.00	2.02

The enzyme activity produced by the isolate cultivated in commercial starch-containing media and the isolate cultured in banana peel waste-containing media were successfully compared. Table 1 shows that 2g of commercial starch produced 1.49 U/mL of amylase activity, while 20g of banana peels produced 2.02 U/mL. Hence, the enzyme activity in media containing banana peels was found higher than that in media containing commercial starch. Thus, the result indicated banana peels had been preferable as a carbon source. Shobhana et al. (2013) also endorsed the use of banana peels as a less expensive carbon source for amylase production. Lokeswari (2010) reported that the media which contain banana agro-residual waste (banana peels) resulted in maximum productivity. Thus, banana peels produced a higher amylase production than any other agricultural waste such as citrus fruits, potatoes, wheat bran, etc. Chandrashekhar Unakal et al. (2012) also reported that production of enzyme by utilization of banana waste employed by fermenting organism gave a considerable interest can be given in using banana waste as an alternative source of amylase production, which is economical and profitable due to the inherent nature of the banana waste itself.

Effect of Different Banana Peels Concentrations on Enzyme Production

The content of banana peels was indicated as a percentage calculated in gram per 100mL volume of Nutrient Broth. As an alternative for commercial starch, the thermostable amylase-producing bacteria were cultivated on a culture including banana peel wastes. Banana peels were present in concentrations ranging from 5-35 percent (w/v). The result of the study as indicated (Figure 3) 25 % banana peels was found to produce the maximum activity of the amylase (2.49 U/mL).

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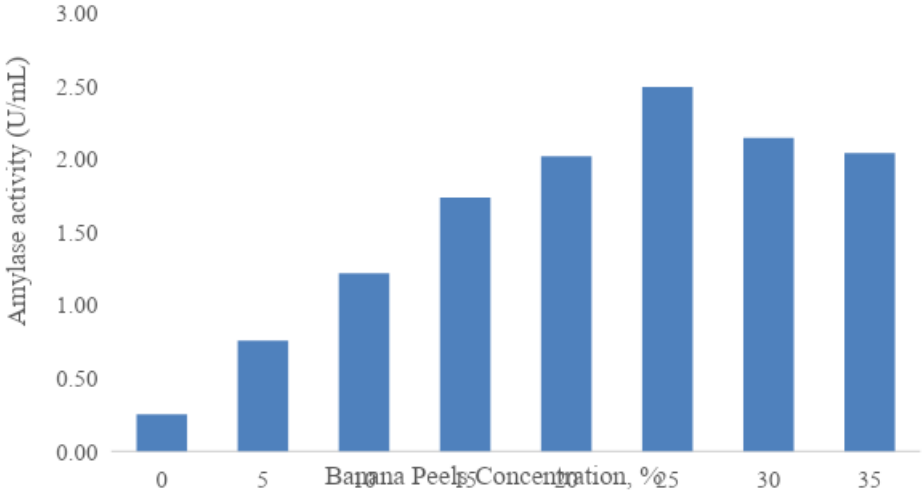

Figure 3: Effect of banana peels as alternative carbon source on the amylase production.

Considering 25g of 25% banana peels concentration produced the highest amylase activity, hence 25% of banana peels were the optimum concentration for the amylase enzyme production. A further increased in the concentration did not increase the amylase activity (Shobhana et al., 2013), in this case 25% as optimum concentration while increasing the banana peels concentration did not affect the growth of organism (Paul & Sumathy, 2013). Given that 25g of 25% banana peels produced the maximum amylase activity, therefore 25% banana peels was the optimal concentration for amylase enzyme synthesis. Further increased in concentration did not boost amylase activity according to Shobhana et al. (2013). From the experiment, with 25% increased of the banana peel concentration did not give any effect on the bacterial growth (Paul & Sumathy, 2013). The amylase activity in the negative control (growth media without banana peel wastes) showed the lowest enzyme activity (0.252 U/mL). The ideal concentration of banana peels, on the other hand, is dependent on the type of banana utilised, as well as the production conditions (temperature, pH, etc.). In the meantime, the amount of starch in a banana depends on how it is ripened (texture, colour, etc.). Green bananas are believed to have more starch than yellow bananas (Shobhana et al., 2013; Paul & Sumathy, 2013). The inoculum size was also found to be equally important factor for the production of amylase (Krishna et al., 2012).

5. Optimization of Process Parameters Using RSM

Once the predicted optimal variables were obtained from optimization using RSM design, the experiments were conducted according to the interest variables, hence quantitatively assayed at 540nm to determine the amylase activity or in this case being called response (Table 2). The results produced were in a form of evaluation, analysis of variation (ANOVA) to determine the

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regression model equation from this relationship, model graphs in variety of representations and post analysis. Using the RSM design called a Central Composite Design (CCD), chosen from the predicted equation fitting for a quadratic surface which was suitable and worked well for process optimization. The layout was designed respectively and measured according to the values recommended and designed by the CCD. According to Table 2, run-6 has the highest activity of amylase at 4.10 U/mL, conducted experimentally at 60°C, pH 6 and 25% (w/v) of banana peels concentration. The lowest amylase activity recorded experimentally gave out at 1.87 U/mL as run-4 (15% substrate concentration; pH 5; 50°C). From the relationship of the designed process parameters and responses ("Experimental") obtained as amylase activity, further quadratic regression model was analysed in order to predict the "Predicted" responses tabulated in Table 2.

Table 2. Summary of response data using RSM design

Run	Block	Parameters			Response, Amylase activity (IU/mL/min)	
		Temp, °C (A)	pH (B)	Peels Conc., % (C)	Experimental	Predicted
1	Block 1	60.00	6.00	25	3.89	3.77
2	Block 1	70.00	7.00	35	3.40	3.30
3	Block 1	50.00	5.00	15	2.48	2.15
4	Block 1	50.00	7.00	15	1.87	1.78
5	Block 1	50.00	5.00	35	3.51	3.36
6	Block 1	60.00	6.00	25	4.10	3.77
7	Block 1	60.00	6.00	25	3.86	3.77
8	Block 1	70.00	5.00	15	2.01	1.90
9	Block 1	70.00	7.00	15	2.05	1.78
10	Block 1	70.00	5.00	35	3.51	3.17
11	Block 1	60.00	6.00	25	3.91	3.77
12	Block 1	50.00	7.00	35	3.57	3.25
13	Block 2	50.00	6.00	25	3.66	3.96
14	Block 2	70.00	6.00	25	3.64	3.86
15	Block 2	60.00	6.00	15	2.33	2.54
16	Block 2	60.00	6.00	25	3.80	3.77
17	Block 2	60.00	5.00	25	2.70	3.04
18	Block 2	60.00	6.00	25	3.71	3.77
19	Block 2	60.00	7.00	25	2.74	2.93
20	Block 2	60.00	6.00	35	3.58	3.90

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REFERENCES

Adrio, J. L., & Demain, A. L. (2014). Microbial enzymes: Tools of biotechnological processes. Biomolecules, 4(1), 117-139.
Bernfeld P (1955). Amylase α and β, In Methods in Enzymology (Colowick SP, Kaplan NO, ed.), Academic Press Inc, New York 1: 149-158.
Bezerra, Vieira, C., Rodrigues, Cruz, A. M., Amante, Regina, E., & Silva, L, H, M. (2013). Nutritional potential of green banana flour obtained by drying in spouted bed. Revista Brasileira de Fruticultura, 35(4), 1140-1146.
Bradley, N. (2007). The response surface methodology. Unpublished master's thesis, Indiana University of South Bend, South Bend. Retrieved February 22, 2016, from https://www.iusb.edu/.
Chandrashekhar Unakal, Radha, I. K., & Basappa, B. K. (2012). Production of a-amylase using banana waste by Bacillus subtilis under solid state fermentation. European Journal of Experimental Biology, 2(4), 1044-1052.
Granato, D., & Calado, V. M. A. (2014). The use and importance of design of experiments (DOE) in process modelling in food science and technology (1st ed.).
Happi, E. T., Bindelle, J., Agneesens, R., Buldgen, A., Wathelet, B., & Paquot, M. (2011). Ripening influences banana and paintain peels composition and energy content. Trop. Anim. Health Production, 43(1), 171-177.
Johannes, T. W., & Zhao, H. (2006). Directed evolution of enzymes and biosynthetic pathways. Current Opinion Microbiology, 9, 261–267.
Krishna, P. R., Srivastava, A. K., Ramaswamy, N. K., Suprasanna, P., & D'Souza, S. F. D. (2012). Banana peel as a substrate for a-amylase production using Aspergillus niger NCIM 616 and process optimization. Indian Journal of Biotechnology, 11, 314-319.
Kumar, A., & Singh, S. (2013). Directed evolution: Tailoring biocatalysis for industrial application. Crit. Rev. Biotechnol.,33, 365–378.
Lee, I. T., Chung, H. W., & Hung, C. C. (2004). Optimization of multiple responses using principal component analysis and technique for order preference by similarity to ideal solution. International Journal of Advanced Manufactured Technology, 27, 407-414.
Lokeswari, N. (2010). Statistical optimization of experimental variables associated with production of alpha amylases by Bacillus subtilis using banana agro-residual wastes in solid-state fermentation. Rasayan Journal of Chemistry, 3(1), 172-178.
Nagarajan, M., Deborah Paripuranam, T., & Umamaheswari, S. U. (2010). Efficient production of alpha-amylase from agro residues using Bacillus subtilis. Journal of Chemical and Pharmaceutical Research, 2(4), 442-448.
Paul, M. S., & Sumathy, V. J. H. (2013). Production of a-amylase from banana peels with Bacillus subtilis using solid state fermentation. International Journal of Current Microbiology and Applied Sciences, 2(10), 195-206. Retrieved February 3, 2016, from http://www.ijcmas.com.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Schiebar, A., & Saldana, M. D. A. (2009). Patato peels: A source of nutritionally and pharmacologically interesting compounds. Food, 3(2), 23-29.
Shaista, K. M., Asghar, K., Rehman, M. J. A., & Adedyo, O. (2003). Bio-processing of banana peel for alpha amylase production by Bacillus subtilis. International Journal of Agricultural Biology, 5(1), 36-39.
Shaktimay Kar, Tapan Kumar Datta, & Ramesh Chandra Ray. (2010). Optimization of thermostable a-amylase production by Streptomyces erumpens MTCC 7317 in solid-state fermentation using cassava fibrous residue. Brazilian Archives of Biology and Technology, 53(2), 301-309.
Shobhana, A. J., Pooja, K. K., Komal, K. B., & Sayali, A. C. (2013). Amylase production from potato and banana peel waste. International Journal of Current Microbiology and Applied Science, 2(11), 410-414. Retrieved February 3, 2016, from http://www.ijcmas.com.
Swetha Sivaramakrishnan, Dhanya Gangadharan & Kesavan Madhavan Nampoothiri. 2007. In R. S. Carlos, & P. Ashok, Alpha amylase production by Aspergillus oryzae employing solid state fermentation. Journal Scientific Industrial Res., 66, 621 626.
Vengadaramana, A. (2013). Industrial important microbial alpha-amylase on starch-converting process. Scholar Academic Journal of Pharmacy (SAJP), 2(3), 209-221.

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Influence of Storage Temperature on the Quality of *Geniotrigona thoracica* Honey

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Abstract

Stingless bee honey is well-known for its high content of moisture compared to Apis mellifera honey. This study aimed to investigate the influence of different temperatures used to reduce moisture content in honey using clay pots. The Geniotrigona thoracica honey was kept in clay pots for 10 days at 25 °C and 35 °C, and the changes in its properties were evaluated on the moisture content, total soluble solids, viscosity, pH, free acidity, and colour. Honey stored at 35 °C reduced moisture content by <20% in 3 days while honey at 25 °C took 7 days. Free acidity was found higher (113 meq/kg) in the sample stored at 35 °C for 3 days compared to honey stored at 25 °C for 7 days (106 meq/kg). From this study, the suitable temperature and the use of clay pots was proved to reduce the moisture content in honey.

Keywords: *clay pots, Geniotrigona thoracica, moisture content, physicochemical, stingless bee honey*

INTRODUCTION

The sting bees or stingless bees produced a natural product called honey, and it is well-known for its rich nutritional content and medicinal properties (Rao, Krishnan, Salleh, & Gan, 2016). By owing to the fact that stingless bee honey contained more health benefits than honey bees honey (Amin et al., 2018), many opportunities in research field available.

The distinctive characteristics of stingless bee honey from ordinary honey was it contained higher moisture and acidity (De Almeida-Muradian et al., 2013; Alvarez-Suarez et al., 2018), which were feared to cause rapid fermentation that can deteriorate the honey quality (Nordin et al., 2018; Silva et al., 2016; Subramanian et al., 2007). The International Honey Commissions (IHC) had set the limit for moisture content of a good quality of honey must <20%. Therefore, several methods had been used by the bee farmer to lessen the level of moisture in honey such as the use of a dehumidifier, conventional heating, and open tray drying. Previous studies also discovered several methods for reducing the moisture content in stingless bee honey but found that the thermal treatment caused the increase in hydroxymethylfurfural (HMF) content (Yap, Chin, Yusof, & Chong, 2019; Zarei, Fazlara, & Tulabifard, 2019).

In this present study, the use of clay pots was implemented to store the stingless bee honey to reduce its moisture content. The moisture from the honey diffused through the clay pots' wall and

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evaporated to the surrounding air due to the porosity of the clay pots. The aim of this work was to evaluate the physicochemical properties of Geniotrigona thoracica honey after storing in clay pots at different temperatures.

RESEARCH METHODOLOGY

Material and sampling

The G. thoracica honey was freshly harvested from Orchard 10, UPM, Malaysia in October 2018 and immediately transported to the laboratory. About 50 g of honey were filled into 40 cylindrical clay pots (Figure 1) and divided into half to be placed in an incubator at 25 ± 2 °C (room temperature; RT) and 35 ± 2 °C (elevated temperature; ET), respectively, until the moisture reduced to <20 %.

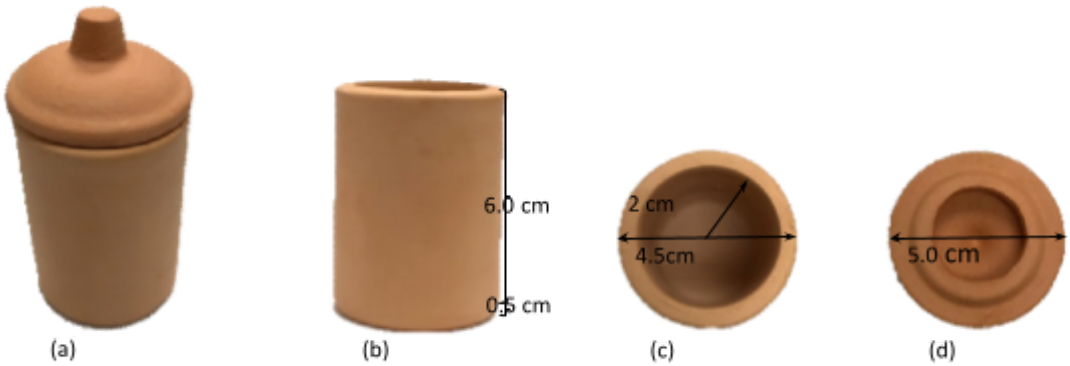

Figure 1. The dimension of the cylindrical-shaped clay pot

Physicochemical determinations

1. Moisture content and total soluble solids (TSS) content

The moisture content and TSS content of honey samples were measured using an Abbe refractometer by obtaining the refractive index for moisture content calculation to the method mentioned in (Ghazali et al., 2019), while for TSS content obtained in term of °Brix value from the refractometer.

2. Viscosity

Honey viscosity was determined using a rheometer (AR-G2, TA Instruments, New Castle, USA) equipped with a 60-mm diameter cone and plate geometry (30 mm truncation gap, 1˚ cone angle). The analysis were performed as a steady-state flow with a shear rate range of 1–1000 s-1.

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3. pH and free acidity

AOAC method 962.19 was used in determination of pH and free acidity of honey. Honey solution of 10 g of honey mixed with 75 mL distilled water was prepared and the pH meter (Sartorius, PB-10) was used to measure the pH value. Titration of the solution using 0.1 M NaOH until pH 8.30 was carried out and the volume of the NaOh used was noted. To obtain the free acidity value which expressed as miliequivalent acid per kg of honey (meq/kg), the volume (mL) of 0.1 M NaOH used to titrate the solution was multiplied by 10.

4. Colour

An UltraScan Pro spectrophometer (HunterLab, Reston, VA, USA) was used to measure the colour parameters (L*, a*, and b*). The Pfund method was used to measure the colour intensity of the honey (Sousa et al., 2016). About 5 g of honey was diluted in 10 mL of ultrapure water to prepare the honey solution and a UV-VIS spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, Piscataway, NJ, USA) was used to the determine the solution absorbance at 636 nm. The value in the Pfund scale was obtained by inserting the absorbance value in equation 1.

$mmPfund = -38.7 + 371.4 \times Abs$ (1) (Where Abs is absorbance).

Statistical analysis

Microsoft Excel was used to analyse all the collected data which were repeated three time. The data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The moisture content of the honey stored at RT and ET were shown in Figure 2. Initially, the honey contained 26.03 ± 0.09 % of moisture which fell in the range of the moisture content of stingless bee honey in Malaysia (Jibril, Abu Bakar, Ismail, & Manivannan, 2016). At ET, the moisture content of the honey decreased drastically and reached moisture level <20% on day 3 while for the honey sample in RT the moisture content reduced to <20% on day 7. The results indicated that the temperature influenced the rate of evaporation of moisture from the clay pots wall. Meanwhile, the TSS content of the honey samples (Table 1) increased when the moisture content decreased. The initial value of the TSS content of the honey was 72.5 ± 0 °Brix. Samples in ET had the highest TSS content on day 3.

The honey initial viscosity was 0.238 ± 0.012 (Table 1). The value of the viscosity increased after storage for both conditions and the values corresponding with the moisture content decrement (Yanniotis et al., 2006). The honey viscosity stored in ET had the highest increment with a value of 2.64 ± 0.29 Pa.s on day 3 compared to honey in RT which had the viscosity of 1.1 ± 0.1 Pa.s on day 7.

On day 0, the value of honey acidic pH (3.42 ± 0.01) was shown in Table 1. Samples in RT showed a slight decrease in pH value after 7 days of storage. Honey free acidity on day 0 (96 ± 2 meq/kg) was lower than the reported value for Thailand stingless bee honey (164 ± 162 meq/kg) (Chuttong et al., 2016). The free acidity of honey samples in ET increased rapidly until day 3 and

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had the highest free acidity value compared to the samples stored at RT after 7 days. The higher value of free acidity in ET samples might due to the higher temperature than RT that could influence the chemical reactions between sugars and amino acids (Samira, 2016).

The colour parameter of L* indicates the lightness of the honey samples increased for both samples in different storage temperatures. The a* (redness/ greenness) and b* (yellowness/blueness) for both samples in different storage temperatures also showing the increasing trend. For colour intensity, the colour of the honey was dark (>114 mmPfund) according to the USDA. Both honey samples stored in RT and ET showed increasing values in colour intensity based on the Pfund scale and these results correspond to the decrease in moisture content. Colour intensity of the honey is related to pigments presented in honey which has antioxidant activities such as flavonoids and carotenoids, therefore the honey in our studies contained high antioxidants due to its high colour intensity (Kek, Chin, Yusof, Tan, & Chua, 2014).

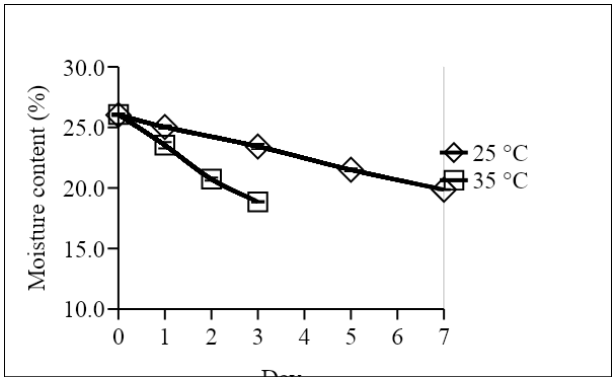

Figure 2. Moisture content of G. thoracica honey at 25 and 35 °C

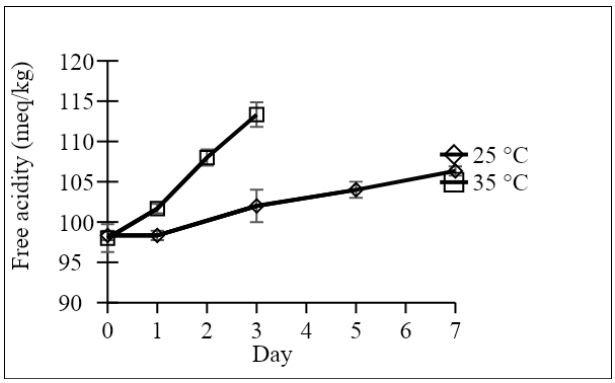

Figure 3. Free acidity of G. thoracica honey at 25 and 35 °C

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Table 1. Physicochemical properties of G. thoracica honey at 25 and 35 °C

Storage temperature (°C)	Day	TSS	Viscosity (Pa.s)	pH	L*	a*	b*	Colour intensity (mmPfund)
25	0	72.5 ± 0	0.238 ± 0.012	3.42 ± 0.01	29.6 ± 0.1	0.58 ± 0.02	4.57 ± 0.12	150 ± 2
	1	73.5 ± 0.1	0.465 ± 0.028	3.4 ± 0	28.4 ± 0	1.01 ± 0.07	5.09 ± 0.03	150 ± 1
	3	74.8 ± 0.1	0.636 ± 0.01	3.42 ± 0	29.3 ± 0	0.80 ± 0.01	5.01 ± 0.05	155 ± 1
	5	77 ± 0.2	0.782 ± 0.04	3.43 ± 0	29.8 ± 0	0.78 ± 0.05	4.82 ± 0.12	158 ± 1
	7	78.6 ± 0	1.1 ± 0.1	3.39 ± 0	30.1 ± 0	0.70 ± 0.06	5.07 ± 0.11	162 ± 1
35	0	72.5 ± 0	0.238 ± 0.012	3.42 ± 0.01	29.6 ± 0.1	0.58 ± 0.02	4.57 ± 0.12	150 ± 2
	1	75.0 ± 0.2	0.465 ± 0.028	3.44 ± 0	28.4 ± 0.0	0.49 ± 0.13	3.81 ± 0.12	156 ± 1
	2	77.7 ± 0.1	1.1 ± 0.1	3.42 ± 0	31.8 ± 0	0.92 ± 0.06	6.27 ± 0.07	157 ± 1
	3	79.5 ± 0	2.64 ± 0.29	3.43 ± 0	34.2 ± 0	1.31 ± 0.1	8.14 ± 0.15	160 ± 1

CONCLUSIONS

The clay pots did help in lowering the level of moisture content of honey and the difference in storage temperature influenced the rate of moisture reduction through the clay pots' wall. Although the honey that kept in clay pots at room temperature (RT) had reduced the moisture content slower (7 days) compared to ET (3 days), the honey in RT showed better honey quality even though stored at a longer period than in ET. Therefore, honey stored in RT was a better condition to store honey in clay pots to reduce its moisture which is also easier for the bee farmer to handle and cost-effective.

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REFERENCES

Alvarez-Suarez, J. M., Giampieri, F., Brenciani, A., Mazzoni, L., Gasparrini, M., González-Paramás, A. M., … Battino, M. (2018). Apis mellifera vs Melipona beecheii Cuban polifloral honeys: A comparison based on their physicochemical parameters , chemical composition and biological properties. LWT - Food Science and Technology, 87, 272–279. https://doi.org/10.1016/j.lwt.2017.08.079

Amin, F. A. Z., Sabri, S., Mohammad, S. M., Ismail, M., Chan, K. W., Ismail, N., … Zawawi, N. (2018). Therapeutic properties of stingless bee honey in comparison with European bee honey. Advances in Pharmacological Sciences, 2018, 6179596.

Chuttong, B., Chanbang, Y., Sringarm, K., & Burgett, M. (2016). Physicochemical profiles of stingless bee (Apidae : Meliponini) honey from South East Asia (Thailand). Food Chemistry, 192, 149–155. https://doi.org/10.1016/j.foodchem.2015.06.089

De Almeida-Muradian, L. B., Stramm, K. M., Horita, A., Barth, O. M., Da Silva de Freitas, A., & Estevinho, L. M. (2013). Comparative study of the physicochemical and palynological characteristics of honey from Melipona subnitida and Apis mellifera. International Journal of Food Science and Technology, 48(8), 1698–1706. https://doi.org/10.1111/ijfs.12140

Ghazali, N. S. M., Yusof, Y. A., Ling, C. N., Othman, S. H., Manaf, Y. N. A., & Baroyi, S. anis H. M. (2019). The Application of Clay Pot for Moisture Reduction of Geniotrigona thoracica Stingless Bee Honey. International Journal on Advanced Science Engineering Information Technology, 9, 2028–2034.

Jibril, F. I., Abu Bakar, M. H., Ismail, S., & Manivannan, L. (2016). Physicochemical characteristics of Malaysian stingless bee honey from Trigona species. International Medical Journal Malaysia, 17, 187–191.

Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W., & Chua, L. S. (2014). Total phenolic contents and colour intensity of Malaysian honeys from the Apis spp. and Trigona spp. bees. Agriculture and Agricultural Science Procedia, 2, 150–155. https://doi.org/10.1016/j.aaspro.2014.11.022 Nordin, A., Sainik, N. Q. A. V., Chowdhury, S. R., Saim, A. Bin, & Idrus, R. B. H. (2018). Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. Journal of Food Composition and Analysis, 73, 91–102. https://doi.org/10.1016/j.jfca.2018.06.002

Rao, P. V., Krishnan, K. T., Salleh, N., & Gan, S. H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees : a comparative review. Brazilian Journal of Pharmacognosy, 26, 657–664. https://doi.org/10.1016/j.bjp.2016.01.012

Samira, N. (2016). The effect of heat treatment on the quality of Algerian honey. Researcher, 8. https://doi.org/10.7537/marsrsj080916.01

Silva, P. M. da, Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey : Chemical composition , stability and authenticity. Food Chemistry, 196, 309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

Subramanian, R., Hebbar, H. U., & Rastogi, N. K. (2007). Processing of honey : a review.

ISSN 2828-1500 (Online) | 2828-1985(Print)

International Journal of Food Properties, 10, 127–143. https://doi.org/10.1080/10942910600981708

Yanniotis, S., Skaltsi, S., & Karaburnioti, S. (2006). Effect of moisture content on the viscosity of honey at different temperatures. Journal of Food Engineering, 72, 372–377. https://doi.org/10.1016/j.jfoodeng.2004.12.017

Yap, S. K., Chin, N. L., Yusof, Y. A., & Chong, K. Y. (2019). Quality characteristics of dehydrated raw Kelulut honey. International Journal of Food Properties, 22(1), 556–571. https://doi.org/10.1080/10942912.2019.1590398

Zarei, M., Fazlara, A., & Tulabifard, N. (2019). Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon, 5, eD1894. https://doi.org/10.1016/j.heliyon.2019.e01894

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Improving the security of the organization from the shadow IoT using Blow-fish encryption algorithm

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Abstract

Smart cities, traffic congestion, waste management, structural health, security, emergency services, logistics, retail, industrial control, and health care are just a few of the applications that the Internet of Things (IoT) can help with. The Internet of Things (IoT) is a mega-technology that can connect to anything, anybody, at any time, place, platform, and network. It has a significant impact on the entire blockchain of enterprises, smart objects, and devices, systems, and services provided by heterogeneous network connection (HNC) and is being developed as a smart pervasive framework for smart devices. Because IoT devices link to complicated equipment, interact with hostile surroundings and are deployed on a variety of unregulated platforms, they confront several security risks and challenges. Because the Internet of Things (IoT) has the capacity to integrate any sort of network or sophisticated system, it may be vulnerable to vulnerabilities inherent in the separate systems that make up the integrated network. The purpose of this research paper is to investigate the security issues that individual systems responsible for IoT interconnection face, as well as their impact on the overall IoT system.

Keywords: *IoT, Shadow IOT, Congestion, HNC*

INTRODUCTION

Physical items can share data, coordinate operations, adapt swiftly to environmental changes and effectively utilize their resources thanks to the Internet of Things. Smart homes, smart cities, healthcare, agriculture, and environmental monitoring are all examples of IoT environments that have become more prevalent in recent years. The Internet of Things (Ashton, 2009) consists of many dispersed sensor nodes and actuators that can gather important information from the environment for individual users via wireless media. The data is forwarded to a gateway node with high-performance computing resources that can be trusted. According to IDC, by 2025, there will be over 41 billion linked IoT devices, generating 79.4 zettabytes (ZB) of data. This can have two effects: an increased likelihood of getting shadow or rogue IoT devices added to the network and an increase in the number of IoT devices added to the network. Second, there will be a massive data influx that will put data security concerns to the test. The inherent restricted computing capabilities of IoT devices, weak network protocols, vulnerable environment, and users' willingness to utilize default device credentials make them more vulnerable to security threats.

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Because data is transmitted through an insecure and fragile medium, it's critical to keep it safe from dangers like unauthorized access, manipulation, and unlawful eavesdropping. While impersonating legitimate users, malicious attackers attempt to enter, edit, and delete data in order to obtain sensitive data. The majority of these vulnerabilities might be addressed by using IoT-specific authentication methods. Mutual authentication (Moon et al., 2016) is a critical component in ensuring the integrity of both the device and the application. A number of secure IoT mutual authentication and key exchange techniques have been developed, with differing levels of security. Confidentiality, data integrity, availability, and other security requirements should be given via a dependable and efficient authentication mechanism that can withstand various security threats while consuming minimal communication and processing resources on the IoT node.

Artificial intelligence is also being used by researchers to develop IoT-based applications for efficient energy management and distribution, smart homes, and health care, among other things. These metaheuristic approaches, which have increased in popularity among optimization scholars, are effective in the vast majority of real-world situations. To optimize IoT-driven applications and network optimization, several researchers have used metaheuristic and heuristic algorithms that mimic biological and other physical phenomena. They use tried-and-true genetic processes to build frameworks for search algorithms that require the least amount of problem knowledge.

The traditional cryptographic technique cannot be implemented on resource-constrained nodes in order to provide an effective authentication system. As a result, we plan to use elliptical curve cryptography to create a lightweight and secure three-factor authentication framework for IoT. Mutual authentication is achieved between nodes using publish-subscribe patterns such as message queue telemetry transfer (Saqib et al., 2020). The general design allows mutual authentication between the subscriber, broker, and publisher, allowing the subscriber to access publisher data via the broker.

Simple and easy–to–break passwords are used in the classic password-based remote user authentication process. For IoT-based critical applications, multifactor remote user authentication approaches (Das, 2011, Chang et al., 2010) that use a combination of identities and passwords entered in a smart card are recommended. An adversary will find it difficult to guess the identity and password since they have high entropies.

Motivation and contribution

The Internet of Things framework is a collaboration between a remote user, a gateway, and a sensor node. In IoT jargon, the sensor node is referred to as a publisher, the remote user as a subscriber, and the gateway as a broker. The majority of authentication protocols are only intended for mutual authentication between a remote user and a gateway node. Researchers have not fully solved mutual authentication between the gateway and the IoT node, to our knowledge. Because mutual authentication is missing, attackers can use this flaw to insert rogue or shadow IoT devices into the network and perform active and passive attacks.

The sensor node is referred to as a publisher in IoT lingo, while the remote user is referred to as a subscriber and the gateway is referred to as a broker. The majority of authentication protocols are

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designed to allow a remote user and a gateway node to mutually authenticate. Researchers have not yet successfully handled mutual authentication between IoT nodes and gateways, to our knowledge. Because there is no reciprocal authentication, attackers can use this flaw to introduce rogue or shadow Internet of Things devices onto the network and launch active and passive assaults.

In this context, our suggested three-factor authentication architecture is built on the publish-subscribe pattern, which is similar to message queue telemetry transport (MQTT), and aims to provide the following benefits:

1)It uses three-factor authentication based on a password, identity, and low-cost digital signature to provide mutual entity authentication of the gateway with both remote users (subscribers) and IoT nodes (publishers), making it ideal for implementing IoT-based critical applications in the health care domain, for example.
2) Session key creation based on nonces is dynamic, meaning that it can change from session to session, making the scheme immune to known session key assaults and ensuring pure forward secrecy.
3) The architecture is designed using computationally cheap hash chains and safe elliptical curve cryptography.
4) The scheme has been properly confirmed using the Scyther tool and is resistant to the majority of commonly used attacks.

The following is the structure of the remainder of the paper: The literature review for the suggested protocol is illustrated in Section II. Section III uses elliptical curve encryption to create a secure, lightweight, signature-based three-factor authentication framework for IoT. The suggested framework's security is examined informally in Section IV. Section V shows how to use the Scyther simulation tool to perform formal security verification on the framework. The performance of the framework is compared in Section VI to that of other relevant current protocols. The paper is finally summarized in Section VII.

LITERATURE REVIEW

The various key agreement and authentication mechanisms important to sensor networks and IoT security are explained in this section.

Yeh et al. (2011) proposed an authentication method for distant users based on elliptical curve cryptography in 2011. However, when compared to other procedures, the computational cost was significant. In 2012, Xue et al. (2013) introduced a time-related credibility-based framework that provided strong authentication between the parties and agreed on a common key for continued communication. However, Xue et al. (2013)'s approach was discovered to be vulnerable to a variety of attacks, including smart card theft and server spoofing.

Chang et al. developed a secure and reliable authentication technique in 2013 that protected users' privacy. Das and Goswami (2013), on the other hand, established in 2013 that their approach could not provide multiple levels of security or safe authentication. Furthermore,

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conventional authentication approaches are ineffective for distributed systems with multiple servers, such as the IoT framework, because remote users who want to utilize the IoT framework's services must know the same number of identities and passwords as the number of servers. (Kumari et al., 2017, Chatterjee et al., 2018).

In 2014, Turkanovi et al. proposed an important arbitration and authentication mechanism for the Internet of Things architecture. However, in 2016, Amin and Biswas (2016) demonstrated that Turkanovic et al. design. 's process contains numerous security flaws, including a DOS attack, sensor node capture attack with malicious node formation, inefficient login and authentication phases, hash function calculation problem, identity theft attack, and offline identity and password guessing attack.

Amin and Biswas devised a security mechanism for multi-gateway WSNs. In 2016, Amin and Biswas suggested distributed cloud framework authentication architecture based on smart cards. Registered users can have secure access to secret data from all private cloud servers using this protocol.

In 2016, Das et al. (2016) proposed a three-factor multi-gateway WSN user authentication mechanism. Das et al. proposed a multi-gateway framework for WSNs since generic WSNs add a lot of overhead to the gateway and use a lot more power than multi-gateway wireless sensor networks. They also demonstrated that the system they proposed is resistant to a variety of cryptographic attacks, including sensor capture and impersonation. However, Das et al. technique .'s are vulnerable to user tracking attacks, according to Kazmi et al. (2019), because the session key is not the same for all three participants.

Amin and Biswas's protocol is vulnerable to sensor capture, disclosure of session key, desynchronization attack, impersonation attack, and offline guessing attack, according to Wu et al. (2017). They also demonstrated that the technique proposed by Amin and Biswas is vulnerable to user tracking attacks and that mutual authentication is not achieved. For multiple-gateway WSNs, Wu et al. proposed a key exchange and mutual authentication system. Srinivas et al. (2017) discovered security flaws in Amin and Biswas' scheme in 2017. Srinivas et al. also demonstrated that sensor nodes have limited battery, memory, and power. Srinivas et al. followed up with a more reliable and effective remote user authentication mechanism for multi-gateway WSNs ideal for IoT frameworks.

In 2017, Bae and Kwak (2020) proposed a reliable and efficient smartcard-based authentication technique in a multi-gateway IoT framework to reduce communicational and computing overhead. Their authentication approach, on the other hand, is vulnerable to attacks such as traceability, impersonation, anonymity, spoofing of gateway nodes, and disclosure of session keys, and it does not provide secure mutual authentication.

In 2017, Kazmi et al. (2019) proposed the concept of Smart Grid, in which appliances are made intelligent enough to communicate with one another via Electromagnetic Compatibility and even control a smart house's power use. By combining two existing heuristic approaches, this paper offers a new hybrid algorithm known as harmony search differential evolution (HSDE). Enhanced differential evolution and harmony search are the algorithms that were used. Peak to average

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ratio, power cost, user comfort, and energy usage are all factors that are considered while evaluating their performance.

On the basis of a wireless sensor network, Mishra et al. (2018) suggested a multimedia communication authentication mechanism for the IoT framework. The efficiency of this plan is very good. The wireless sensor network framework, on the other hand, has a history of security issues. Wu et al. proposed an authentication architecture for WSN in order to address this security issue. To satisfy security requirements such as confidentiality in IoT, the authentication technique is based on key exchange and biometrics for WSNs.

Hassan et al. (2018) examined recent advances in edge computing technologies and their implications for the Internet of Things. They outlined the necessary conditions for a successful edge computing deployment in the IoT and looked at a few key edge computing scenarios.

Shin and Kwon (2019) found security flaws in the Jung et al. technique. 's (Jung et al., 2017) and proposed a three-factor authentication and key exchange mechanism for wireless sensor networks. The proposed architecture uses the XOR operation and hash functions to meet a variety of security requirements.

3. Methodology:

The first step in preventing or resolving a shadow IoT problem is to gain visibility.

According to the Shadow IoT security issues, the IoT devices linked to the network are unknown, resulting in just a threat.

To reduce the shadow IoT security concerns, take the following steps:

- Sanctioned
- Authorized (not sanctioned yet irrelevant)
- Prohibited (not sanctioned and dangerous)

Block Diagram:

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Working Definition of Shadow IOT:

Shadow IoT refers to the Internet of things (IoT) devices or sensors that are in use without the knowledge of IT within a business.

Employees used personal smartphones or other mobile devices for work functions before the days of bringing your own device (BYOD) laws.

"Shadow IoT is a continuation of shadow IT on a much larger scale," explains Mike Raggo, CSO of 802 Secure.

"It is due to the increasing number of devices per employee, as well as the sorts of gadgets, functionalities, and purposes."

Consider the case of Shadow IoT Involved in real-time:

1. Germany's telecoms regulator, the Federal Network Agency, banned a connected doll (i.e., a doll connected to the Internet), dubbed "My Friend Cayla," since it was designated as an "illegal espionage device."

2. In 2017, hackers used an internet-connected fish aquarium to get access to business networks and steal 10 GB of data from a North American casino.

3. Wiki-leaks released information about a CIA gadget known as Weeping Angel, which reveals how an agent may turn a Samsung smart TV into a live microphone.

Blow-fish is a symmetric encryption technique that was created to take the role of DES. It divides messages into 64-bit chunks and encrypts each one separately.

The fact that Blowfish is freely available in the public domain is one of the key reasons for its continued appeal. That isn't to say that it isn't still a good encryption method. Many people believe it has never been defeated.

Blowfish has been used to encrypt user passwords and secure online payments, among other things. It's regarded as one of the most user-friendly and adaptable encryption algorithms available.

CONCLUSION AND FUTURE RESEARCH

The technique serves as a foundation for a more comprehensive methodology for a variety of scenarios. It serves as a foundation for furthering the energy efficiency service for smart coasters and other devices.

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REFERENCES

M. Abramovici, “Smart Products,” in CIRP Encyclopedia of Production Engineering, T. I. A. f. Produ, L. Laperrière, and G. Reinhart, Eds., Berlin, Heidelberg: Springer Berlin Heidelberg, 2014, pp. 1–5.
W. Kritzinger, M. Karner, G. Traar, J. Henjes, and W. Sihn, "Digital Twin in manufacturing: A categorical literature review and classification," IFAC-PapersOnLine, vol. 51, no. 11, pp. 1016–1022, 2018, doi: 10.1016/j.ifacol.2018.08.474.
R. Stark and T. Damerau, "Digital Twin," in CIRP Encyclopedia of Production Engineering, Berlin, Heidelberg: Springer Berlin Heidelberg; Imprint: Springer, 2019, pp. 1–8.
F. Tao et al., "Digital twin-driven product design framework," International Journal of Production Research, vol. 57, no. 12, pp. 3935–3953, 2019, doi: 10.1080/00207543.2018.1443229.
A. Tukker and U. Tischner, "Product-services as a research field: past, present and future. Reflections from a decade of research," Journal of Cleaner Production, vol. 14, no. 17, pp. 1552–1556, 2006, doi: 10.1016/j.jclepro.2006.01.022.

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Food Cost Analysis During Lockdown Based on Activity-Based Costing and Food Frequency Questionnaire Methods in Batununggal Village, Bandung City

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Abstract

The emergence of the debate about the level of effectiveness and efficiency of vaccination versus Lockdown policies currently attracts the attention of the authors. The author believes that the lockdown policy will be effective to be implemented in the future. This study was aimed to determine the type and amount of food needs of residents in Batununggal Village, Bandung Kidul District, Bandung City. And obtain a mathematical model to predict the cost of food security if the Lockdown policy scenario is implemented. Urban areas are used as subjects in this study due to the vulnerability of food availability in cities when a pandemic condition occurs where food supply from the supply area (rural) is disrupted. In this study, the author uses an exploratory method, activity-based costing, and a food frequency questionnaire which aims to obtain information about the number of basic food needs for 100 residents of Batununggal Village, Bandung City, as the research sample. The results of the study found that the cost of food per person is Rp. 219,848 per pax for 14 Days. With the following mathematical equation Food cost during 14 days lockdown = IDR 219,848 X, X = Person.

Keywords: *Food Cost, Lockdown, Activity-based costing, food frequency questionnaire*

INTRODUCTION

COVID-19 (coronavirus disease 2019) is a disease that can cause pneumonia, acute respiratory syndrome, kidney failure, and even death. The number of sufferers and cases of death due to Coronavirus infection every day continues to increase significantly, so that this incident is called a pandemic. When this proposal was made, the number of positive cases in Indonesia had exceeded 385,980 cases (Sari, 2020).

Lockdown is one of the options to minimize the virus spread. Many studies related to lockdowns related to food security and health have been carried out, including awareness and attitudes towards food waste, food purchasing behavior, and household food expenditure estimates (Jribi et al., 2020), the impact of lockdowns on food security caused by the limited number of workers, transportation, farmer morale and agricultural coordination (Inegbedion, 2021), the impact of lockdown on eating behavior deviations (Ramalho et al., 2021), nutritional adequacy (Batlle-Bayer

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et al., 2020), physical and mental health (Röhr et al., 2020), (Shoesmith et al., 2021), (Gambin et al., 2021).

In general, there is controversy regarding the lockdown policy. On the one hand, lockdown is considered effective and efficient to minimize the spread of the virus and save the country's economy (Alvarez et al., 2020), (Sonuga-Barke, 2021), (Dutta & Husain, 2020), but on the other hand, lockdown is considered to have a negative impact on the supply chain and the country's economy (Joshi et al., 2020), (Dutta & Husain, 2020), (Allen, 2021). Based on the above, the author is interested in researching the impact of the lockdown if it is implemented in Indonesia, especially how much food costs must be incurred by the Indonesian government if the lockdown is implemented.

The Indonesian government has a different policy from the Chinese government, which has implemented a centralized lockdown policy and succeeded in reducing the number of its spread in Wuhan. For the Government of Indonesia, the centralized lockdown policy is predicted to require very high costs, can disrupt economic growth, and have many technical obstacles in the field. At present, both the central and regional governments have made various efforts to break the chain of the spread of Covid-19, starting from the formation of the Covid Task Force, PSBB, studies on mass vaccinations, there are even local governments that have implemented Lockdown policies, however, until now, The efforts mentioned above have not produced satisfactory results, on the other hand, the acceleration of resolving Covid-19 must be carried out by the government considering that the longer the time will be directly proportional to the costs incurred both for handling Covid-19 and costs for restoring the national economy.

The Indonesian government's efforts to break the Covid-19 chain have many challenges, including budget problems, the decline in people's income if the Covid-19 policy is actually implemented like in Wuhan, different regional government policies both in terms of method and timing of policy implementation, lack of compliance the community towards the regulations in their respective regions, the boredom of the community over the pandemic and the PSBB which has been running for almost ten months, as well as other challenges. This is very understandable considering that this pandemic came suddenly and beyond prediction. Based on the above, many parties, including the government, believe that the alternative mass vaccination policy is the most appropriate policy to be implemented at this time. Based on the latest data obtained by the author, the government increased the PEN funds (covid management) in July 2021 to IDR 744.75 Trillion from the previous IDR 699.43 Trillion, of which IDR 187.8 Trillion was allocated for social protection (bisnis.com, 2021) and it is estimated that will increase in line with the planned increase in covid data and the government's plan to extend the PPKM. Meanwhile, the estimated cost of the lockdown, according to the government, is estimated at IDR 18.7 trillion per day for 34 provinces in Indonesia (Kompas.com, 2021), so that the current PEN cost is sufficient to carry out a lockdown for 40 days (744.75 T: 18.7 T). = 39.7 days), where experts say that it is enough to do a 15-day lockdown so that the government can make savings on the 2021 State Budget.

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This study was aimed to determine the type and number of basic needs of residents in Batununggal Village, Bandung Kidul District, Bandung City. And obtain a mathematical model to predict the cost of food security if the Lockdown policy scenario is implemented. The emergence of the debate about the effectiveness and efficiency of vaccination versus lockdown policies currently attracts the attention of the authors. The author believes that the vaccination policy will be effective if it is followed by a lockdown policy in the future (as explained above), so it is very important to find a mathematical model for the cost of food security if a lockdown policy is taken in the future. Batununggal Village has very diverse social conditions, including diversity in income levels, education, age, population density, and others, so it is very suitable to be used as a subject representing urban areas (BPS Kota Bandung 2019). Urban areas are used as subjects in this study due to the vulnerability of food availability in cities when a pandemic condition occurs where food supply from the supply area (rural) is disrupted. It is hoped that the results of this study will become input for stakeholders in determining effective policies in breaking the chain of the spread of Covid-19.

The formulation of the problem in this study is how is the cost of family food during the lockdown based on the activity-based costing method and the food frequency questionnaire in Batununggal Village, Bandung City? As well as a simple mathematical model to determine the cost of family food costs during the lockdown period?

The purpose of this study was to determine the cost of family food during the lockdown based on the activity-based costing method and food frequency questionnaire in Batununggal Village, Bandung City? And get a simple mathematical model to determine the cost of family food costs during the lockdown in Batununggal Village?

LITERATURE REVIEW

Risk And Risk Management

Risk is an undesirable situation, and if it occurs, it will cause losses both in material and non-material forms (Supardi, 2019). Risk management is the application of management functions in handling risks, especially risks faced by organizations/companies, families, and communities. This includes activities in the form of planning, organizing, compiling, coordinating, and supervising (Djojosoedarso, 1999).

Covid-19

COVID-19 (coronavirus disease 2019) is a disease caused by a new type of coronavirus, namely Sars-CoV-2, which was first reported in Wuhan, China, on December 31, 2019. This COVID-19 can cause acute respiratory symptoms such as fever above 38 °C, coughing, and shortness of breath for humans. In addition, it can be accompanied by weakness, muscle aches, and diarrhea. In patients with severe COVID-19, it can cause pneumonia, acute respiratory syndrome, kidney failure, and even death. The number of sufferers and cases of death due to Coronavirus infection every day continues to increase significantly so that this incident is called a pandemic; when this proposal was made, the number of positive cases in Indonesia had exceeded 385,980 cases (Sari, 2020).

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Lockdown

According to Oxford University Press, the definition of a lockdown is an official order to control the movement of people or vehicles within an area due to a dangerous situation. Meanwhile, according to Lindsay Wiley, Lockdown is an effort to create a geographical quarantine, or also known as cordon sanitaire, which is to create a barrier and try to stop people from entering or leaving (from a certain area) with the exception of shipping goods or people to guard important needs. Based on the two definitions above, it can be concluded that lockdown is a security policy package against a threat, in this case, the spread of COVID-19. This policy must be complete with security guarantees for social needs such as food supply, health, education, and others even though they are being isolated. (Febrian & Santosa, 2020).

Food Security

According to the Food Law No. 7 of 1996, which was updated in the Law of the Republic of Indonesia Number 18 of 2012, food security is a condition of meeting food needs for households which are reflected in the availability of sufficient food, both in quantity and quality, safe, equitable and affordable. (UU RI Nomor 18 Tahun, 2012). Meanwhile, according to FAO, food security is a situation where all households have physical and economic access to food for all their family members, where households are not at risk of losing both accesses. To realize food security, it is necessary to strengthen the coordination and synchronization of related parties in planning, policy, development, and control. (Hanafie R., 2010)

Activity-Based Costing (ABC)

The activity-Based Costing method is a cost information system oriented to providing complete information about activities to enable policymakers to process activities. (Mulyadi, 2014). The provision of complete information in the ABC method covers all stages of activities, namely the planning stage, implementation stage, and logistic support stage. (Ahmad Dunia dan Wasilah, 2012). Based on the two definitions above, the ABC method is the most appropriate method to be used by the author in determining the type of activity in the household in full, which will then be used as the basis for calculating the cost of food security that occurs in the household during the lockdown.

Food Frequency Quistionnaire (FFQ)

The Food Frequency Questionnaire (FFQ) is a questionnaire that provides an overview of the consumption of energy and other nutrients in the form of a person's consumption frequency. These frequencies include daily, weekly, monthly, and yearly, which are then converted into consumption per day. FFQ provides an overview of individual eating patterns or habits of nutrients. Food ingredients and foods listed in the FFQ can be made according to the needs of researchers and research facilities (Umi, 2007).In this study, the authors use the Simple or Non-Quantitative FFQ, considering the data needed is a list of data along with the amount of food that is generally consumed by one family on a regular basis with daily, weekly, monthly, and yearly frequencies without involving nutrition, portions, and others.

METHODOLOGY

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The above problems will be solved by several methods, namely exploratory methods, analytical descriptive, Activity Based Costing (ABC), and Food Frequency Ratio (FFQ). The exploratory method is a research method that aims to dig up information (Arikunto, 2011), where this study aims to dig up information about the number of basic food needs of families in Batununggal village, Bandung.

Analytical descriptive is a method that is characterized by focusing on solving problems that exist in the present or actual with the data collected and then explained and analyzed. (Sedarmayanti, 2011)

The analysis in this study also uses the ABC and FFQ methods, where the ABC method will be used in determining the type of activity in the household completely and then will be used as the basis for calculating the cost of food security that occurs in households during a lockdown. Meanwhile, the FFQ will be used during data collection using a questionnaire. The results of the FFQ will then be analyzed using a simple statistical method to make an expenditure cluster for each household, then the feasibility of each cluster will be analyzed using the ABC Return method to recalculate the feasibility of the data that has been collected.

The final result of this research is to obtain a model in the form of a mathematical equation to predict the cost of family food security during the lockdown period.

The total population based on 2018 data is 56,151 people (BPS 2019). While the sample used in this study is calculated using the Slovin formula with an error rate of 10%, which is equal to:

$n = N / \{1 + N (e)2 \}$

$n = 56.151 / \{1 + 56.151 (0.10)2 \}$

n = 99.82 atau 100 People

The sampling technique in this study used the Cluster Random Sampling Technique, where the sample was determined based on the domicile group or region of the members of the research population.

Data collection techniques in this study are observation, interviews, questionnaires, FGD, and triangulation, namely data collection techniques that combine data from various collection techniques and existing data sources.

In this research, the data collection method required is through:
a. The Preliminary Survey is preceded by
a.1. Literature Study on family expenses.
a.2. Creating a family cluster that will be used as a target sample includes families with prosperous, simple, and pre-prosperous economic levels.
a.3. Conduct initial interviews with targeted families to determine the type and amount of daily, weekly, monthly, and yearly expenses.
a.4. FGD with a team of lecturers to determine the distribution of clusters and the contents of the questionnaire.
b. Questionnaire distribution.

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c. Questionnaire results are processed quantitatively with the method of calculating the ratio of food adequacy for each family or person to determine the type of food need and the amount, then the average need for each person is calculated, and the rupiah value is determined using the ABC method so that an equation can be made for the above calculations.
d. FGD with family representatives and a team of lecturers to minimize calculation differences that may occur as a result of inflation variables, shipping costs, and others that may occur.

FINDINGS AND DISCUSSION

This study was conducted to analyze the cost of family food during the lockdown based on the method of activity-based costing and food frequency questionnaire in Batununggal Village, Bandung City? So the author gets a simple mathematical equation model to determine the cost of family food costs during the lockdown in Batununggal Village. Family expenditure to be breakdown as follows:

a. Average electricity expenditure per month.
b. LPG gas purchases on average per month.
c. Purchase of drinking water on average per month, if purchased.
d. Purchase of clean water on average per month, if purchased.
e. Purchase of rice on average per month.
f. Average sugar purchases per month.
g. Purchase of cooking oil on average per month.
h. Expenditures for seasonings (onion, chili, pepper, salt, vetsin and others) are on average per month.
i. Expenditure for purchasing eggs on average in one month.
j. Expenditure for purchasing fish on average in one month.
k. Expenditure for purchasing meat on average in one month.
l. Expenditure for purchasing chickens on average in one month.
m. Expenditure for purchasing vegetables on average in one month.
n. Expenditure for purchasing tofu and tempeh is an average of one month.
o. Expenditure for purchasing fruits on average in one month.
p. Expenditures for purchasing bath soap, washing, washing floors, baygon and others are averaged in one month.
q. Expenditures for the purchase of supplies of medicines (wood puti, betadine, etc.) on average in one month.

Based on data collection in the field, it was found that 114 were filled in by respondents and then 100 questionnaires were selected that represent the population, with a description of 100 respondents, 182 respondents' children, 72 respondents' spouses, and 52 other dependents in the respondent's house, so that the total population was 406. person. Which will be the current divisor. The average income of respondents is known to be around 3,670,200 per family/respondent and the average family expenditure for food needs is currently Rp. 1,912,678.
Based on the FFQ method, the current demand for food is shown in the following Table 1 and Table 2. So that the food cost per person during the lockdown is Rp. 219,848 for 14 days. So if the current total population of Indonesia is 271,349,899 people x Rp. 219,848 obtained lockdown fee of Rp. 59,655,757,843,757 (59 Trillion) which is much cheaper than the costs incurred by the current government. Where the calculation above is still fairly high because the cost of lockdown in the area is likely to be smaller than the need in urban areas.

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The mathematical equation for estimating lock down is as follows:

Food cost during 14 days lockdown = IDR 219,848 X
X = Person

The above equation is very useful for predicting the inventory that must be provided by the government along with the logistics costs and logistics arrangements.

Tabel 1. Lockdown Cost Breakdown based on FFQ method

No	Description	Total Per Month (a)	divider (3+4+5+100) (b)	Per capita c = a / b	Per Day d = a / 30	Lockdown Cost e = d x 14
1	Montly Income	324.900.000	406	800.246	10.830.000	151.620.000
2	Spouse Monthly Income	367.200.000		904.433	12.240.000	171.360.000
3	Children	182				
4	Spouse	72				
5	Other	52				
6	Drinking water (Gallon)	638		2	21	298
7	Rice (Kgs)	1.819		4	61	849
8	Sugar (Kgs) :	213		1	7	100
9	Cooking Oil (ltr) :	425		1	14	198
10	eggs (Kg)	331		1	11	154
11	Fish (Kgs)	200		0	7	93
12	Beef (Kgs)	153		0	5	71
13	Chicken (Kgs)	322		1	11	150
14	Vegetables (kgs)	420		1	14	196
15	Clean water (Gallon)	601		1	20	280

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Tabel 2. Lockdown Cost Breakdown based on ABC method

No	Description	Total Per Month (a)	divider (3+4+5+100) (b)	Per capita c = a / b	Per Day d = a / 30	Lockdown Cost e = d x 14
1	Drinking water	7.623.500		18.777	254.117	3.557.633
2	Rice	20.490.000		50.468	683.000	9.562.000
3	Sugar	3.197.000		7.874	106.567	1.491.933
4	Cooking Oil	8.548.500		21.055	284.950	3.989.300
5	Eggs	7.443.000		18.333	248.100	3.473.400
6	Fish	6.200.150		15.271	206.672	2.893.403
7	Beef	13.511.000		33.278	450.367	6.305.133
8	Chicken	11.351.168		27.959	378.372	5.297.212
9	Fruits	11.939.280		29.407	397.976	5.571.664
10	Vegetables	14.083.000		34.687	469.433	6.572.067
11	Electricity	25.577.000		62.998	852.567	11.935.933
12	Clean Water	6.132.000		15.103	204.400	2.861.600
13	Bumbu (Rp)	8.845.250		21.786	294.842	4.127.783
14	Bath soap	20.443.000		50.352	681.433	9.540.067
15	Baby needs	9.366.000		23.069	312.200	4.370.800
16	Tahu Tempe	6.894.000		16.980	229.800	3.217.200
17	Gas	9.624.000		23.704	320.800	4.491.200
	Total Cost	**191.267.848**		**471.103**	**6.375.595**	**89.258.329**
	Total Income - Total Expenditure	**175.932.152**		**433.330**	**5.864.405**	**82.101.671**

Food Cost During Lockdown per person (IDR)	**219.848**

CONCLUSION AND FUTURE RESEARCH

Based on the results of the study, the following conclusions can be drawn:

1. The cost of food per person during the lockdown based on the activity-based costing method and food frequency questionnaire in Batununggal Village, Bandung City is Rp. 219,848,-
2. A simple mathematical model to determine the cost of family food during the lockdown period in Batununggal Village is as follows:
   Food cost during 14 days lockdown = IDR 219,848 X

The suggestions in this study are as follows: the government should adopt the lockdown method to stop the Covid-19 pandemic because the cost is much cheaper than the current method. The implementation should follow Germany, where the public is given cheques rather than purchasing their respective lockdown supplies. This is to minimize changes in the logistics pattern of food delivery.

REFERENCES

Ahmad Dunia dan Wasilah, F. A. (2012). Akuntansi Biaya. Jakarta:: Salemba.
Arikunto, S. (2011). Prosedur Penelitian : Suatu Pendekatan Praktik. Jakarta: Rineka.
bisnis.com. (2021, 7 17). https://ekonomi.bisnis.com. Retrieved from ekonomi.bisnis.com: https://ekonomi.bisnis.com/read/20210717/9/1418943/membengkak-total-anggaran-penanga

ISSN 2828-1500 (Online) | 2828-1985(Print)

nan-covid-jadi-rp74475-triliun?utm_source=Desktop&utm_medium=Artikel&utm_campaign=BacaJuga_1
Djojosoedarso, S. (1999). Prinsip-Prinsip Manajemen Risiko Dan Asuransi. Jakarta: Salemba Empat.
Dunia, Ahmad, F., & Wasilah, A. (2012). Akuntansi Biaya. Jakarta: Salemba.
Fahmida, U. (2007). Nutritional Assessment. SEAMEO-TROPMED RCCN. Jakarta: Universitas Indonesia.
Febrian, R., & Santosa, B. (2020, March 16). VOI Indonesia. Retrieved from https://voi.id/berita/3690/pengertian-i-lockdown-i-dan-negara-negara-yang-sudah-melakukannya
Ghozali, I. (2013). Aplikasi Analis Multivariat dengan Program IBM SPSS 21 ed.7. Semarang: Universitas Diponegoro.
Hanafie, R. (2010). Peran Pangan Pokok Lokal Tradisional dalam Diversifikasi Konsumsi Pangan. JSEP (Journal of Social and Agricultural Economics), 275.
Hanafie, R. (2010). Peran Pangan Pokok Lokal Tradisional dalam Diversifikasi Konsumsi Pangan. J-SEP, 275.
Kompas.com. (2021, 6 24). nasional.kompas.com. Retrieved from kompas: https://nasional.kompas.com/read/2021/06/24/11132481/ini-perkiraan-biaya-lockdown-yang-pernah-diungkap-jokowi?page=all
Mulyadi. (2014). Akuntansi Biaya Edisi-5. Yogyakarta: Universitas Gajah Mada.
Pratama, W. P. (2021, July 17). Bisnis.com. Retrieved from https://ekonomi.bisnis.com/read/20210717/9/1418943/membengkak-total-anggaran-penanganan-covAllen, D. W. (2021). Covid-19 Lockdown Cost/Benefits: A Critical Assessment of the Literature. In International Journal of the Economics of Business. https://doi.org/10.1080/13571516.2021.1976051
Alvarez, F., Argente, D., & Lippi, F. (2020). A Simple Planning Problem for COVID-19 Lockdown. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.3569911
Batlle-Bayer, L., Aldaco, R., Bala, A., Puig, R., Laso, J., Margallo, M., Vázquez-Rowe, I., Antó, J. M., & Fullana-i-Palmer, P. (2020). Environmental and nutritional impacts of dietary changes in Spain during the COVID-19 lockdown. Science of the Total Environment, 748. https://doi.org/10.1016/j.scitotenv.2020.141410
Dutta, M., & Husain, Z. (2020). What cost decisiveness? A cost benefit analysis of the lockdown to contain COVID-19 in India. MedRxiv.
Gambin, M., Sękowski, M., Woźniak-Prus, M., Wnuk, A., Oleksy, T., Cudo, A., Hansen, K., Huflejt-Łukasik, M., Kubicka, K., Łyś, A. E., Gorgol, J., Holas, P., Kmita, G., Łojek, E., & Maison, D. (2021). Generalized anxiety and depressive symptoms in various age groups during the COVID-19 lockdown in Poland. Specific predictors and differences in symptoms severity. Comprehensive Psychiatry, 105. https://doi.org/10.1016/j.comppsych.2020.152222
Inegbedion, H. E. (2021). COVID-19 lockdown: implication for food security. Journal of Agribusiness in Developing and Emerging Economies, 11(5). https://doi.org/10.1108/JADEE-06-2020-0130

ISSN 2828-1500 (Online) | 2828-1985(Print)

Joshi, A., Bhaskar, P., & Gupta, P. K. (2020). Indian economy amid COVID-19 lockdown: A prespective. In Journal of Pure and Applied Microbiology (Vol. 14). https://doi.org/10.22207/JPAM.14.SPL1.33

Jribi, S., Ben Ismail, H., Doggui, D., & Debbabi, H. (2020). COVID-19 virus outbreak lockdown: What impacts on household food wastage? Environment, Development and Sustainability, 22(5). https://doi.org/10.1007/s10668-020-00740-y

Ramalho, S. M., Trovisqueira, A., de Lourdes, M., Gonçalves, S., Ribeiro, I., Vaz, A. R., Machado, P. P. P., & Conceição, E. (2021). The impact of COVID-19 lockdown on disordered eating behaviors: the mediation role of psychological distress. Eating and Weight Disorders. https://doi.org/10.1007/s40519-021-01128-1

Röhr, S., Reininghaus, U., & Riedel-Heller, S. G. (2020). Mental wellbeing in the German old age population largely unaltered during COVID-19 lockdown: results of a representative survey. BMC Geriatrics, 20(1). https://doi.org/10.1186/s12877-020-01889-x

Shoesmith, E., Shahab, L., Kale, D., Mills, D. S., Reeve, C., Toner, P., de Assis, L. S., & Ratschen, E. (2021). The influence of human–animal interactions on mental and physical health during the first COVID-19 lockdown phase in the U.K.: A qualitative exploration. International Journal of Environmental Research and Public Health, 18(3). https://doi.org/10.3390/ijerph18030976

Sonuga-Barke, E. J. S. (2021). Editorial: 'No pain - No gain' – Towards the inclusion of mental health costs in balanced "lockdown" decision-making during health pandemics. In Journal of Child Psychology and Psychiatry and Allied Disciplines (Vol. 62, Issue 7). https://doi.org/10.1111/jcpp.13435

Allen, D. W. (2021). Covid-19 Lockdown Cost/Benefits: A Critical Assessment of the Literature. In International Journal of the Economics of Business. https://doi.org/10.1080/13571516.2021.1976051

Alvarez, F., Argente, D., & Lippi, F. (2020). A Simple Planning Problem for COVID-19 Lockdown. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.3569911

Batlle-Bayer, L., Aldaco, R., Bala, A., Puig, R., Laso, J., Margallo, M., Vázquez-Rowe, I., Antó, J. M., & Fullana-i-Palmer, P. (2020). Environmental and nutritional impacts of dietary changes in Spain during the COVID-19 lockdown. Science of the Total Environment, 748. https://doi.org/10.1016/j.scitotenv.2020.141410

Dutta, M., & Husain, Z. (2020). What cost decisiveness? A cost benefit analysis of the lockdown to contain COVID-19 in India. MedRxiv.

Gambin, M., Sękowski, M., Woźniak-Prus, M., Wnuk, A., Oleksy, T., Cudo, A., Hansen, K., Huflejt-Łukasik, M., Kubicka, K., Łyś, A. E., Gorgol, J., Holas, P., Kmita, G., Łojek, E., & Maison, D. (2021). Generalized anxiety and depressive symptoms in various age groups during the COVID-19 lockdown in Poland. Specific predictors and differences in symptoms severity. Comprehensive Psychiatry, 105. https://doi.org/10.1016/j.comppsych.2020.152222

Inegbedion, H. E. (2021). COVID-19 lockdown: implication for food security. Journal of Agribusiness in Developing and Emerging Economies, 11(5). https://doi.org/10.1108/JADEE-06-2020-0130

ISSN 2828-1500 (Online) | 2828-1985(Print)

Joshi, A., Bhaskar, P., & Gupta, P. K. (2020). Indian economy amid COVID-19 lockdown: A prespective. In Journal of Pure and Applied Microbiology (Vol. 14). https://doi.org/10.22207/JPAM.14.SPL1.33

Jribi, S., Ben Ismail, H., Doggui, D., & Debbabi, H. (2020). COVID-19 virus outbreak lockdown: What impacts on household food wastage? Environment, Development and Sustainability, 22(5). https://doi.org/10.1007/s10668-020-00740-y

Ramalho, S. M., Trovisqueira, A., de Lourdes, M., Gonçalves, S., Ribeiro, I., Vaz, A. R., Machado, P. P. P., & Conceição, E. (2021). The impact of COVID-19 lockdown on disordered eating behaviors: the mediation role of psychological distress. Eating and Weight Disorders. https://doi.org/10.1007/s40519-021-01128-1

Röhr, S., Reininghaus, U., & Riedel-Heller, S. G. (2020). Mental wellbeing in the German old age population largely unaltered during COVID-19 lockdown: results of a representative survey. BMC Geriatrics, 20(1). https://doi.org/10.1186/s12877-020-01889-x

Shoesmith, E., Shahab, L., Kale, D., Mills, D. S., Reeve, C., Toner, P., de Assis, L. S., & Ratschen, E. (2021). The influence of human–animal interactions on mental and physical health during the first COVID-19 lockdown phase in the U.K.: A qualitative exploration. International Journal of Environmental Research and Public Health, 18(3). https://doi.org/10.3390/ijerph18030976

Sonuga-Barke, E. J. S. (2021). Editorial: 'No pain - No gain' – Towards the inclusion of mental health costs in balanced "lockdown" decision-making during health pandemics. In Journal of Child Psychology and Psychiatry and Allied Disciplines (Vol. 62, Issue 7). https://doi.org/10.1111/jcpp.13435

id-jadi-rp74475-triliun

Sahara, W. (2021, June 24). Kompas.com. Retrieved from https://nasional.kompas.com/read/2021/06/24/11132481/ini-perkiraan-biaya-lockdown-yang-pernah-diungkap-jokowi?page=all

Sari, H. P. (2020, October 24). Kompas.com. Retrieved from https://nasional.kompas.com/read/2020/10/24/15304701/update-24-oktober-tambah-4070-kasus-positif-covid-19-jadi-385980

Sedarmayanti. (2011). Metodologi Penelitian. Bandung: CV Mandar Maju.

Supardi, E. (2019). Ekspor Impor: Teori Dan Praktikum Kegiatan Ekspor Impor Untuk Praktisi Logistik dan Bisnis. Yogyakarta: Deepublish.

Uma, S. (2010). Research Methode for Business.

Umi, F. d. (2007). Nutritional Assesment. SEAMEO-TROPMED RCCN Universitas Indonesia.

UU Nomor 18 Tahun 2012 Pasal 1 Nomor 4 Undang-Undang Republik Indonesia Tentang Pangan. (n.d.).

UU RI Nomor 18 Tahun. (2012). UU Nomor 18. Pasal 1 Nomor 4 Undang-undang Republik Indonesia tentang pangan.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Available online at: https://proceeding.researchsynergypress.com/index.php/ihsatec
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Web-Based Platform for Don Bosco High School – Senior High School – Technical Vocational Education Track in Adoption of Hybrid Learning

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Abstract

In the last two decades, the hybrid or blended learning paradigm has emerged as a viable alternative to traditional classroom instruction. This study, such elements associated with adopting blended learning addressed numerous results, implications, and possible future paths for Senior High School where Technical Vocational Education in the Philippines progressively interacts and develops with each other. This study aimed at developing a web-based platform or portal for DBHSP – Don Bosco High School Paranaque to resolve some classroom challenges such as conventional teaching, which is time-consuming, and the perennial lack of classrooms in public schools by providing additional/alternative teaching tools. To this end, this study further endeavored to determine the level of project effectiveness of the web-based portal in terms of the ISO 9126-1 software quality model main characteristics, namely: functionality, reliability, usability, efficiency, maintainability, and portability. The data processing was analyzed using Fourth Generation Techniques (4GT), dissemination of innovations, and Technology Acceptance Model (TAM), which is an information system theory that describes how users come to embrace and use technology. According to the paradigm, when users are presented with new technology, a variety of factors impact their decision about how and when they will use it. Hybrid learning predates modern instructional technologies. The authors conclude that its evolution will be inevitably linked to current information communication technologies and an encouraging environment for technology adopters teaching academics in a senior high school – Technology Vocational Education that is simulating some aspects of human thought perception processes. To evaluate the effectiveness, the author contends that Hybrid Learning integrates access, progress, and students' impression of their learning environments. The research technique used descriptive and developmental methodologies, and the DBHS – Senior High School were purposefully selected to benefit from the web-based approaches to teach both on and off-campus learners.

Keywords: *hybrid, blended learning, innovations*

INTRODUCTION

Breakthrough such as technology and innovations introduces key basic changes in accomplishing huge upgrades in profitability. Used to help both educating and learning, innovation imbues classrooms with computerized learning devices, for example, PCs and handheld gadgets; extends course offerings, encounters, and learning materials; bolsters learning 24 hours per day, seven days; assembles 21st-century abilities; builds understudy engagement and inspiration, and quickens learning. Innovation likewise has the ability to change educating by introducing another model of associated instructing. One of the advancements of innovation is the Internet. The

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Internet is formed by enjoying two words that denote a global network: (international) and net (network) (Salamh, 2005).

The educational system has also profited from the advantages provided by the Internet. The Internet, which provides learners with access to knowledge as well as the ability to communicate by written, audio, and video means, has experienced fast growth all around the world. New internet-based teaching strategies have erased traditional place and time barriers, allowing students to access the material whenever and wherever they desire (Murphy, 2003). Because the learner can access information at any time and from any location, the Internet has become an indispensable part of education. Courses/subjects offered using technological tools can be considered a form of enriched education, and this includes web-based online courses and other types of internet-supplemented courses (Scida&Saury, 2006). According to Salamh (2005), web-based education is a novel education paradigm that may be utilized to promote the acquisition of new information skills as well as the enrichment of students' learning habits and experiences. Many educational strategies, including presentations, conversations, demonstrations, answer-questions, brainstorming, case studies, cooperative learning, and problem-centered learning, can be carried out in web-based environments. Learners can get experience through researching, writing, observing, listening, and performing tasks in this manner (Picciano, 2006). It may be claimed that due to the increasing popularity of computers and, in particular, the Internet, online learning instructional settings are rapidly becoming more prevalent. However, the online teaching-learning environment lacks numerous advantages that the face-to-face environment has, which led to the notion of blended learning. According to Ross and Gage (2006), online higher education students are less satisfied with completely online courses than with traditional courses. As a result, combining online learning with traditional learning environments could be far more effective in solving educational problems and meeting educational goals (Murphy, 2003). Furthermore, Graham (2006) contends that blended learning was established for its potential benefits in terms of providing more effective education, ease, and access to the teaching-learning environment. Many researchers employ blended learning in very diverse ways in international literature, referring to it as hybrid learning and mixed learning. Young (2002) defines blended learning as a form of education that integrates online and face-to-face learning activities in a planned, pedagogically valuable way, with certain face-to-face activities substituted by online ones.

Don Bosco High School Paranaque – Senior High School is envisioned to be a premier institution for Technical-Vocational Education in Paranaque City, Philippines; thus, the school is committed to producing graduates who are equipped with lifelong skills and competencies, desirable values, and work ethics in order that they may become responsible and productive citizens of the community. In line with the core values of DBHSP – Don Bosco High School Paranaque, the school is committed to providing effective instruction to the students and efficient service to its clientele. Based on the interview, one of the classroom challenges is that conventional teaching in the classroom is not time-wise. Therefore the researcher developed an alternative way of learning. Hybrid or Blended learning will provide a big convenience for the instruction to achieve its target

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by combing the face-to-face interaction in traditional learning and time, place, and material richness provided by Web-Based Platform learning.

A Web-Based platform gives Internet users a single, personalized access point to a network-based campus. The most interesting portals in the context of education are horizontal. Horizontal means providing practically everything an individual user within the organization requires doing his or her function. Authentication and access are determined by the individual's function or roles inside the company. Horizontal enterprise portals (HEPs) are adaptable and adjustable. They are designed to provide access to practically everything that an individual user affiliated with the campus needs to manage his or her relationship with the school, and if effectively built, they can replace most of the user's computer "desktop." These users can include students, faculty, staff, parents, prospective students, alumni, and members of the community at large.

LITERATURE REVIEW

The ideas enriched the researcher's insight on the subject and provided a broader perspective in the pursuit of the study. Views, concepts, theories, and erudite researchers related to the present investigation were considered the presentation of the review.

State of the Art

The reviewed literature was found to have a bearing on the present study. They served as the foundation for conceptualizing the research problem, research design, and methodology for the study.

E-learning

When one enters the field of integrated e-learning, there are numerous options available (also referred to as hybrid or mixed-mode learning). Many scholars, researchers, and educators believe that blended e-learning provides the best synchronous and asynchronous e-learning possibilities. Combining face-to-face sessions with online sessions is one of the most popular blended–learning modalities. Because the in-person portion provides more opportunities for social interaction, access to nonverbal cues that support affinity-seeking efforts, casually building relationships, familiarity and trust, addressing and diffusing misunderstanding of Formative feedback for all participants, knowledge or communications, and more, this format offers many benefits (Garrison & Vaughan 2008; Kim & Bonk 2006). Furthermore, online sessions offer better schedule flexibility (since not all sessions must be synchronous and in-person), time savings (due to no travel to a central place), self-direction, peer learning and collaboration, and student responsibility for learning (Finger et al., 2010; Garrison & Vaughan 2008; Kim & Bonk, 2006). Lest readers think that blended learning is the ideal answer for e-learning, it likewise has issues. Similarly, as different sorts of e-learning require much arranging and cautious outline, the same is valid for mixed learning (Finger et al., 2010; Garrison & Vughan 2008). Notwithstanding the standard plan requests, one should likewise consider which activities best fit the up close and personal or remote sessions and mastermind or change them in view of the evaluation. Arranging, coordination, and facilitation become key concerns in blended learning. (King 2017)

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Distance learning has many names and forms:
- Distributed learning refers to the fact that learning resources and users are distributed.
- Distance learning entails a significant physical distance between users (which is not always the case)
- A three-dimensional simulated environment can be used for virtual learning.
- Mobile Learning, which is usually delivered to portable devices such as tablets or smartphones.
- Blended or Hybrid learning, which mixers traditional face-to-face instruction with some form of distance learning delivery system and beyond.

Asynchronous e-Learning
This broad term refers to learning that is delivered non-simultaneously via online technology. This form of delivery does not necessitate the teacher and users to be connected at the same time. One of the most notable benefits of this format is the lack or elimination of time constraints. Some learners may be able to watch videos and answer questions in the middle of the night, while others may be able to do so in the afternoon or evening. However, there are many other opportunities to customize the format for asynchronous e-learning:
- The class size is not fundamentally limited. (consider MOOCs)
- Peer learning and collaboration provide a wide range of opportunities. (Small and large groups, discussion forums, joint initiatives, and so forth.).
- There are numerous methods for presenting or discovering knowledge (e.g., video and audio clips, presentations, web pages, articles, books, dialogue, interactivity, music, movies, animations, graphics, simulations, etc.).

Synchronous e-Learning
Synchronous e-learning occurs when learners and instructors connect through technology at the same time. Given the great benefits of flexibility that synchronicity provides, why anyone would persevere through scheduling and technical difficulties.

Impact of Hybrid/Blended Instructional
In the study of Rawlins, Troy A.; Ali, Rifath (2017); A conventional classroom environment makes a worldview in which college teachers must have the capacity to rapidly distinguish and suit contrasts among understudy adapting necessities to accomplish good scholarly execution scores while all the while working inside college arrangements in regards to course deviation(s) or alteration(s) in dates and times. Educators in on-grounds classrooms use PowerPoint slides in addresses or trade modules, cluster assignments, and flipped classrooms while using appropriate informational mechanical stages to associate with understudy learning and update execution scores in classes. Overall, these instructional framework strategies have been successful in attaining positive understudy execution scores for family unit understudies in Eastern Kentucky University's (EKU) Occupational Safety and Health (OSH) degree program courses. However, EKU's understudies of the Muslim faith who were selected in OSH 261 Principles of Occupational Safety and Health achieved lower than average execution scores than their residential partners inside a standard on-campus instructional outline. This scientist assumes the real reason for

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Muslim understudies bring down scores in OSH 261 is identified with poor class participation caused by going to a week-by-week Friday religious service called Jumu'ah. In spite of the way that there is a school procedure regarding course support, there is no understanding, avoidances, or plans offered which provide guidance to a religious cabin for understudies of Muslim certainty. Instructors, rather, are dependent on sorting out imaginative instructional arrangement courses of action, which meet understudy adjusting needs (instructional strategy) towards extending insightful execution scores while working inside school approach for course investment in the midst of class dates and times. In this easygoing mixed technique pilot examination of 96 (N = 96) Muslim understudies enrolled in OSH 261 were introduced to creamer or blended approach to managing instructional arrangement over three semesters called Adobe Connect to address both the understudy adjusting needs and to follow the school game plan with respect to understudy participation to help augments in academic execution scores. The quantitative outcome of this pilot study revealed that understudies of Muslim certainty had their ordinary insightful execution scores increased by 5% after Adobe Connect was implemented at the close of each semester.
Faces of Blended Learning

The blended learning model—the mix of web-based learning and physical tutoring—isn't new. Be that as it may, as this model keeps on advancing and developing, it's creating new sorts of learning encounters. Mixed learning started decades back as a reasonable answer for filling holes in access to educational modules content. Schools that couldn't provide specific advanced or optional coursework found an answer in online courses, which could fairly convey substance to understudies whenever and wherever commonly coming to crosswise over the region and state lines. In spite of the fact that understudies kept on taking conventional face-to-face classes, they could supplement these courses with online courses.

Three Trends in Blended Learning (Horn and Fisher 2017, p. 59-63)
1. Schools Continue to Adopt Station Rotation.
2. Schools Explore Ways to Unlock Flexible Pacing.
3. The Flex Model in high schools challenges traditional structures.

METHODOLOGY

The research technique used descriptive and developmental methodologies for Don Bosco High School Paranaque – Senior High School. The research began by identifying the current system of Don Bosco High School of Paranaque – Senior High School TVE or Technical Vocational Education Track. This is done by interviewing, observing, and recording stakeholders' personal experiences. The system was aimed at solving problems encountered by teachers and students and also suggestions for improving the present system. Furthermore, the study aims to determine the systems acceptance and quality level. Thus, a descriptive method is used using the survey instrument and test cases. The study designed a new model of web platform using a hybrid learning system for Don Bosco High School – Senior School, Technical Vocational Education track (DBHSP – SHS TVE Track), tested its acceptance, and evaluated its quality based on research development method.

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Project Development

Nowadays, another procedure, known as the Fourth Generation Technique (4GT), is being utilized to rapidly create data frameworks. This procedure makes utilization of various programming advancement apparatuses. The designer needs to indicate just a couple of attributes of the product at an abnormal state. The instruments at that point naturally build up the code for the given details.

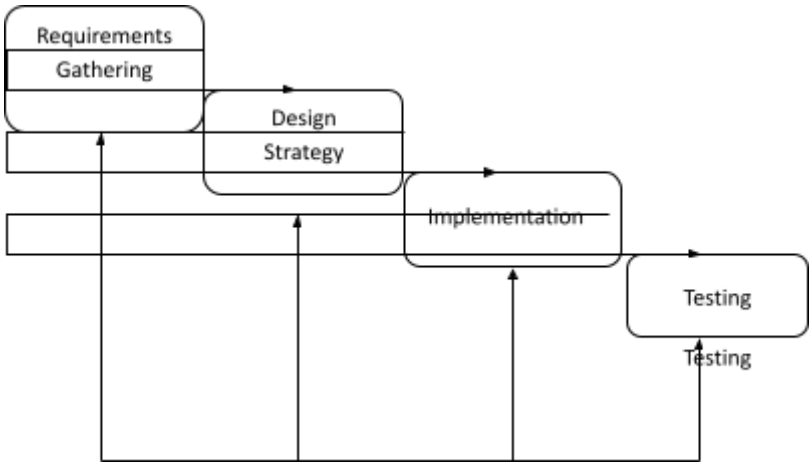

Figure 1. Fourth Generation Techniques (4GT)

Project Design

Web-Based Portal Data Flow Diagram is utilized as a stage to make a review of the E-learning without broadly expounding, which can later be explained. It typically comprises general application dataflow and procedures of the e-learning process. It contains the majority of the user flow and their substances such as all the streams of Student, Activity Log, Files, Teachers, Subject Teacher. The majority of the beneath charts have been utilized for the representation of information handling and structures outline of the E-Learning procedure and working stream.

The author discusses the project technical descriptions, testing and evaluation findings, data analysis, and interpretation based on the results of the distributed surveys. This section also shows the expectations and observations of each respondent towards the system. The author explains the *Level of Software Quality Characteristics* in terms of functionality, dependability, usability, efficiency, maintainability, and portability.

Project Technical Description

This technical description covers the design, the preparation of all required operation and construction studies, the supply of all required metering, IT, communication, and equipment, the installation of such equipment, and finally, the acceptance of the operational system.

The Web-Based Platform for Don Bosco High School Paranaque is developed and designed using an open-source course management system. MOODLE stands for "Modular

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Object-Oriented Dynamic Learning Environment. Developed by Martin Dougiamas, it is used by thousands of educational institutions worldwide to provide an organized interface for e-learning or Internet-based learning. Moodle core includes the entire infrastructure required to create a Learning Management System.

Testing Result

Software testing is an area that is being examined and given utmost importance of the fast-paced world of technology. There are various stages at which testing is done to ensure the quality of delivery.

Legend:

Range	**Equivalent**	**Interpretation**
3.50 – 4.00	Strongly Agree	Strongly Acceptable
2.50 – 3.49	Agree	Acceptable
1.50 – 2.49	Disagree	Unacceptable
1.00 – 1.49	Strongly Disagree	Strongly Unacceptable

Evaluation Result

Summary of Level of Software Quality Characteristics

This summarizes the level of functionality, reliability, usability, efficiency, maintainability, and portability of the system with the sub-characteristics.

Table 1. The Summary of Level of Software Quality Characteristics

FUNCTIONALITY	SA	A	D	SD	**Sub-Characteristics**	Weighted Mean
	4	3	2	1		
Q1	1	4			Suitability	3.60
Q2	1	4			Accurateness	3.20
Q3	5				Security	4.00
RELIABILITY	SA	A	D	SD	**Sub-Characteristics**	Weighted Mean
	4	3	2	1		
Q4		5			Maturity	3.00
Q5	1	4			Fault Tolerance	3.20
Q6	3	1	1		Recoverability	3.40
USABILITY	SA	A	D	SD	**Sub-Characteristics**	Weighted Mean
	4	3	2	1		
Q7	3	2			Understandability	3.60
Q8		5			Learnability	3.00
Q9	1	4			Operability	3.20
Q10	1	4			Attractiveness	3.20

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EFFICIENCY	SA 4	A 3	D 2	SD 1	**Sub-Characteristics**	Weighted Mean
Q11	2	3			Time Behavior	3.40
Q12	2	3			Resource Utilization	3.40
MAINTAINABILITY	SA 4	A 3	D 2	SD 1	**Sub-Characteristics**	Weighted Mean
Q13	1	3	1		Analyzability	3.00
Q14	1	4			Changeability	3.20
Q15	2	3			Stability	3.40
Q16	3	2			Testability	3.60
PORTABILITY	SA 4	A 3	D 2	SD 1	**Sub-Characteristics**	Weighted Mean
Q17	2	3			Adaptability	3.40
Q18	2	3			Installability	3.40
Q19	2	3			Conformance	3.40
Q20	2	3			Replaceability	3.40

Table 1 shows the summary result of the evaluation conducted among different IT Professionals composed of IT Teachers from DBHS – Don Bosco High School Paranaque and government schools, Programmer, IT staff, and Web Developer.

FINDINGS AND DISCUSSION

The finding of the study revealed that the level of overall Software Quality Characteristics obtained a mean of 3.36 and was interpreted as acceptable. The level of functionality was strongly acceptable (WM = 3.60). For the rest of the characteristics, it was acceptable, i.e. reliability (WM = 3.20), usability (WM = 3.25), efficiency (WM=3.40), maintainability (WM = 3.30) and portability (WM = 3.40

CONCLUSION AND FURTHER RESEARCH

Based on the findings of the study, it can be concluded that web-portal or web-based platform Software Quality Characteristics were acceptable to the respondent-evaluators.

Recommendations:
1. Develop and provide more information about the course/subject based on DepEd Curriculum Guide. In addition, it is recommended to yearly update the content of the portal and its references.
2. Enhance the system flexibility based on uses and functions by providing more activities for the students

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3. Improve the systems Graphical User Interface (GUI) and color harmony to be a lot easier to use for students and other users.
4. Enhance system performance and operation by providing good internet connectivity for the students and other users' fast access to the portal.
5. Maintain system portability by checking and updating computer specifications, performance, and backups.

REFERENCES

Al-Azawei, A.Al-Bermani, A. , Lundqvist, K. (2016). Journal of Information TechnologyEducation. Vol. 15, p109-130.
Bath, D. &Bouke, J. (2010). Getting started with blended learning, Griffith University ISBN: 978-1921760-24-2
Forest, E (2017). Educational Technology: An Overview, Viewed November 6, 2017 https://EducationalTechnology.net/Educational-Technology-AnOverview/
Guest, G. Bunce, A & Johnson, L. (2006). How many interviews are enough? An experiment with data saturation and variability first published February 1, 2006 http://Journals.Sagepub.com/DOI/ABS/1.1177/152822X05279903
Horn, M.B. ,Fisher, J.F. (2017). ASCD / www . Ascd . Org 60 Educational Leadership / March 2017 Copyright of Educational Leadership is the property of Association for Supervision & Curriculum Development 59-63 (, p. 59-63)
Horn, M.B. ,Staker, H (2014).Using Disruptive Innovation to Improve Schools, San Francisco: Jossey-Bass. Copyright © 2014.
Insook L., Junghoon L., Eunmo S (2006.) A Study on the Development of Collaborative Learning Model, The Journal of Korean association of computer education, vol. 9 , no. 2, (2006), pp. 27-36 Information Technology Curriculum Integration, DOI: 10.4018/978-1-59904-881-9.ch132
Rawlins, Troy A.; Ali, Rifath (2017). Evaluating of the Impact of Hybrid or Blended Instructional Design on Muslim Students; Online Journal of Distance Learning Administration, v20n1 Spr.
https://www.ed.gov/oii-news/use-technology-teaching-and-learning viewed November 6,2017
https://techterms.com/definition/moodle viewed January 18, 2018
http://www.softwaretestinghelp.com/web-application-testing/ viewed January 18, 2018
https://www.guru99.com/web-application-testing.html viewed January 20, 2018
http://what-when-how.com/portal-technologies-and-applications/benefits-and-limitations-of-portals/viewed January 20, 2018

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Integrating of Voice Recognition Email Application System for Visually Impaired Person using Linear Regression Algorithm

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Abstract

The outcome of this study will surely help visually impaired people who face difficulties in accessing the computer system. Voice recognition will help them to access email. This study also reduces the cognitive load taken by visually impaired users to remember and type characters using a keyboard. If this system is implemented, self-esteem and social and emotional well-being of the visually impaired users will be lifted up for they will now feel they are being valued in society and has fair treatment and access to technology main function of this study is to use a keyboard of the user that will respond through voice. The purpose of this study is to help a visually impaired person to use the modern application to interact with voice recognition systems with the use of email into different types of modern gadgets, Line computers, or mobile phones. In terms of functionality of the application, the proponents will use a set of APIs,' or Application Program Interface such as Google Speech-to-text and text-to-speech application and it will process through Email System and also the SNMTP or Simple Network Management Protocol will be used for mailing services, in programming software, the proponent will be using PHP for the backend of a web interface. For the creation of a Web Base UI, HTML and CSS will be used. Voice typing and Dictation Speech Interaction models using windows dictation engine. The proponent used a descriptive research design in this study. Descriptive research design is being used by the proponents to describe the characteristics of a population or phenomenon of visually impaired persons being studied. Descriptive research is mainly done because the researchers want to gain a better understanding of a topic. It focuses on providing information that is useful in the development. The research is based on a mixed method focused on producing such informative outcomes that can be used. Based on the results of the surveys, conclusions were drawn: The majority of the respondents were male adultery period ranging from ages 32-41.all are working as massage therapists. The majority of the respondents rated the overall function of the application as Excellent and rated the level of security of the application as Secured.

Keywords: *Food Cost, Lockdown, Activity-based costing, food frequency questionnaire*

INTRODUCTION

Nowadays, not all people are aware of how a typical Blind person suffers in their everyday living, according to (Global Data on Visual impairments 2012). Many of them are always problematic on how they live with difficulties with normal daily activities such as driving, reading, socializing, and walking. And to boost their confidence for interacting and accessing different technologies. To define what is Visual impairment describes a person who has a vision loss and someone who cannot see at all or has a partial vision loss, completely blind, and others called legal blindness. Based on the World Health Organization Prevention of Blindness and Deafness 2008 edition. Globally there are 2.2 billion people having a near or distance vision impairment, and the estimated numbers of visually impaired persons in 2012 were 286 million worldwide, of which

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246 million had low vision, and 39 million were blind. Of those who are blind, 90%. According to (Althomali T (2012), The main cause of blindness and vision impairment is they have cataracts, uncorrected refractive errors in their eyes, and caused by things like infection, genetics, or injury. The vision of the visually impaired and blind measures 20/200 or worse. However, the majority of people ages 50 years have a vision impairment and blindness, so vision loss can affect people of all ages.

Moreover, Internet has made the life of people so easy that people today have access to any information they want while sitting at their home. One of the main fields that the Internet has revolutionized is "communication." While talking about communication over the Internet, the first thing that comes to our mind is Email. Emails are considered to be the most reliable way of communication over the Internet, for sending or receiving some important information.

Voice Recognition can be of great value to blind people as it empowers them to independently complete tasks which they would normally not be able to accomplish, boost their self-confidence and Visually impaired people can do a lot of things such as reading emails or managing their own accounts without help from others with the use of voice recognition, Voice recognition serves as an alternative to typing on a keyboard. It gives users the option to use a speech-to-text system, which means users talk to the computer and their input is interpreted and converted to electronic text. Put simply, you talk to the computer, and your words appear on the screen. The proponent believes that the increasing number of blind persons in the Philippines will begin building a demand for the new trends in technology. It seems that the time is right to begin developing high-technology services to meet the needs of this growing population of the literate blind, not only for them to consider themselves into the millennials but also a big chance to boost their social status and self-esteem. This study is in the hopes of better serving this hidden population of blind people. We hope to gain new ideas and make new innovations that will benefit all those who are blind in the Philippines.

LITERATURE REVIEW

Based on a conducted Survey article on the Use of Mobile Applications for People who Are Visually Impaired (Griffin, Banda, Ajuwon 2017), The few studies indicated and conducted on the person with visual impairments or who are blind and have low vision are concerning in the mobile application usage. The use of the mobile application has increased globally completed; the online survey the summary with the total of 259 participants having visual impairments. They use Descriptive statistics and bivariate tests methods where they used to examine all associations for the demographic's characteristics and mobile app use. The result of the online survey is that all the participants are rated special apps with useful (95.4%) and accessible (91.1%) tools for individuals with visual impairments. In the middle age adult group, More than 90% are strongly agreed with the practicality of special apps, while in the old adult group, are significantly higher percentage was observed. Some of the participants with a low vision as an addition to the result considered special appl has a less accessible for using apps. For the final results of this study, this population is satisfied with us of mobile apps, and they would like to see the improvements. Furthermore, the result shows that the persons who have visual impairments frequently use

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application design to them to accomplish different daily activities. The development of the application for the visually impaired and blind is used to refine and test the existing apps, so the study provides preliminary information regarding the app's usage for the visually impaired and blind.

Moreover, based on the study, Ecommerce Based Online Shopping for Visually Impaired People using Speech Recognition) on pointing out the use of online shopping for the visually impaired, For Visually impaired people, it is difficult to do online shopping (Kunal, Nawkhare 2017) We are developing a system which helps blind people to choose the color of clothes along with categories such as material, size, patterns, etc. by using Automatic speech recognition module—translating of spoken words into text using deep learning method". According to their study, it successfully replaced Gaussian mixture speech recognition and feature coding at an increasingly larger scale. The main aim of this project is to propose an online shopping application especially for blind people through which they can choose whatever they want by simply speaking a sentence, and to implement this. We are going to integrate the deep learning method and clustering.

On Other studies related to Speech- Voice recognition (Gros, Mihelic, Pavesic 2017), "HOMER is a voice-driven text-to-speech system developed for blind or visually impaired persons for reading the Slovenian texts." Based on their study, users can obtain texts from the special corpora organized on the computer network server at the information center of the Association of the Slovenian Blind and Visually Impaired Persons. The system consists of three main modules. The text-to-speech module enables speech synthesis from arbitrary Slovenian text input. The speech recognition module performs speaker-independent isolated word recognition, and the dialogue module controls the different tasks of the HOMER system and obtains texts from the source text corpora. Presently, the system runs under Linux and requires a Pentium/133 PC with a minimum of 32 MB of RAM and an additional standard 16-bit sound card. While according to M. Bazzani and E.M. Mumolo (Alcatel FACE Res. Center, Pomezia, Italy), they stated: "PC-based telephone communications system for deaf-blind people is a system has been developed that allows deaf-blind people to communicate with others by a standard telephone set." Based on their study, the system will use speech processing technologies integrated with a PC (personal computer). The handicapped person can activate or receive a telephone communication, entering sentences by typing on a Braille terminal and receiving the messages on a Braille tactile display. A text-to-speech subsystem converts the typed sentences into voice, and a connected-word voice recognizer converts the vocal answer into text and then into a Braille message. Results of a field trial of the system carried out in an Italian community of handicapped people are reported.

METHODOLOGY

The researcher used a descriptive research design in the project. Descriptive research design is being used by the proponents to describe the characteristics of a population or phenomenon of visually impaired persons being studied. Descriptive research is mainly done because the researchers want to gain a better understanding of a topic. It focuses on providing information that is useful in the development. The research is based on a mixed method focused on producing such informative outcomes that can be used.

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The respondents of this study were the visually impaired persons who can able to speak and hear to be able to verify all the important information on his/her email account and use the voice recognition application properly.

The proponent used one method for data gathering is the interview, as the core and umbrella of this study. Interviews on the respondents, which are some of the visually impaired persons who use a desktop computer, a visually impaired person who is engaged in office works who has problems in sending mails, a visually impaired person who also has experiences in using voice recognition AI like Siri. For computing the percentage of the respondents, Statistical Tools and Instruments are being used for determining the sampling Population, Frequency, Mean, Standard Deviation, and Annova Analysis for the Validation and distribution of the instruments used.

FINDINGS AND DISCUSSION

Table 1. The Demographic Profile of the Respondents in Terms of Age Bracket

Age Bracket	Frequency (f)	Percentage (%)	Rank
18 – 25	0	0%	5
26 – 31	11	22%	3
32 – 41	21	42%	1
42 – 49	13	26%	2
50 above	5	10%	4
Total No. Respondents (N)	50	100%	

As shown from the table, out of 50 respondents in terms of age bracket, 32-41 got the highest frequency of 21 or 42 % is rank 1, 42-49 with a frequency of 13 or 26 % is rank 2, 26-31 with a frequency of 11 or 22 % is in rank three while 50 above with a frequency of 5 or 10 % is in rank 4, and 18-25 got no frequency of 0 or 0 % is in rank 5.

Table 2. The Demographic Profile of the Respondents in Terms of Gender

Gender	Frequency (f)	Percentage (%)	Rank
Male	34	68%	1
Female	16	32%	2
Total No. Respondents (N)	50	100%	

As shown from the table, out of 50 respondents in terms of gender, the frequency of Male is 34, which is equivalent to 68%, which is rank 1, while the frequency of females is 16 or 32% which is in rank 2.

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Table 3 – The Demographic Profile of the Respondents in Terms of Job/Designation

Job/Designation	Frequency (f)	Percentage (%)	Rank
Singer	12	24	2
Massage therapist	16	32	1
Student	10	20	3
Radio announcer	2	4	5
Factory worker	2	4	5
Call center agent	8	16	4
Total No. Respondents (N)	50	100%	

As shown from the table, out of 50 respondents in terms of the Job description, the frequency of Massage therapist got the highest of 16 or 32 %, which is rank 1, Singer with a frequency of 12 or 24 %, which is rank 2, Students with a frequency of 10 or 20% which is in rank 3 while Call center agent with a frequency of 8 or 16% which is in rank 4 and both Radio announcer and Factory worker got the frequency of 2 or 4% which is in rank 5.

Table 4 . The Demographic Profile of the Respondents in Terms of Highest Educational Attainment

HIGHEST EDUCATIONAL ATTAINMENT	Frequency (f)	Percentage (%)	Rank
College Level	3	6%	3
Vocational	32	64%	1
High School Graduate	0	0%	4
High School Level	0	0%	4
Elementary Graduate	0	0%	4
Elementary Level	15	30%	2
Total No. Respondents (N)	50	100%	

As shown from the table, out of 50 respondents in terms of Highest educational attainment, the frequency of Vocational got the highest of 32 or 64 %, which is rank 1, Elementary level with a frequency of 15 or 30 %, which is rank 2, College level with a frequency of 3 or 6 % which is in rank 3 while High school graduate, high school level, and elementary graduate got no frequency.

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Table 5 – The Demographic Profile of the Respondents in Terms of Experience using any kind of gadget.

Experience using any kind of gadget	**Frequency (f)**	**Percentage (%)**	**Rank**
1 to 5 years	10	20%	2
6 to 10 years	30	60%	1
Not at all	10	20%	2
Total No. Respondents (N)	50	100%	

As shown from the table, out of 50 respondents in terms of experience using any kind of gadgets, the frequency of 6 to 10 years got the highest of 30 or 60 % which is rank 1, 1 to 5 years got a frequency of 10 or 20 % which is rank 2, while those who do not have experience using any kind of gadget got a frequency of 10 or 20 % which is also rank 2.

Part II. Evaluation of the Respondents

Table 1. The evaluation of the respondents in terms of Accessibility no. 1

Criteria Accessibility	How would you rate the Log-in method in terms of bounding g-mail accounts?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	21	42%	1
Very Good	14	28%	3
Good	15	30%	2
Fair	0	0%	4
Poor	0	0%	4
Total no. Respondents (N)	50	100%	
Mean	4.12		
Verbal Interpretation	Very Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accessibility. Excellent got the highest frequency of 21 or 42%, which is in rank 1, Good with a frequency of 15 or 30% which is in rank 2, Very good with a frequency of 14 or 28% which is in rank three while Fair and Poor got no verbal interpretation which is rank 4.

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Table 2 – The evaluation of the respondents in terms of Accessibility no. 2

Criteria **Accessibility**	How would you rate the accessibility of the application in terms of creating and sending messages through voice recognition?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	21	42%	1
Very Good	14	28%	3
Good	15	30%	2
Fair	0	0%	4
Poor	0	0%	4
Total no. Respondents (N)	50	100%	
Mean	4.12		
Verbal Interpretation	Very Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accessibility. Excellent got the highest frequency of 21 or 42% which is in rank 1, Good with a frequency of 15 or 30% which is in rank 2, Very Good with a frequency of 14 or 28% which is in rank 3 while Fair and Poor got no verbal interpretation which is rank 4.

Table 3. The evaluation of the respondents in terms of Accessibility no. 3

Criteria **Accessibility**	How would you rate the applicant's ability in terms of accessing the sent messages, inbox, and drafts by listening to it?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	16	32%	2
Very Good	22	44%	1
Good	8	16%	3
Fair	4	8%	4
Poor	0	0%	5
Total no. Respondents (N)	50	100%	
Mean	4		
Verbal Interpretation	Very Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accessibility. Very Good got the highest frequency of 22 or 44% which is in rank 1, Excellent with a frequency of 16 or 32% which is in rank 2, Good with a frequency of 8 or 16% which is in rank 3 while Fair with a frequency of 4 or 8% which is in rank 4 and Poor got no verbal interpretation which is rank 5.

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Table 4. The evaluation of the respondents in terms of Accessibility no. 4

Criteria Accessibility	How would you rate the accessibility of the application in terms of retrieving messages through voice recognition?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	9	18%	3
Very Good	12	24%	2
Good	24	48%	1
Fair	5	10%	4
Poor	0	0%	5
Total no. Respondents (N)	50	100%	
Mean	3.5		
Verbal Interpretation	Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accessibility. Well got the highest frequency of 24 or 48%, which is in rank 1, Very Good with a frequency of 12 or 24%, which is in rank 2, Excellent with a frequency of 9 or 18%, which is in rank 3 while Fair with a frequency of 5 or 10% which is in rank 4 and Poor got no verbal interpretation which is rank 5.

Table 5. The evaluation of the respondents in terms of Accessibility no. 5

Criteria Accessibility	How would you rate the accessibility of the application in terms of locating information easily by tapping the screen?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	24	48%	1
Very Good	17	34%	2
Good	9	18%	3
Fair	0	0%	4
Poor	0	0%	4
Total no. Respondents (N)	50	100%	
Mean	4.3		
Verbal Interpretation	Very Good		

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As shown from the table, out of 50 respondents in verbal interpretation in terms of accessibility. Excellent got the highest frequency of 24 or 48% which is in rank 1, Very Good with a frequency of 17 or 34% which is in rank 2, Good with a frequency of 9 or 18% which is in rank 3 while Fair and Poor got no verbal interpretation which is rank 4.

Table 6. The evaluation of the respondents in terms of Accuracy no. 1

Criteria **Accuracy**	How would you rate the accuracy of every data and information produced by the application?		
Likert Scale	Frequency f(x)	Percentage (p)	Rank
Excellent	3	6%	4
Very Good	15	30%	2
Good	24	48%	1
Fair	8	16%	3
Poor	0	0%	5
Total no. Respondents (N)	50	100%	
Mean	3.26		
Verbal Interpretation	Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accuracy. Good got the highest frequency of 24 or 48% which is in rank 1, Very Good with a frequency of 15 or 30% which is in rank 2, Fair with a frequency of 8 or 16% which is in rank 3 while Excellent with a frequency of 3 or 6% which is in rank 4 and Poor got no verbal interpretation which is rank 5.

Table 7 – The evaluation of the respondents in terms of Accuracy no. 2

Criteria **Accuracy**	How would you rate the accuracy of the voice navigation guide (VNG) in guiding the user to use the application?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	11	22%	2
Very Good	24	48%	1
Good	8	16%	3
Fair	5	10%	4
Poor	2	4%	5
Total no. Respondents (N)	50	100%	
Mean	3.74		
Verbal Interpreter	Good		

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As shown from the table, out of 50 respondents in verbal interpretation in terms of accuracy. Very Good got the highest frequency of 24 or 48% which is in rank 1, Excellent with a frequency of 11 or 22% which is in rank 2, Good with a frequency of 8 or 16% which is in rank 3 while Fair with a frequency of 5 or 10% which is in rank 4 and Poor with a frequency of 2 or 4% which is in rank 5.

Table 8. The evaluation of the respondents in terms of Accuracy no. 3

Criteria **Accuracy**	How would you rate the accuracy of the words and phrases decoded by the application in terms of receiving voice input from the user?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	16	32%	2
Very Good	25	50%	1
Good	7	14%	3
Fair	2	4%	4
Poor	0	0%	5
Total no. Respondents (N)	50	100%	
Mean	4.1		
Verbal Interpretation	Very Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accuracy. Very Good got the highest frequency of 25 or 50% which is in rank 1, Excellent with a frequency of 16 or 32% which is in rank 2, Good with a frequency of 7 or 14% which is in rank 3 while Fair with a frequency of 2 or 4% which is in rank 4 and Poor got no verbal interpretation which is rank 5.

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Table 9 – The evaluation of the respondents in terms of Accuracy no. 4

Criteria **Accuracy**	How would you rate the accuracy of the pronunciation or diction of the speech recognition in terms of providing voice output back to the user?		
Likert Scale	**Frequency f(x)**	**Percentage (p)**	**Rank**
Excellent	23	46%	1
Very Good	15	30%	2
Good	12	24%	3
Fair	0	0%	4
Poor	0	0%	4
Total no. Respondents (N)	50	100%	
Mean	4.22		
Verbal Interpretation	Very Good		

As shown from the table, out of 50 respondents in verbal interpretation in terms of accuracy. Excellent got the highest frequency of 23 or 46%, which is in rank 1, Very Good with a frequency of 15 or 30%, which is in rank 2, Good with a frequency of 12 or 24%, which is in rank 3, while Fair and Poor got no verbal interpretation which is rank 4.

CONCLUSION AND FUTURE RESEARCH

Conclusion

Based on the results of the surveys, conclusions were drawn:

1. Majority of the respondents were male.
2. Majority of the respondents were in their adultery period ranging ages 32-41.
3. Majority of the respondents were working as massage therapists.
4. Majority of the respondents, in terms of highest educational attainment, took vocational courses or training on a special course.
5. Majority of the respondents rated the overall function of the application as Excellent.
6. Majority of the respondents rated the security of the application Secured.

Recommendation

Based on the results from the surveys of the android based voice recognition email application, the proponents can say that the proposed application is given highly recommended ratings by the respondents who are blind or visually impaired persons. The respondents were very overwhelmed upon using the application, for they felt cared for and loved knowing that there are some developers who really want to develop a new trend in computer technology for people like them. But there are some suggestions and feedback received from the respondents

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REFERENCES

Griffin-Shirley N, Banda DR, Ajuwon PM, et al. A Survey on the Use of Mobile Applications for People who Are Visually Impaired. Journal of Visual Impairment & Blindness. 2017;111(4):307-323. doi:10.1177/0145482X1711100402

Abhishek Thakur, Rajesh Kumar, Naveen Kumar, (Dept. of ECE, IGCE, Abhipur, Mohali, Punjab, India, "Automatic Speech Recognition System for Hindi Utterances with Regional Indian Accents" https://pdfs.semanticscholar.org/7117/6b84ca0ec49b6c9f7120d35469ba6e7b8859.pdf

Jerrick Chua, Unisse chua, Cessar de Padua, Janelle Isis Tan, and Mr. Danny Cheng who studies college of Computer Studies at De La sale University of Manila, "A Filipino Hands-free Text Messaging Application" https://pdfs.semanticscholar.org/2127/7e621115cbadc074bdbbf9538bcbf090dae5.pdf?_ga=2.94376604.1890603161.1552798848-910769576.1552798848

Miguel Carlos Pascual, John LlyodLagura, Rehum Jay Rabadan, and AlexanderTabac from electronics and engineering graduates of De La Salle University (DLSU),"SMS for Blind Using Braille" https://aboutphilippines.org/Video-descriptions/SMS-for-the-Blind.pdf

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The Chemical Composition, Microbiology and Micronutrients Changes of Fresh Barracuda Fish and Smoked Barracuda Fish using Different Smoking Methods

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Abstract

Fish play an essential role in human nutrition and ensure about 20% of protein intake for one-third of the world's population, especially in developing countries. Fish is consumed because of its nutritional benefits, such as protein, essential amino acids, fats, and micronutrients (vitamins and minerals). Micronutrients can prevent disease disorders due to micronutrient deficiencies. But behind its nutritional advantages, fish are very easy to spoil. Fish preservation and processing methods explore ways to stop or slow down spoilage. One method of preserving and processing fish that can be applied is smoking. This study aimed to evaluate the moisture content, total fat, heavy metals, vitamin A, and microbiology of fresh and smoked barracuda fish with different smoking methods, namely traditional smoking, and liquid smoke. Fresh barracuda fish is smoked using the traditional smoking method and liquid smoke. Fresh and smoked barracuda fish were then analyzed, including water content, total fat content, heavy metals (Cd, Hg, Sn, As), histamine, micronutrients (vitamins A and D), and microbial contamination. The levels of heavy metals, histamine, and microbial contamination have met the quality standard of smoked fish (SNI 2725: 2013). Vitamin A in fresh barracuda and smoked barracuda was < 15.85 mcg/100 g, while vitamin D was not detected in either fresh barracuda or smoked barracuda.

Keywords: *heavy metals, histamine, microbiology contamination, smoked fish, vitamin*

INTRODUCTION

Fish play an important role in human nutrition and ensure about 20% of protein intake for one-third of the world's population, especially in developing countries (Bene et al., 2007). Fish is consumed in various parts of the world because of its nutritional benefits, such as protein, essential amino acids, vitamins, minerals, and fats (Geoffroy et al., 2018). Fish protein has a nutritional value which is very important for pregnant women for proper fetal development and will promote proper mental development and immunity to disease among growing children (NAFDAC, 2003). Micronutrients such as vitamins and minerals can also prevent disease disorders

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due to micronutrient deficiencies (Mohanty et al., 2016). But despite its nutritional advantages, fish are very susceptible to spoilage at high environmental temperatures in the tropics within 12-20 hours (Clucas, 1981). Fish preservation and processing methods explore ways to stop or slow down spoilage (Olokor et al., 2007). One method of preserving and processing fish that can be applied is smoking.

Smoking is a traditional fish preservation method that aims to extend the shelf life, improve the taste of the final product, give color and taste to the product, and has a bacteriostatic and antioxidant role (Kristinsson et al., 2008). Smoked fish quality is influenced by raw materials, salting method, salt concentration, smoke composition, and smoking method (Adeyeye, 2016; Alcicek & Atar, 2010; Stolyhwo & Sikorski, 2005). The proximate and fat composition of smoked fish is highly dependent on its content and composition in the fresh fish used for smoking. Other factors that can affect the fat composition of smoked fish are the preparation of smoking raw materials when smoking and storage of smoked fish (Goulas & Kontominas, 2005). Food processing methods have been found to affect the composition of foods as well as to lose nutrients in processed foods. The nature of the diet and the effect of the processing method on the fish to be processed should be considered.

Therefore, this study aimed to evaluate the moisture content, total fat, heavy metals, vitamin A, and microbiology of fresh and smoked barracuda fish with different smoking methods, namely traditional smoking, and liquid smoke.

LITERATURE REVIEW

Fresh barracuda fish weighing as much as ±10 kg were procured from the Fish Auction Hall in Demak, Central Java, Indonesia, as the raw material. Barracuda fish samples were taken to Diponegoro University, Semarang, Indonesia, for smoking by two different methods.

Smoking Process

Fresh barracuda fish were cleaned and washed using clean water. Then, the fish were gutted. All of the fish that were gutted were then washed and drained. The traditional smoking method used a smoking furnace for approximately ±15 minutes. The liquid smoke method was carried out by immersing the fish in a 5% liquid smoke solution for 30 minutes, draining it for 30 minutes, then heating it gradually, at a temperature of 40-45°C for 1 hour; 60-70°C for 1 hour; and 90°C for 1 hour (Swastawati et al., 2017).

Moisture and Total Fat Content

The proximate analysis consisting of moisture was carried out according to the AOAC 925.09 2005 method, and total fat content was carried out to the AOAC 960.39 2005. (AOAC, 2005).

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Heavy Metals

Analysis of heavy metals (Cd, Hg, Sn, As) was performed following the methods reported by National Standardization Agency for Indonesia number 2354.5:2011, the testing method using an atomic absorption spectrophotometer (National Standardization Agency for Indonesia, 2011).

Histamine Levels

Histamine analysis was carried out according to the histamine testing from Shimadzu (2013) and Hitachi Technical Data. The analysis begins with the preparation of a standard histamine series of at least six concentration points in the linearity range of 0.1-500 mg/L into a 50 ml volumetric flask. The sample weighed 5 g of the test portion and added 10 ml of TCA 5%, then vortexed. The test sample solution was quantitatively transferred into a 25 ml volumetric flask and sonicated. TCA 5% was added up to the mark and homogenized. The test sample solution was transferred into a 2 ml tube and then centrifuged. Supernatant 1 ml was added with 0.4 ml of 1 N NaOH and calibrated with distilled water, then homogenized. The test sample solution was filtered with a 0.45 µm syringe filter into a 2 ml tube. The series solution and the test sample 200 µL were put into each 2 ml vial, then added 900 µL MPA, 440 µL OPA, and 50 µL AABA, then vortexed. The solution was injected into the HPLC system. Histamine levels are calculated by the following formula:

$$\text{Histamine levels (mg/kg)} = \frac{\frac{(Rasio\ spl-a)}{b}\ x\ FP\ x\ V}{W\ spl}$$

a = Intercept of the standard calibration curve
b = Slope of the standard calibration curve
FP = Dilution factor
V = Final volume of test solution (ml)
W_{spl} = Weight of the test portion weighing (g)

Vitamine A and D Levels

Analysis of vitamins A and D was carried out according to the AOAC 2001.13.2011 method procedure (AOAC, 2011). The procedure begins by making a standard series of vitamins A and D, at least six points of concentration in a 10 ml volumetric flask. The solid sample was weighed in a 100 ml glass beaker, then a solution of ethanol 95%, KOH 50%, and pyrrolic acid was added and stirred until homogeneous. The solution mixture was heated in a water bath at 80°C for 45 minutes. Then the solution was cooled to room temperature. The solution was put into a 100 ml volumetric flask and added with glacial acetic acid, and diluted with THF: ethanol (1:1) solution to the mark, then homogenized. The solution was filtered using a 0.45 m GHP/PTFE filter syringe into a 2 ml amber vial, then 20 µL was injected into the HPLC system, with a maximum wavelength of 325 nm for vitamin A and 264 nm for vitamin D. Vitamin levels were calculated using a standard calibration curve, with the equation of the line: Y = bx + a, with the following formula:

$$\text{Vitamin Levels (µg/100 g or µg/100 ml)} = \frac{\frac{(Luas\ area\ spl-a)}{b}\ x\ V\ x\ FP\ x\ 100}{W\ spl\ atau\ V\ spl}$$

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a = Intercept of the standard calibration curve
b = Slope of the standard calibration curve
FP = Dilution factor
V = Final volume of test solution (ml)
W_{spl} = Weight of the test portion weighing (g)
V_{spl} = Sample pipetting volume (ml)

Microbiological Contamination

Microbiological analysis of fresh barracuda and smoked barracuda included determination of total plate count (TPC), *Escherichia coli*, and *Salmonella* sp. TPC determination was carried out on fresh and smoked barracuda fish samples using the Petri dish count method based on Indonesian National Standard number 01-2323.3-2015. Analysis of *Escherichia coli* using the most probable number (MPN) method was based on 01-2332.1-2015. Determination of *Salmonella* sp. was based on the method of Indonesian National Standard number 01-2332.2-2006.

RESULTS AND DISCUSSIONS

Moisture and Total Fat Content

The moisture and total fat content of fresh barracuda and smoked barracuda using different smoking methods are shown in Table 1.

Table 1 Moisture and total fat content of fresh barracuda and smoked barracuda

	Treatments		
	Fresh barracuda fish	Traditional smoking methods	Liquid smoke methods
Moisture content (%)	74.35±0.275^{c}	72.02±0.424^{b}	67.75±0.262^{a}
Total Fat (%)	0.32±0.000^{a}	0.73±0.007^{c}	0.64±0.014^{b}

Data are the average of two replication ± standard deviation
Data followed by different letters show significant differences (≤0.05)

Different smoking methods were able to reduce the water content of fresh barracuda fish, from 74.35 to 72.02% in the traditional method and 67.75% in the liquid smoke method. The decrease in water content can reduce microbial activity and extend the shelf life of the product (Cardinal, 2001). Smoked barracuda fish in both smoking methods has a water content that still exceeds the Indonesian national standard for smoked fish, which is a maximum of 60% (BSN, 2013). Both smoked fish smoked using traditional smoking methods, and liquid smoke provided higher levels of fat compared to raw materials, which were 0.73 and 0.64%, respectively (Table 1). The increase in fat is caused by the water content lost during the smoking process. Smoking, heating, and high

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salt concentrations can cause chemical and physical changes that increase protein digestibility. This change also reduces thermolabile compounds and polyunsaturated fatty acids (Arason et al., 2014). The increase in total fat content in smoked barracuda correlated with a decrease in water content. An inverse correlation between fat and water content for many fish species has been widely reported (Ljubojevic et al., 2016).

Heavy Metals

The heavy metals analyzed in this study are Hg, Cd, As, and Sn. The heavy metal content of fresh and smoked barracuda fish is showed in Table 2.

Table 2 The heavy metals content of fresh and smoked barracuda fish

Logam berat (mg/kg)	Treatments		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
Hg	0.90 ± 0.000[b]	0.18 ± 0.007[a]	0.16 ± 0.021[a]
Cd	ND	ND	ND
As	6.21 ± 0.346[a]	7.46 ± 0.721[a]	6.795 ± 0.417[a]
Sn	ND	ND	ND

ND = Not detected
Data are the average of two replication ± standard deviation
Data followed by different letters show significant differences (≤0.05)

Heavy metal levels of Hg in fresh barracuda fish were 0.90 mg/kg, while in traditional smoked and liquid smoked fish were 0.18 mg/kg and 0.16 mg/kg, respectively. The highest concentration of heavy metal As was detected in smoked fish using the traditional smoking method, which was 7.46 mg/kg, and the lowest was 6.21 mg/kg in fresh fish. There was a slight variation in As levels in both fresh and smoked fish, and it was not statistically significant ($p > 0.05$), while heavy metals Cd and Sn were not detected in the three samples. The levels of Hg, Cd and Sn metals are below the maximum limit of the Indonesian national standard, which is a maximum of 0.5 mg/kg while (National Standardization Agency for Indonesia, 2013). Cadmium (Cd) is known as an endocrine disruptor and can cause the development of prostate cancer and breast cancer in humans (Saha & Zaman, 2012).

Histamine Levels

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Histamine analysis results showed that histamine was not detected in fresh or smoked barracuda fish samples (traditional smoking and liquid smoke). This can be caused by histamine levels that are too small because the fresh barracuda fish used as raw material is still in fresh condition. Fresh fish does not contain free histamine but contains the amino acid L-histidine. Histamine is formed in fish by certain bacteria capable of producing the enzyme histidine decarboxylase, which can convert free histidine into histamine (FAO/WHO, 2013). Histamine was not detected in barracuda fish samples smoked using liquid smoke due to the presence of phenolic compounds that act as antibacterials (Dien et al., 2019).
Various countries have set legal limits for the consumption of foods containing histamine. The maximum limit exceeds 50 mg/kg (FDA, 1998), the maximum limit exceeds 100 mg/kg (European Commission, 2003; South African Bureau of Standards, 2001), and the maximum limit for histamine consumption exceeds 200 mg/kg (Australian Food Standards Code, 2001).

Vitamin A and D
Vitamin A in fresh barracuda fish was < 15.85 mcg/100 g, while in smoked barracuda fish smoked using traditional methods and liquid smoke, no vitamin A was detected (Table 3). This is caused by the heating in the smoking process, so that it can cause the loss of important nutrients. Smoking fish contributes to the physical loss of lipids and micronutrients due to the dripping of fat and more water from the fish (Roos et al., 2003). Vitamin A in G. barracuda fish smoked using a smoking drum, and the kiln was 11.41 mg/100 g and 13.93 mg/100 g, respectively (Adeyeye et al., 2017). Vitamin A has antioxidant activity, improves vision and bone growth. Fish species, in general, can easily convert carotenoids into vitamin A (Aremu et al., 2013).

Table 3 Vitamin content of fresh and smoked barracuda fish

Vitamin (mcg/100 g)	Perlakuan		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
Vitamin A	< 15.85	< 15.85	< 15.85
Vitamin D	ND	ND	ND

ND = Not detected
Data are the average of two replication

Vitamin D is a fat-soluble vitamin and has two main forms, namely vitamin D2 and vitamin D3. Vitamin D is synthesized in the skin and partly comes from food sources (Macdonald, 2012). Fresh and smoked barracuda fish samples in this study, no vitamin D was detected (Table 3). Previous studies have found that fatty fish, such as salmon, bluefish, mackerel, and tuna, are good sources of vitamin D (Lu et al., 2007). Vitamin D levels in the skin of various types of fish, such as trevally, Atlantic salmon, yellowfin tuna, bream, blackfish, and rainbow trout, are found in the range of 1.8

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to 30 g/100g (Pierens & Fraser, 2015). The level of vitamin content may vary due to several factors such as environment, season, climate, age, nutrition, and species (Mattila et al., 1995). Vitamin D deficiency can be a risk factor for chronic diseases, including cancer, autoimmune, and cardiovascular disease (Grant & Garland, 2002; Munger et al., 2006; Zittermann, 2006). Vitamin D insufficiency (vitamin D serum
< 50 nmol/L) has a worldwide prevalence (Holick & Chen, 2008).

Microbial contamination

Microbial quality of smoked fish samples using traditional smoking and liquid smoke methods were 4.9x102 colonies/g and 6.0x102 colonies/g, respectively. The total number of microbes in barracuda fish after the smoking process decreased when compared to fresh barracuda fish (8.2x102 colonies/g). The results of the analysis of variance and Duncan's further test (Table 3) showed that smoked barracuda fish smoked using traditional smoking methods and liquid smoke did not significantly affect the total microbial count. The decrease in the total number of microbes in the sample was caused by the presence of bactericidal and bacteriostatic smoke components (Swastawati et al., 2007). Smoked fish products smoked by different methods (traditional and liquid smoke) have met quality standards because the total plate number value is below the maximum limit of the Indonesian national standard number 2725: 2013, which is 5.0 x 104 colonies/g or with a log value of 4.69 (BSN, 2013).

Table 3 Microbial contamination of fresh and smoked barracuda fish

Microbial contamination	Treatments		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
TPC (colony/g)	8.2×10^{2b}	4.9×10^{2a}	6.0×10^{2a}
Escherichia coli (APM/g)	< 1.8	< 1.8	< 1.8
Salmonella sp (negative/g)	Negatif	Negatif	Negatif

Data are the average of two replication

Escherichia coli is an indicator of sanitation. Sanitation facilities affect the presence of *Escherichia coli* bacteria in food. The content of Escherichia coli bacteria in smoked fish samples using different smoking methods is < 1.8 MPN/g. This is influenced by the heating process during the smoking process, resulting in the death of *Escherichia coli* bacteria. Escherichia coli is a mesophilic bacterium with a growth temperature of 7°C to 50°C and an optimum temperature of around 37°C (Adams & Moss, 2008). Both smoked fish products have met the quality standard of smoked fish on the Indonesian national standard number 2725: 2013 for *Escherichia coli* a maximum of < 3 APM/g (National Standardization Agency for Indonesia, 2013).

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Fresh and smoked barracuda fish samples (traditional methods and liquid smoke) were found to be negative/g on the pathogenic bacteria Salmonella sp., according to the Indonesian national standard number 2725: 2013 (National Standardization Agency for Indonesia, 2013). *Salmonella* sp. can grow at an optimum temperature of 37°C and a maximum of 45.6°C. *Salmonella* is sensitive to heat and dies at 70°C, so smoking at 70°C or more is sufficient to kill *Salmonella* bacteria in all parts of the food being cooked (Hu & Kopecko, 2003; Jay et al., 2005).

CONCLUSIONS

Barracuda fish has good potential to be processed into smoked fish. Smoked barracuda fish is smoked with different methods, namely the traditional smoking method and liquid smoke. It is safe for consumption because of the heavy metal content, histamine has met the quality standard of smoked fish. Smoked fish samples also contain microbiological content, such as ALT, Escherichia coli bacteria, and pathogenic bacteria Salmonella sp. below the limit determined by the Indonesian national standard number 2725:2013. Vitamin A levels in fresh barracuda fish and smoked barracuda fish were < 15.85 mcg/100 g, while vitamin D was not detected in either fresh barracuda fish or smoked barracuda fish.

REFERENCES

Adeyeye, S.A.O., Fayemi, O.E., Adebayo-Oyetoro, A.O. 2017. Amino acid, vitamin and mineral profiles of smoked fish as affected by smoking methods and fish types. Journal of Culinary Science and Technology. 1-14.

Alcicek, Z., & Atar, H.H, 2010. The effects of salting on chemical quality of vacuum packed liquid smoked and traditional smoked rainbow trout (Oncorhyncus mykiss) fillets during chilled storage. Journal of Animal and Veterinary Advances, 9: 2778-2783.

Arason, S., Nguyen, M.V., Thorarinsdottir, K.A., & Thorkelsson, G. 2014. Chapter 6: Preservation of fish by curing. In Seafood Processing: Technology, Quality and Safety. Editor: Boziaris, I.S. John Wiley & Sons, Ltd.

Aremu, M.O., Namo, S.B., Salau, R.B., Agbo, C.O., & Ibrahim, H. 2013. Smoking methods and their effects on nutritional value of African catfish (Clarias gariepinus). The Open Nutraceuticals Journal, 6: 105-112.

Associaton of Official Analytical Chemists (AOAC). 2005. Official method on analysis of the association of official analytical of chemist. Arlington: The Association of Official Analytical Chemist, Inc.

Associaton of Official Analytical Chemists. 2011. Vitamin A (retinol) in foods. Liquid Chromatography. AOAC 2001.13.2011. Arlington: The Association of Official Analytical Chemist, Inc.

Australian Food Standards Code. 2001. Part D: Fish and fish products. Standards D1 and D2. Version 18.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Bene, C., Macfayden, G., & Allison, E.H. 2007. Increasing the contribution of small-scale fisheries to poerty alleviation and food security. FAO Fisheries Technical Paper no. 481. FAO, Rome.

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Morkore, T., Thomassen, M., & Vallet, J.L. 2001. Relation of smoking parameters to the yield, colour, and sensory quality of smoked Atlantic salmon (Salmo salar). Food Research International. 34: 537-559. Doi: 10.1016/S0963-9969(01)00069-2

Clucas, I.J. 1981. Fish handling, preservation and processing in the Tropic Part 1. Tropical Institute, London.

Dien, H.A., Montolalu, R., dan Berhimpon, S. 2019. Liquid smoke inhibits growth of pathogenic and histamine forming bacteria on skipjack filets. IOP Conference Series: Earth and Environmental Science, 278(1), 12018.

European Food Safety Authority. 2008. Polycyclic aromatic hydrocarbons in food. Scientific opinion of food panel on contaminants in food chain adopted on 9 June 2008. EFSA Journal, 724: 1-114.

FDA. 1998. FDA and EPA guidance levels. In fish and fishery products hazards and controls guide (pp. 245-248, Appendix 5). Department of Human Health and Human Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 2013. Public health risks of histamine and other biogenic amines from fish and fishery products. Meeting report.

Geoffroy, O.E., Elie, M., Lambert, C.H., Youssouf, A., Armand, B.G., Philippe, A.L. 2018. Proximate composition of cultured (Oreochromis niloticus) and (Clarias gariepinus) based on commercial feed in Benin. International Journal of Agricultural Environmental and Biotechnology, 3(5): 176-183.

Goulas, A.E., & Kontominas, M.G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel (Scomber japonicas): biochemical and sensory attributes. Food Chemistry, 93: 511-520.

Grant, W.B., & Garlant, C.F. 2002. Evidence supporting the role of vitamin D in reducing the risk of cancer. Journal of International Medicine, 252(2): 178-180.

Hitachi Technical Data. Analysis of amino acids by OPA post column method.

Holick, M.F., & Chen, T.C. 2008. Vitamin D deficiency: a world wide problem with health consequences. The American Journal of Clinical Nutrition, 87(4): 1080S-1086S.

Hu, L., & Kopecko, D.J. 2003. Campylobacter species. Miliotis, MD., Bier, J.F (Editor). International Handbook of Foodborne Pathogens. New York (US): Marcel Dekker Inc.

Jay, J.M., Martin, J., & David, A.G. 2005. Modern food microbiology. New York (US): Springer.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Ljubojevic, D., Radosavljevic, V., Pelic, M., Dordevic, V., Zivkov, B.M., & Cirkovic, M. 2016. Fatty acid composition, chemical composition and processing yield of traditional hot smoked common carp (Cyprinus carpio, L.). Iranian Journal of Fisheries Sciences, 15(4): 1293-1306.
Lu, Z., Chen, T.C., Zhang, A., Persons, K.S., Kohn, N., Berkowitz, R., Martinello, S., & Holick, M.F. 2007. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D?. Steroid Biochemistry and Molecular Biology, 103: 642-644.
Macdonald, H.M. 2012. Contributions of sunlight and diet to vitamin D status. Calcified Tissue International, 92(2): 163-176.
Mattila, P., Piironen, V., Uusi-Rauva, E., & Koivistoinen, P. 1995. Cholecalciferol and 25-Hydroxycholecalciferol contents in fish and fish products. Journal of Food Composition and Analysis, 8(3): 232-243.
Mohanty, B.P., Sankar, T.V., Ganguly, S., Mahanty, A., Anandan, R., Chakraborty, K., Paul, B.N., Sarma, D., Dayal, J., Venkateshwarlu, G., Mathew, S., Asha, K.K., Mitra, T., Karunakaran, D., Chanda, S., Shahi, N., Das, P., Das, P., Akhtar, M.S., Vijayagopal, P., & Sridhar, N. 2016. Micronutrient composition of 35 food fishes from India and their significance in human nutrition. Biological Trace Element Reseacrh, 174(2): 448-458. Doi: 10.1007/s12011-016-0714-3.
Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S., & Ascherio, A 2006. Serum 25-Hydroxyvitamin D levels and risk of multiple sclerosis. JAMA, 296(23): 2832-2838.
NAFDAC. 2003. National agency for food and drug administration and control. Consum. Saf. Bull. 2(2): 1394-1596.
National Standardization Agency for Indonesia. 2006. Microbiological test method-Part 2: Determination of Salmonella in fishery products - Indonesian National Standard Number 01-2332.2-2006. Jakarta: Badan Standardisasi Nasional.
National Standardization Agency for Indonesia. 2011. Determination of heavy metal levels of Lead (Pb) and Cadmium (Cd) in fishery products - Indonesian National Standard Number 2354.5:2011. Jakarta: National Standardization Agency.
National Standardization Agency for Indonesia. 2013. Smoked fish using hot smoking method – Indonesian Standard Number 2725:2013. Jakarta: National Standardization Agency
National Standardization Agency for Indonesia. 2015. Microbiological test method-Part I: Determination of Coliform and Escherichia coli in fishery products - Indonesian National Standard Number SNI 2332.1:2015. Jakarta: National Standardization Agency.
National Standardization Agency for Indonesia. 2015. Determination of total plate count (TPC) on fishery products – Indonesian National Standard Number 01-2332.3-2015. Jakarta: National Standardization Agency.
National Standardization Agency for Indonesia. 2016. Chemical test method part 6: Determination of mercury (Hg) heavy metal content in fishery products – Indonesian National Standard Number 2354.6: 2016. Jakarta: National Standardization Agency.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Olokor, J.O., Ihuahi, J.A., Omojono, F.S., Fabiyi, B.A., & Adelomo, E.O. 2007. Handbook of practical fisheries technology division. National Institute of Fresh Water Fisheries Research (NIFFR). New Bussa, Niger Sate: Rem-Thomas. pp 13-42.

Patange, S.B., Mukudan, M.K., dan Ashok K.K. 2005. A simple and rapid method for colorimetric determination of histamine in fish flesh. Food Control, 16(5): 465-472. doi:10.1016/j.foodcont.2004.05.008.

Pierens, S.L., & Fraser, D.R. 2015. The origin and metabolism of vitamin D in rainbow trout. The Journal of Steroid Biochemistry and Molecular Biology, 145: 58-64.

Roos, N., Islamy, M., Thilsted, S.H. 2003. Small indigenous fish species in Bangladesh: contribution to vitamin A, calcium dan iron intakes. Journal of Nutrition, 133(11 Suppl 2): 40218-40268.

Saha, N., & Zaman, M.R. 2012. Evaluation of possible health risks of heavy metals by consumption of foodstuffs available in the central market of Rajshahi City, Bangladesh.

Shimadzu Application News No. L449. 2013. High speed, high resolution analysis (Part 46) analysis of pore-column derivatized biogenic amines by the Nexera SIL-30AC autosampler. First Edition. Juni 2013

Stolyhwo, A., & Sikorski, Z.E. 2005. Polycyclic aromatic hydrocarbons in smoked fish: a critical review. Food Chemistry, 91: 303-311.

Swastawati, F., Sumardianto, & Indiarti, R. 2006. Quality comparation of smoking catfish using pine liquid smoke with different concentration. Jurnal Saintek Perikanan, 2(1): 29-39.

Swastawati, F., Cahyono, B., & Wijayanti, I. 2017. Changes in the quality characteristics of tuna (Euthynnus affinis) with the traditional smoking method and the application of liquid smoke. Jurnal Info, 19(2): 55-64.

Zittermann, A. 2006. Vitamin D and disease prevention with special reference to cardiovascular disease. Progress in Biophysics and Molecular Biology, 92(1): 39-48.

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Ensure the Proper Wearing of Face Masks Using Machine Learning to Fight Covid-19 Virus

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Abstract

In this pandemic time, wearing face masks is mandatory to all because of the possibility that a person can get COVID-19 virus through their mouth, nose or eyes, which could possibly happen when a person has a direct or close contact to a person with that virus. But, despite the strict implementation, some people disregard the proper wearing of face masks and unaware the risks of possible virus transmission for such negligence. In this paper, it will demonstrate how a Convolutional Neural Network (CNN) can detect if a person is wearing a face mask or not and the additional parameter to support to detect if the face mask is properly worn by a person by considering the facial landmarks thru face recognition using Histogram of Oriented Gradients (HOG) feature descriptor with a linear SVM machine learning algorithm. Two (2) processes are involved in proper wearing of face masks detection. It needs to pass in Face Mask Detection to proceed to the next process which is the Face detection wherein the result of checking should return false to confirm the proper wearing of the face mask of a person.

Keywords: *Convolutional Neural Network (CNN), Histogram of Oriented Gradients (HOG) and SVM machine learning algorithm*

INTRODUCTION

The world stops when covid-19 strikes. Many countries impose total lockdown to stop the threat of this deadly virus. People were restricted in going outside and issued an order to stay at home. But it seems that it's too late for some preventive action because even powerful countries like the United States, Japan and France were unable to control the virus thus they faced the deadly covid-19 which the numbers of infected is continuously rising each day until today. Protocol was implemented to attempt to control the spread of this virus. The vaccine is unavailable yet, the only thing the people can do is to follow the health protocol issued by the government. The Inter-Agency Task Force or IATF is the agency formed by the executive of the Philippine government to respond to emerging infectious diseases in the Philippines . The said agency imposed protocols such as wearing a face mask and face shield, social distancing and always sanitizing hands to ensure the safety of people against the COVID-19 virus. They always remind the public to follow and strictly observe the issued health protocols. But as we observed, people do not properly use the face mask which the right way to use is that it should cover both your nose, mouth and chin. Despite that there's a strict implementation in wearing face masks in public places but it's a bad response from the others when the authority is not around. They will only use the face mask if the authority advised them to use it and remove it once the authority leaves them. They wear face masks but not in the proper way and maybe they are unaware that the virus could enter the nose, eyes or mouth of a person. The paper objective is to present a method that can

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detect if a person is properly using the face mask in response to fight against the COVID-19 virus. It will be discussed additional evaluation to support the accuracy of detecting proper wearing of face masks.

LITERATURE REVIEW

Ejaz and Islam (2019) work to boost the accuracy of recognition for masked faces. They proposed multiple approaches for this like Multi-Task Cascaded Convolutional Neural Network (MTCNN) to deal with face detection problem, then Google FaceNet embedding model for countenance extraction and the classification task will be processed by the Support Vector Machine (SVM). The approach has been found with attractive outcomes and noteworthy performance on masked face recognition after conducting experiment. Joshi et al. (2020) proposed also a framework which will identify the faces and their corresponding facial landmarks present within the video frame using the MTCNN face detection model. The neoteric classifier will process the facial images and cues thru the utilization of the MobileNetV2 architecture as an object detector for identifying masked regions. The proposed framework was tested on a dataset of collection of videos that captures the movement of individuals in publicly spaces while complying with COVID-19 safety protocols. The methodology shows the effectiveness in detecting facial masks because of precise and accuracy of results. The tactic of considering facial landmarks like nose, mouth and eyes in face recognition is crucial in validating proper wearing of face masks. Histogram of Oriented Gradients (HOG) is essentially a feature detector that's often accustomed extract features from image data. It's widely employed in computer vision tasks for object detection like face recognition. Yuan et al. (2019) demonstrate an efficient expression identification method based on facial landmark feature points. Humans may be able to transfer information and show emotion through their visage, which is a powerful nonverbal means for them to do so. The geometric positions of different critical components of the face are the subject of their research. The facial area is first recognized via an image or video. The major features of the face will then be retrieved, and the location of the face will be corrected at the same time. The relative position of the face is used to put a group of critical points. The aforementioned procedure is an excellent technique to not only prevent the effects of changing the environment and thus the lightings, but also to increase the popularity of facial expressions. However, they used the HOG feature extraction approach of facial features in a different way in their research. The results suggest that the proposed method can extract crucial information and obtain greater recognition accuracy in their experiment.

RESEARCH METHODOLOGY

The researcher used developmental methods to come up with the desired algorithm. This research type was used to develop a system that will detect the proper wearing of face mask to fight the COVID-19 virus using Machine Learning (ML). The developmental approach was used since the study aims to establish processes that needs to generate system/ algorithms. The study creates approach that consists of two (2) principal processes: Detecting if a person is wearing a mask and if the person is properly wearing the mask. The researcher builds a CNN model using TensorFlow to detect if a person is using a face mask and apply Histogram of Oriented Gradients

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(HOG) feature descriptor with a linear SVM machine learning algorithm to detect if a person is properly wearing the face mask.

FINDINGS AND DISCUSSION

The Figure 1 shows the learning curves (training and validation accuracy and loss) of the model are the following for 30 epochs of training. It shows ~99% accuracy on our test set.

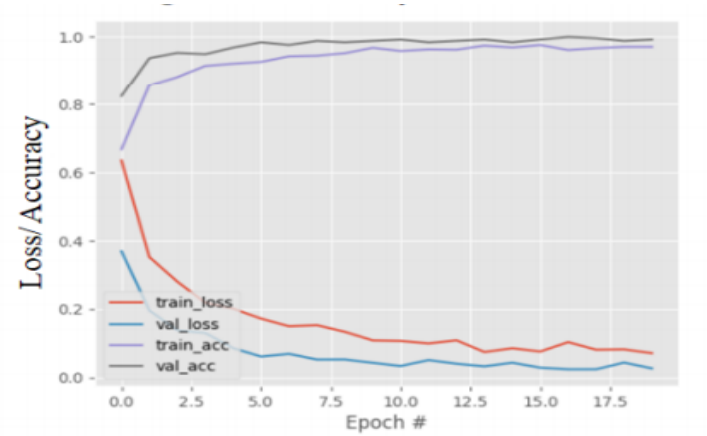

Figure 1. Learning Curves

In this image, the model we have was able to detect that the person is wearing a mask and displays the label for that. We notice in the image that the mask is not properly worn because it should cover the nose. In using the face mask, make sure it fully covers the mouth, nose, and chin. Additional parameter was inserted to address the issue. The image presents a person wearing a face mask (it was confirmed by the model) but he failed to properly wear the mask. Face detection based on facial landmarks like eyes, nose and mouth will be the option to confirm and support the accuracy of detection of properly wearing face masks. Below is the evaluation done on face detection using the Histogram of Oriented Gradients (HOG) feature descriptor with a linear SVM machine learning algorithm.

In image A, it returns a result of “No face detected” since nose and mouth was covered by the face mask. Unlike in image B, since it's not fully covered the nose thus it returns a result of “Face detected”. This option will confirm the face mask detection that passed in the first process was properly worn if Process B returns a result of True or “No face detected”.

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A **B**

CONCLUSION AND FURTHER RESEARCH

Health protocols that are implemented by the government like wearing face masks should be followed by its people in this pandemic time. Machine Learning (ML) algorithms can be considered in support for the implementation of the said health protocols. In this paper, the two (2) processes discussed such as Face Masks Detection and Face Recognition used Convolutional Neural Networks (CNN) and Histogram of Oriented Gradients (HOG) feature descriptor with a linear SVM machine learning algorithm which successfully support to meet the goal of the study. Based on the results obtained, the additional parameter is able to support to make it more accurate the detection of proper wearing of face masks. The additional method detects faces based on the facial landmarks like nose, eyes, and mouth. A result of false or no face detected from Process B will be used to confirm that the face mask was properly worn.

Recommendations

Based on the significant findings and conclusions of this research, the following recommendations are offered:

1. The researcher encourages the government (local and national) to support the application of the algorithm in detecting the proper used of face mask in fighting the spread of COVID-19 virus to human.
2. Future researchers may use other algorithms as reinforcement to improve the accuracy of the algorithm.

REFERENCES

Peng, B. & Gopalakrishnan, A.K. 2019. A Face Detection Framework Based on Deep Cascaded Full Convolutional Neural Networks, 2019 IEEE 4th International Conference on Computer and Communication Systems (ICCCS), Singapore, p. 47-51.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Joshi, A.S. 2020. Deep Learning Framework to Detect Face Masks from Video Footage, 2020 12th International Conference on Computational Intelligence and Communication Networks (CICN), Bhimtal, India, p. 435-440.

Mady, H. & Hilles, S.M.S. 2018. Face recognition and detection using Random forest and combination of LBP and HOG features, 2018 International Conference on Smart Computing and Electronic Enterprise (ICSCEE), Shah Alam, p. 1-7.

Yuan, C. 2017. Expression recognition algorithm based on the relative relationship of the facial landmarks, 2017 10th International Congress on Image and Signal Processing, BioMedical Engineering and Informatics (CISP-BMEI), Shanghai, p. 1-5.

Yang, P. 2018. Faceness-Net: Face Detection through Deep Facial Part Responses, IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. 40, no. 8, p. 1845-1859.

Nair, P. & Cavallaro, A. 2009. 3-D Face Detection, Landmark Localization, and Registration Using a Point Distribution Model, IEEE Transactions on Multimedia, vol. 11, no. 4, p. 611-623.

Khan, 2019. Facial Recognition using Convolutional Neural Networks and Implementation on Smart Glasses, 2019 International Conference on Information Science and Communication Technology (ICISCT), Karachi, Pakistan, p. 1-6.

Ejaz, M.S. & Islam, M.R. 2019. Masked Face Recognition Using Convolutional Neural Network, 2019 International Conference on Sustainable Technologies for Industry 4.0 (STI), Dhaka, Bangladesh, p. 1-6.

Rahman, M.M. 2020. An Automated System to Limit COVID-19 Using Facial Mask Detection in Smart City Network., 2020 IEEE International IOT, Electronics and Mechatronics Conference (IEMTRONICS), Vancouver, BC, Canada, p. 1-5.

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Capabilities of Computer Algorithm Utilizing Artificial Neural Networks and its Implications to Economy: A PublicPolicy Analysis

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Abstract

The massive abundance of studies pertinent to Artificial Neural Networks (ANN) has produced exciting and dulcet effects on different industries and academic disciplines. Albeit the findings of the studies relating to ANNs invite potential enterprising opportunities, it is an incontestable fact that these enterprising opportunities, like fruits of the ANNs, valiantly interpose an economic threat to the working manpower. Employment retrenchment is portending as companies opt to enjoy the benefit yielded from the application and use of ANN mechanisms. ANNs will overshadow and replace the working manpower. This study is a meta-analysis that profoundly discourses on the implications of economic issues embedded in the application and use of ANNs. The findings hereof are critical and material considerations in the craft of effective public policy measures that necessarily balance the economic impact of the ANNs to working manpower. Thus this study aims to answer two primary inquiries; (1) what are the economic implications of the application and use of ANNs? and (2) what public policy measures balance the economic downsides of the application and use of ANNs?

Keywords: *Artificial Neural Networks, Algorithm, Central Processing Unit, Artificial Intelligence*

INTRODUCTION

The artificial neural network is an arithmetic logic model for integrating sensory information received so that an action or response is generated between input and output. It is a computer architecture in which several processors interconnect between neurons in a human brain, which can learn by trial-and-error methods. Thus, ANNs can handle complex forms of data to generalize the indication of what is going to happen.

Artificial Neural Networks have known to be inefficient before the fusion of advances in artificial intelligence, IoT, etc. They gradually become powerful and are one of the most popular machine learning models and become a part of artificial intelligence because of the undefined result no other model can make it. To go upgrading of and innovation in technology which enhance human-machine relationships is the primary concern of having an artificial neural network. Because we face challenges in 4IR, accept that the opportunities and challenges are multifaceted and complex. Using automation and artificial intelligence are some of the 4th Industrial Revolution technology transfers. Artificial neural networks are the mainstream success of

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machine learning and artificial intelligence. Thus, this study focuses on the Capabilities of Computer algorithms like the Human Brain Utilizing Artificial Neural networks.

LITERATURE REVIEW

According to Wankar (2014), Artificial Neural Networks have characteristics in common, like the system unit of the computer interconnected with the input/output devices, including a processor and other interrelated technical support within the system.

According to Kumar (2017), Neural Network is a state that gives information or signal processing associated with a mathematical model based on the biological neural networks. Artificial neural networks are known for the function of iterative algorithms for creating networks, in feedbacks, dendrite, which can operate in supervised and unsupervised in interconnected neurons that provide an exciting alternative for complex problem solving and other applications. To address the issues of pattern recognition, prediction, optimization, etc., researchers from different disciplines have designed artificial neural networks in solving the problem, issues, and other concerns.

According to Sannaki (2016), the Artificial Neural Network is a fundamental means for providing a trial and error in modeling information processing capabilities in the artificial nervous system that plays a role in cognitive science. ANNs are a step-by-step procedure like computer algorithms that can learn by giving instructions on skills, knowledge, or experience acquired. Because of this feature, they are often well suited for modeling complex and not linear processes. They are greatly developed beyond an initial stage and able to compute like human beings.

According to Mijwil M.M., a piece of information such as the unprogressive programming in the past practices or established were usually stored in the whole network storage. The loss of data and other information in one place does not prevent the network from functioning.

Incomplete knowledge capability works: After ANN training, the missing data and incomplete information may produce inefficient outputs resulting in the loss of performance.

- Have fault-tolerance. The weak cells of an artificial neural network do not preclude it from generating output. This feature makes the networks fault-tolerant.
- Have a distributed memory. For ANN to learn, it is necessary to find out or come to a decision about the accuracy in terms of holistic testing of the network system according to the desired output by showing it to the networks. The network's success is dependent on the selected instances, but it doesn't mean that the networks in all aspects can produce false output.
- Gradual corruption: The gradual corruption of the network depends upon the periodic inspection from concerned maintenance. But it does not immediately corrode.
- The capability of machine learning. By looking at the same process, it can make decisions and learn events to Artificial Networks.
- The capability of parallel processing. An artificial neural network can withstand for a long period of time provides proper security and maintenance to ensure enough strength to perform more than one job simultaneously.

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RESEARCH METHODOLOGY

This is a meta-analysis study that employed a qualitative research method. Meta-analysis is a statistical approach to develop a systematic overview of the effects of points of a study. The process of gathering the data of this study was divided into four phases: Data import, Screening of articles, Extraction of data, and Statistical analysis. Each of the phases was subjected to critical review.

FINDINGS AND DISCUSSION

Result

Artificial neural networks are the essential tools used in machine learning. The artificial neural networks tend to replicate the way that the human learns in real-time. The input, hidden, and output layers are essential elements in the Artificial Neural Network that transform the raw data into something that the output layer can use.

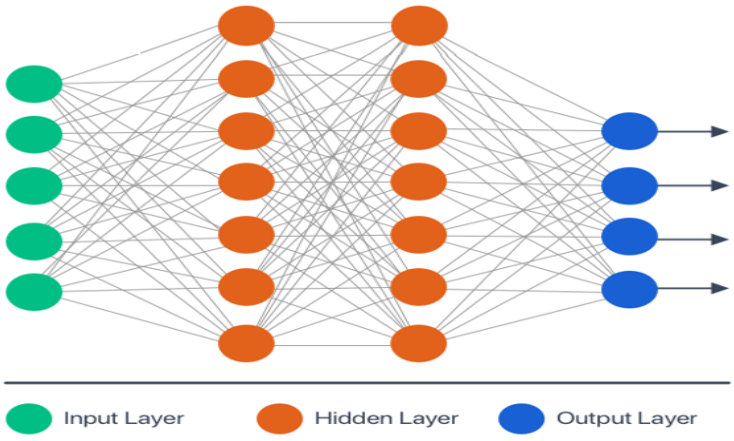

Figure 1. Artificial Neural Network

The artificial neural network has a learning ability that makes them so powerful and essential for various tasks. It is a piece of a computing design to stimulate in such a way the human brain analyzes and processes information. It is how a biological neuron works by adding together the values of the inputs it receives. If it is more than the threshold, it sends its signal to its output to which other neurons receive, and the process is called a feedforward network. Feedforward is a neural network that operates only in one direction from the input layer pass through the hidden layer, and goes forward to the output layer. The cell is a node in the layer-able to receive conveying nerve impulses from the sense organs to the nerve centers within the neural networks.

To know an Artificial Neural Network, one must go beyond what it has done wrong and is doing right, which is called feedback. It is how we learn what it has done wrong and doing

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right, and this is also what ANNs need to know. It is where you start to see similarities to the human brain.

If you want to learn Volleyball, and if you strongly hit the ball, it will go outside the court and lose the points. But if you hit it enough over the net onto the other side in the court, you could gain a point. It is a classic example of feedback where you lose the score or potentially earn points. And this is what we learn about right or wrongdoing. It is what a neural network needs to know.

Feedback is the process in which neural networks have learn something the way the human brain learns. Backpropagation is just a way of the spreading of something that uses a step-by-step procedure of

the disappearance of all cells within the neural networks and determines the loss of every node, who is responsible for providing and updating the weights as well as to minimizing the node with higher errors, functioning from the result in the artificial neural network, in the hidden neurons, and pass through the input layer going backward. Backpropagation is an algorithm used in the training of neural network simulation or design to calculate a graded difference in psychological activity along an axis.

The primary function of artificial neurons is to take into one's mind and conceptualize a model of biological neurons and neural networks.

It receives more than one inputs and adds all the data it receives to produce an output.

The type of artificial neural network where data in the datasets is labeled is called Supervised Learning. A supervisory signal through supervised learning has a pair of inputs with the desired output value.

Independent learning is the step-by-step procedure used to draw a conclusion from datasets comprising input information without labeled responses. You can do a lot of unique research because there is so much unlabeled data in the world. Basically, cluster analysis is the most common unsupervised learning method used for exploratory data analysis, searching the number of hidden neurons.

Artificial Neural Networks are an alternative to conventional programmed computing is based on the operation of the brain is most likely depending upon their generalization capability, especially the ability to handle unseen data. This time, ANN's ability to generate output or decisions is subject to comparison to what the human brain can do.

DISCUSSION

In our daily life and current situation, ANNs are rapidly developing, and they do continuous tests and evaluations on their advantages and the problems encountered during utilization. Don't forget that the disadvantages of artificial neural networks, which are a branch of science, are gradually excluded one by one on their advantages that are increasing day by day.

The artificial neural network has a learning ability that makes them so powerful and essential for various tasks. It is a piece of computing designed to stimulate in such a way the human brain analyzes and processes information. Artificial Neural networks are the type of computer algorithm models in which many non-linear processing elements align in parallel networks. These networks are the basis of the current understanding of biological nervous systems that have proven useable in pattern or sequence recognition problems.

Advantages of Artificial Neural Networks

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According to Mijwil M.M., information such as the unprogressive programming in the past practices or established were usually stored in the whole network storage. The loss of data and other information in one place does not prevent the network from functioning.
Incomplete knowledge capability works: After ANN training, the missing data and incomplete information may produce inefficient outputs resulting in the loss of performance.
Have fault-tolerance. The weak cells of an artificial neural network do not preclude it from generating output. This feature makes the networks fault-tolerant.
Have a distributed memory. For ANN to learn, it is necessary to find out or come to a decision about the accuracy in terms of holistic testing of the network system according to the desired output by showing it to the networks. The network's success is dependent to the selected instances, but it doesn't mean that the networks, in all aspects, can produce false output.
Gradual corruption: The gradual corruption of the network depends upon the periodic inspection concerning maintenance. But it does not immediately corrode.
The capability of machine learning. By looking at the same process, it can make decisions and learn events to Artificial Networks.
The capability of parallel processing. An artificial neural network can withstand for a long period of time provides proper security and maintenance to ensure enough strength to perform more than one job simultaneously.

Disadvantages of Artificial Neural Networks

- According to Mijwil, Maad, the disadvantage of the Artificial Neural Network is hardware dependent. Being hardware-dependent equipment, it requires a processor with parallel processing.
- Unexplained behavior of the network. It is the most significant issue of ANN. Artificial Neural Network produces a probing solution. It will not provide a clue as to why? How? It abridges the problem of the network.
- Determination of network structure. To determine the network structure, it is the usual practice to take trial and error in doing some experimental study.
- The difficult revelation of issues and problems of the network. In revealing issues and problems of artificial neural networks can work with the arithmetic logic unit in the computer system. The converted numeric value of the problem will be forwarded to the neural network. To directly influence the behavior of the network, will display mechanism to be determined, depending on the user's ability.
- Unknown duration of the network. The training will be complete if it reduces to a specific error in the sample. This value does not give us optimum results.

CONCLUSION AND FURTHER RESEARCH

An artificial Neural Network is a computer algorithm mimicking the human brain. It is like a human being, learning through the training experience. Because of this feature, they are often well suited for modeling complex and non-linear processes. The synaptic connections that exist between the neurons involve adjustment from learning in biological systems.
Artificial Neural Networks are responsible for the mathematical representation of something to animate the structure of biological neural networks and functionalities. ANNs are well

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advanced and able to compute and behave like human beings. Artificial Neural networks are the types of computer algorithm models in which many non-linear processing elements align in parallel networks. These are usually based on the current understanding of biological nervous systems that have proven useable in the pattern or sequence recognition problems in the current situation. Artificial Neural Network will be mimicking how the human brains perform in decision making

REFERENCES

Alameer, Z., 2019b. Improve Adaptive Neuro-fuzzy Inference System based on a modified slap swarm algorithm using the genetic algorithm to forecast crude oil price. Nat. Resource. Res., 1–16.

Dacombe, J.; An introduction to Artificial Neural Networks.https://medium.com/@jamesdacombe/an-introduction-to-artificial-neural-networks-with-example-ad459bb6941b

Essa, F., Abd Elaziz, M., Elsheikh, A.H., 2020. An enhanced productivity prediction model of active solar still using artificial neural network and Harris Hawks optimizer. Appl. Therm. Eng. 170, 115020.

Elaziz, M.A., Ewees, A.A. Improve Adaptive Neuro-fuzzy Inference System based on a modified slap swarm algorithm using the genetic algorithm to forecast crude oil price. Nat. Resources. Res., 1–16.

Faramarzi, A., Heidarinejad, M., Mirjalili, S., Gandomi, A.H., 2020. Marine Predators' Algorithm: a nature-inspired metaheuristic. Expert Syst. Appl., 113377.

Lee J. Lancashire, Christophe Lemaitre, and Graham R. Ball; An introduction to artificial neural networks in bioinformatics application to complex microarray and mass spectrometry datasets in cancer studies; Oxford University, 2009

Mijwil M.M.; Artificial Neural Networks Advantages and Disadvantages;(2018). . https://www.linkedin.com/pulse/artificial-neural-networks-advantages-disadvantages-maad-m-mijwel

Sannaki S. S. & Kulkarni A. A., 2016. A Review Paper on Artificial Neural Network in Cognitive Science; Belagavi, India, 2016

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Android File And Message Encrypted Application Using Advanced Encryption Standard-Vigenere and Electronic Codebook/ Public Key Cryptography Standards/Padding a Hybrid Encryption Algorithm

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Abstract

The study, entitled Android File And Message Encrypted Application Using Advanced Encryption Standard-Vigenere and Electronic Codebook/ Public Key Cryptography Standards/Padding a Hybrid Encryption Algorithm, was a proposed solution about Social Engineering and hacking. With the Data Privacy Act of 2012, the study promotes and inspires. The study's goal is to provide users with security and protection for their personal information. The purpose of this research is to prevent cyber theft. The theft of financial and/or personal information through the use of a computer/device for fraudulent or other illegal purposes is referred to as cyber theft. The objectives were aimed at the system's functionality, and the scope and limitations were considered to determine the study's capability and boundaries. For this case, the study proposed solutions. The first chapter provides a general overview of the application. The project background covered the area, challenge, and how the developers came up with the plan, as well as the study's major argument. The Android SMS and File Manager Encrypted Application employs two distinct hybrid encryption algorithms. The prototype is the model that is appropriate in our system development because the proponents are developing a mobile application. This application promotes the Data Privacy Act, which protects and maintains the customer's or user's right to confidentiality. The survey results are positive, and almost everyone would like to have this type of application that can secure their files and messages. As a result, the proponents conclude that this application is feasible and long-term.

Keywords: *AES, Android, Algorithm, Encryption, Hybrid, Hacking, SMS*

INTRODUCTION

People nowadays rely heavily on mobile phones as their primary mode of communication. SMS and file transfers on smartphones were not as secure as they could have been if users did not lock or password protect our apps. It's difficult to go a week without hearing about a new leak, breach, or privacy blunder, according to www.digg.com. Consumers have realized that for their data to be secure, they must take personal responsibility for it. Because customers spend so much time on their phones, mobile apps are a good place to start. Navigating the murky waters of app store scams, on the other hand, is time-consuming and difficult. There are thousands of privacy-conscious apps to choose from, each with its own set of features and efficacy levels. So, which should you go with? Which is the most efficient? Another question is why cell phones should be protected in the first place. Hackers can use dangerous software, or malware, to get access to information on our computers, although most people are aware of the need to use computer security software. Malware can even infiltrate cellphones, which are effectively small computers that run "mobile operating systems," which may be less visible. As a result, they may be

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vulnerable to the same threats and vulnerabilities that affect computer operating systems. Once a cybercriminal has gained access to your device, malware can steal or even hold your data hostage. In truth, there are many ways to reveal or divulge your private chats, such as social engineering, which involves using misleading strategies or methods to persuade someone into divulging private or personal and crucial information. Encryption is one way to improve security on smartphones, according to the Data Privacy Act. Encryption protects mobile devices in what ways? Encryption performs several functions. A lot more than merely preventing someone from accessing your phone's dataphone, just like the lock screen. Consider a lock screen to be similar to the lock on a person's front door. A lock on a door stops an uninvited guest from entering the house and stealing personal belongings, but the homeowner must consider what the thief would do if the locks were broken and the thief gained access to the house. To protect data fully, we need to have numerous layers of defense. This is known as "defense in depth" in the security field, and encryption provides it. Encrypting data raises security to a whole new level. It renders the phone's information illegible. Even if a hacker managed to get past the locked phone screen, they might still be unable to access personal information.

LITERATURE REVIEW

SMS communication is well-designed to provide end-to-end secure communication over SMS between mobile clients using AES and MD5 encryption. The most well-known information benefit is SMS. SMS technology is used in security-sensitive industries, including e-account management and e-government. Between the versatile client (MS) and the SMS focus, SMS is sent as unencrypted. SMS messages are saved in system administrators' frameworks and can be seen by their employees. SMS does not provide a secure environment for private data transmission. Many Android developers have created a system that encrypts SMS messages using the AES algorithm. Short Message Service (SMS) has evolved into a useful tool and has played an important role in the lives of its users. SMS provides food for installation, portable money-saving, vital updates such as stock and news alarms, activity refreshes, climatic data, and business-related data. Security and impersonation concerns are at the forefront of the examination. The investigation used a fall encryption procedure designed for transmitting SMS through any type of communication channel. The quality of the computation based on the structure was focused on developing an anchored encryption procedure and adapting AES (Advanced Encryption Standard) encryption with two additional encryption layers. It looked into the system's impact on the encryption process. Security Assurance Framework for SMS Using Cascaded Encryption Algorithm is being developed by other developers. Security validation is required in all media transmission lines. Everyone desires to keep any data conveyed across unbound and anchored media transmission lines mysterious. Short Message Service (SMS) has evolved into a useful tool and has played an important role in the lives of its users. SMS provides food for installation, portable money-saving, vital updates such as stock and news alarms, activity refreshes, climatic data, and business-related data. Security and impersonation concerns are at the forefront of the examination.

RESEARCH METHODOLOGY

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The prototype is the model that is appropriate in our system development because the proponents are developing a mobile application. Prototyping is a paradigm in which a prototype is produced, tested, and then reworked as needed until an acceptable prototype is achieved from which the whole system or product can be developed. It's a cycle that allows for system adjustment and repeats the rapid design if it has to be changed or updated. It is intended to communicate the key features or benefits to potential users or other stakeholders in an effective manner. It can be done in something as simple as Balsamiq or even on paper, and the goal is to visualize the main things people will be able to do with the tool as a way of assisting stakeholders in attaching the solution to the problem you are solving. This term is also frequently used by developers when they want to quickly hack together a quick version of the proposed tool to hit and work to understand or even resolve likely technical challenges. In this situation, the proponents will publish our system to the Play Store, and the group will gradually improve the scope and capabilities of the system in response to user feedback and demands. Our group will keep track of their feedback and reviews on our system, and then the proponents will design and update new versions, which will then be uploaded to the Play Store so that users may download new versions of our system.

FINDINGS AND DISCUSSION

The proponents performed a survey to learn about the users' requirements and desires. The Computer Science Students were chosen as the respondents by the researchers. There are 695 students enrolled in Computer Science. The Registrar's Office of Taguig City University provided this information. The Slovin's Formula is applied to the entire population. Hence the total number of respondents to whom the proponents should at least provide survey questionnaires is 255. Because they are more familiar with the function that we are attempting to perform, the proponents chose Computer Science Students as their respondents. In addition, the researchers interviewed a group of Computer Science students and professors. The group answers a few questions on how satisfied they are with their current mobile phone experience and how secure they are. They also added such items to the survey forms they will fill out. The survey results are positive, and nearly everyone would like to have an application like this to protect their files and messages. As a result, the proponents conclude that this use is realistic and long-term. Android SMS and File Manager Encrypted Application is for anyone who owns an Android phone. This is an application that can secure their SMS and File Manager. Encryption is the process of securing a user's message content and files in a file manager. This app will be uploaded to the Google Play Store and reviewed by users. The system's functionalities and capabilities will be maintained if the proponents monitor every user suggestion and review.

CONCLUSION AND FURTHER RESEARCH

The Android SMS and File Manager Encrypted Application employs two-hybrid encryption algorithms: the first is the AES-Vigenere Encryption Algorithm, which encrypts the contents of SMS messages, and the second is the AES/ECB/PKCS5/Padding, which encrypts the bytes

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of files in the file manager. This program promotes the Data Privacy Act, which protects and maintains the right to privacy of the customer or user. The proponents conducted a poll to learn about the needs and desires of the users. The survey results are positive, and almost everyone would like to have a program like this to protect their files and messages. As a result, the proponents conclude that this application is feasible and long-term. Based on the findings and conclusions reached, the following is presented. Android SMS and File Manager Encrypted Application Using AES-Vigenere and AES/ECB/PKCS5Padding a Hybrid Encryption Algorithm will be available for everyone to use and test on the Google Play Store. The proponents will review the user reviews and comments for the application's upkeep. Due to human error, the application will still require some options for password recovery. The application will send you various password recovery options via email. Enhance the program by maintaining and enhancing the system's features: The application will provide a vault for the user's encrypted files to be stored in. This can improve the security of the user's data. They are updating the application's user interface and experience for better accessibility and use by users.

REFERENCES

Albert, J. R. G., Serafica, R. B., & Lumbera, B. T. (2016). Examining
Trends in ICT Statistics: How Does the Philippines Fare in ICT?. Discussion Paper Series No. 2016-16. Philippines: Philippine Institute for Development Studies.

Basharat, I., Azam, F., & Muzaffar, A. W. (2012). Database Security and Encryption: A Survey Study. Volume 47– No.12. Pakistan: National University of Sciences and Technology (NUST).

Cordero-Batac, E. R., & King Kay, C. O. (2018). Data Protection Laws of the World Philippines. Republic Act No. 10173. Philippines: DLA PIPER.

Degabriele, J. P. (2014). Authenticated Encryption in Theory and in Practice. Thesis Paper. London: Information Security Group Department of Mathematics Royal Holloway, University of London

Joshi, M. R., & Pathak, V. M. (2011). A survey of SMS-based Information Systems. Master's Thesis. Finland: University of Eastern Finland School of Computing.

Kaur, E., & Singh, E. N. (2015). SMS Encryption using NTRU Algorithm. Vol. 3, Issue 2. India: International Journal of Advanced Research in Computer Science & Technology.

Lalis, J. T., Gerardo, B. D., & Byun, Y. (2014). Securing Bluetooth Communication with Hybrid Pairing Protocol. Vol. 8, No. 4. Philippines: Institute of ICT, West Visayas State University, Iloilo City.

Nimmya Unnikreshanan, D. K. (2015). End to End Secure SMS Communication: A Literature Survey. Volume 4, Issue3. India: Vidya Academy of Science and Technology Thalakkott

Yadav, R. K. (2013). Cryptography on Android Message Applications – A Review. India: PDM College of Engineering Bahadurgarh.

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The Development of A Cloud-Based University Research Repository Software Using A Configurable Subscription Model

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Abstract

With more research that is added every year of every school calendar, there is no doubt it becomes a file or stack of research hardbound resides on the library. These researches should not only settle on the shelves, making them electronically available as references, or to be cited are the ones it truly deserves. This paper emphasizes the need for a cloud-based research repository to be implemented in every University that can be utilized to serve its purpose. This research repository is based on an online publication and subscription model. Online publication provides reading sources via the internet in which is accessible and more convenient to most people. The repository will also adapt the concept of reconfigurability as the users may have their own preferences with regard to how they publish or subscribe to a paper. These would give them more options on deciding how they would publish and or avail paper references. Research that is within the repository that will be referenced, cited, or downloaded has the corresponding remuneration based on the approval of the University. In this way, more researchers will continue to provide more scholarly output to be published and to gain more citing, downloads, and eventually more remuneration. The repository has the potential to expand as more researchers will be turned in its service and would be beneficial to stakeholders. The respondents in this paper show the acceptability of the process, making them more likely to work in any educational institution. Moreover, as time progress, researchers and organizations would avail to use the software in accordance with their needs as well as the preferences of its user with the configurability of the software, thus providing a continuous educational-business process to all stakeholders. And with the current situation of the global pandemic, heterogeneous access to resources is all being sought.

Keywords: *Online Publishing, Subscription Model, Research Repository, Configurability*

INTRODUCTION

Online publication has been a trend in this era as these provide reading sources via the internet in which is accessible and more convenient to most people. This paper aims to elicit the advantages of having an electronic/cloud-based avenue wherein stakeholders can provide their research works while, on the other hand, researchers who are looking for information that will help on their scholarly work. The University will serve as the admin serving as the bridge between stakeholders. Furthermore, this setting enables the distribution of publication globally and delivers content to any devices such as personal computers, tablets, and smartphones. It does not

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also require physical printing and distribution physically which are costly to most companies/organization or individuals who publishes their works. It has also helped and solved the risk of over-production of reading materials (Hughes, 2014).

Online publication has acted as the source of learning for anyone as this enables the publication of a wide range of sources that are written by authors worldwide. Constantly increasing the number of students is relying on these sources as they can view and read in a more convenient manner. The students would no longer need to go to physical libraries, and these would help them access the source anytime and anywhere. This was probably the most exciting development in terms of bringing design thinking into mainstream library practice (Bell, 2016).

It is apparent today that most universities have their own publication sites in which they are able to offer their own written works such as journals and researches. The schools across America are responding with policies in which allow access to open-source textbooks that are written by the faculty, peer-reviewed, and available online. It also enables the improvement of student engagement by being more interactive (Deseret, 2015).

LITERATURE REVIEW

Cloud-Based Technology

According to an article by LoPresti (2014), most publishing companies are now modernizing their business models. One of these movements in the technology sector is shifting into cloud-based computing, where data and information, processing, and storage are managed using the internet or the company's local data servers.

It is said that there are several ways cloud-based services can help publishers. This includes publication, design, content management, and business services. It is a must that publishers are well-informed on the potential benefits as well as the pitfalls in shifting to the cloud. Emblidge (2015) conducted research about building a publishing studies database that can be made available through subscription from an academic publisher and reflected publishing practices globally. An online academic database was created because there are certain problems with textbooks in publishing studies. On the same note brought about by the 21st Century, online publishing has risen in this technological era (McNaught, 2015). It is really evident that approaches to access resources are shifting now using technology. There are signs that this is changing as more libraries cover research outputs emanating from the universities (Bangani, 2018).

Digital Repository and Open Access

A digital repository can serve as a virtual space for gathering and sharing objects of interest and importance, where they can be searched, studied, and enjoyed at any place with access to the internet (Bogucki, 2021).

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The Open Access (O.A.) movement continues to gain traction. The recent breakdown of negotiations between Elsevier and the University of California system has brought renewed attention to O.A. issues to academic faculty, students, librarians, administrators, and governance bodies. The academic library has a role in explaining O.A. issues, advocating for O.A., and of course for supporting and managing O.A. resources, including institutional repositories, O.A. journal subscriptions, OER, open research data, and other O.A. materials such as grey literature. (Mack, 2020).

Open access overall has not allowed libraries to save money on serials **subscriptions** and has often increased costs through their support of institutional **repositories** and payment of author fees. Continued library support for open access is often more of a philosophical stance without significant cost-saving benefits (Holley, 2018).

Subscriptions and Configurability

In 2015, 86 percent of the articles in eLife and PLOS acknowledge funder support, as do 76 percent of the articles in the largely subscription journals of BioOne. Such findings can inform libraries and funding agencies, as well as publishers, in their consideration of a direct-payment open access model (Wilinsky & Rusk, 2019). This shows the increase in subscriptions in journals. The ever-increasing journal subscriptions are also evident in many universities and research institutions failing to provide access to the much-needed scholarship for the propagation of research and development due to dwindling budget allocations (Tapfuma & Hoskins, 2019). Moreover, it provides covering a large number of researches in less time; thus, it is also essential at the same time to understand the **library subscription** quality, according to Bandi,2021.

The price of journals and subscriptions also varies, constantly rising prices of journal and database **subscriptions**, granting agencies requirements for recipients to submit their research publications into open access **repositories**, and pressure on libraries to create Institutional **Repositories** (I.R.) to promote the institutions' reputations (Dawson & Yang, 2016).

Partnership and consortium are also observed to amplify the support in research. John Wiley and Sons Inc., the Austrian Academic **Library** Consortium, and the Austrian Science Fund announced a three-year combined open access publication and **subscription** agreement. This transformative agreement provides researchers and students at 22 institutions with access to all **subscription** journals published by Wiley. Corresponding authors from KEMÖ-affiliated institutions will also be able to publish unlimited open access articles in Wiley's hybrid journals at no charge to the author. (John Wiley and Sons Inc., 2018)

It is apparent that a growing number of people have shifted to using a digital format of publications. People from the academic community use online publication in distributing studies, reports, and journals in a more accessible manner for both students and teachers.

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These online sources are also more inexpensive than the traditional printed format as this no longer requires reprinting copies based on its demand.

Institutions, including universities, have adapted this mainly to make publications more accessible, which helps widen the audience of their published journals or researches. According to Tilley, E. (2004), "Articles freely available online are more highly cited. For greater impact and faster scientific progress, authors and publishers should aim to make research easy to access." In their study, it was concluded that online papers were 4.5 times more likely to be cited than the offline papers. With their conclusion, no doubt that those who aspire to publish their works preferred using digital means rather than the traditional. For such in a university, as the primary subject in this study, digital publishing helps expand the audience of the researches that were submitted in their library, and not only that they could promote these researches, but they could be beneficial for all the researchers who have a hard time finding resources.

The proponent has also adapted the concept of reconfigurability. According to Barreau, Renard & Fournier (2018), it is important for librarians to understand the heterogeneity of these expectations, as well as local priorities, so that journal access meets users' needs.

RESEARCH METHODOLOGY

The research design used in the study is the descriptive method. The descriptive method is primarily concerned with how the variables that were gathered will be turned into a result that will turn out solutions or answers with regards to the problem of the study.

More importantly, the study focused on how a configurable cloud-based research repository may be used within a university. This will be implemented wherein it will allow the faculty, administrative staff, and students to publish their works, particularly researches, where anyone can access and read these papers.

The proponent distributed survey questionnaires to assess the perception of the respondents about what is their ideal research repository. This includes the assessment for the ff. Problems: 1) Their previous experiences on online subscription; 2)The level of agreement of the respondents on the likelihood of supporting the development of a university research repository; 3) The level of agreement of the respondents on the benefits of an online research repository using a subscription business model; 4) The level of agreement of the respondents on the identified characteristics of the system, and 5) The level of agreement on the implementation of a strong security backbone of the system. The proponent measured the level of agreement based on the Likert Scale measure shown in Table 1.

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Table 1. Likert Scale

SCALE	RANGE	INTERPRETATION
1	1.00 – 1.50	Not Problem
2	1.51 – 2.50	Minor Problem
3	2.51 – 3.50	Moderate Problem
4	3.51 – 4.00	Serious Problem
SCALE	RANGE	INTERPRETATION
1	1.00 – 1.50	Strongly Disagree/ Extremely Unlikely
2	1.51 – 2.50	Disagree/Unlikely
3	2.51 – 3.50	Partly Disagree/Neutral
4	3.51 – 4.50	Agree/Likely
5	4.51 – 5.00	Strongly Agree/Extremely Likely

System Development

The proponent has come up with a configurable research repository that established the results of the gathered data from different sources such as the questionnaire, related studies, and related kinds of literature. This is developed through an Agile Development Methodology, which is an iterative and incremental type of development.

The proponent used PHP programming language for the server-side programming and MySQL for the back end of the prototype. The use of a Model-View-Controller framework was also employed in the development, thus the use of the CodeIgniter Framework. The proponent used The Open Group Architectural Framework (TOGAF), which is shown in figure 1, presenting the Enterprise Architecture. It describes the structure and processes of the developed system and how it is linked to other systems within the organization. It is a high-level approach in system designs typically modeled in four levels which are composed of Business, Application, Data, and Technology levels (Wikipedia.org).

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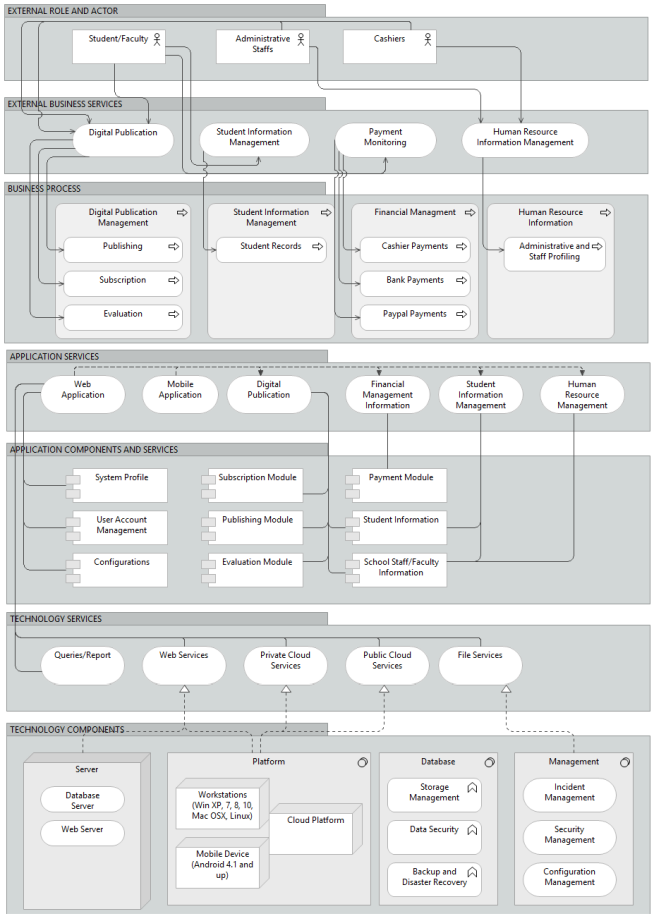

Figure 1. Research Publishing System Enterprise Architecture.

FINDINGS AND DISCUSSION

Based on the analysis and interpretation of the gathered data, the study produced and plotted the following findings:

1. On the assessment of the previous experiences of the respondents on online subscription:
   1.1 The weighted mean of encountering a problem with regards to the cost and payment is *2.44*, interpreted as there were *minor problems* on the previous experience of the respondents.
   This shows that respondents are open to paid subscriptions.
   1.2 The worldwide web (www) presence of the university research repository was considered as a *moderate problem* which obtained an overall mean assessment of *2.63.*
   This shows that respondents preferred to have a research repository that can be accessed through the world wide web.

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1.3 The relevance of material was considered as a *moderated problem* which obtained an overall mean assessment of *2.68.*
This shows that respondents are willing to accept payment subscription as long as the material gathered has relevance to their research or study.

2. On the assessment of the agreement of the respondents on the likelihood of supporting the deployment of the university research repository and its corresponding subscription model, the respondents *likely agreed* on supporting the deployment of a university research repository which obtained an overall assessment of *3.92.*
This shows that most of the respondents would like to have a research repository.
3. On the assessment on the level of agreement of the respondents on the benefits of an online research repository using a subscription business model:
   3.1 The respondents *agreed* on the benefits of an online research repository using a subscription model when it comes to the predictable revenue of the school, obtaining an overall mean assessment of *4.10*.
   3.2 The respondents *agreed* on the benefits of an online research repository using a subscription business model in terms of building longer-term relationships, obtaining an overall mean assessment of *4.08*.
   3.3 The respondents *agreed* on the benefits of an online research repository using a subscription business model in terms of convenience for students/researchers, obtaining an overall mean assessment of *4.10*.
   3.4 The respondents *agreed* on the benefits of an online research repository using a subscription business model in terms of minimizing workload, obtaining an overall mean assessment of *4.10*
   This further supports the advantage of having a research repository that could work both for the researcher and students. The repository could also be an added tool for income generation options for the University.
4. Figure 2 shows the business process flow of the system. As seen, publishers, either a student or a faculty member, may submit research. It will be evaluated by an evaluator and may decide to approve or reject to publish the research. If approved, the school administrator will set the price for the research. After setting the price, the research will be published.

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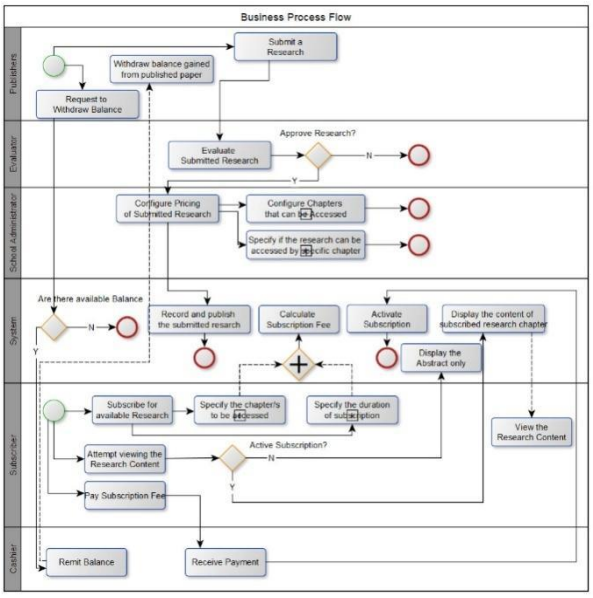

Figure 2. Business Process Flow of the Research Publishing System

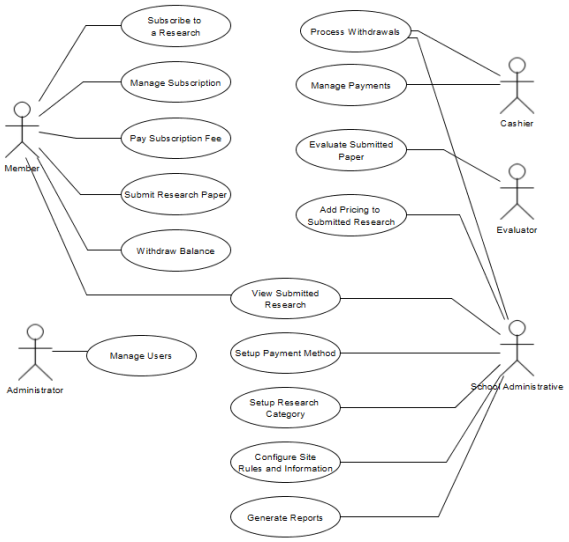

Figure 3. Use-case of the Developed Research Publishing system

Figure 3 shows the use-case overview of the developed system. There are five (5) roles in the developed system. Each of these roles has varied access to the different features of the system.

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5. Assessment of the level of agreement of the respondents on the identified characteristics of the system:
   5.1 The respondents *agreed* on the effectiveness of the identified characteristics of the system in terms of features and functionality, obtaining an overall mean assessment of *4.17.*
   5.2 The respondents *agreed* on the effectiveness of the identified characteristics of the system in terms of navigation, obtaining an overall mean assessment of *4.23.*
   5.3 The respondents *agreed* on the effectiveness of the identified characteristics of the system in terms of control and feedback, obtaining an overall mean assessment of *4.28.*
   5.4 The respondents *agreed* on the effectiveness of the identified characteristics of the system in terms of context and text, obtaining an overall mean assessment of *4.25.*
6. On the assessment of the level of agreement on the implementation of a strong security backbone of the system.
   6.1 The respondents *agreed* on the implementation of a strong security backbone of the system in terms of attack monitoring and prevention, obtaining an overall mean assessment of *4.23.*
   6.2 The respondents *agreed* on the implementation of a strong security backbone of the system in terms of a strong password, obtaining an overall mean assessment of *4.14.*
   6.3 The respondents *agreed* on the implementation of a strong security backbone of the system in terms of two-factor authentication (2FA) on all the accounts, obtaining an overall mean assessment of *4.12.*
   6.4 The respondents *agreed* on the implementation of a strong security backbone of the system in terms of embedding other measures in the system both in application and process, obtaining an overall mean assessment of *4.18.*

CONCLUSION

With the current trend of technology, especially with the way research is going on, it is essential to have an online repository of researches. This will ensure that every research created can be monitored, referenced, viewed, and even cited. Moreover, this study shows that online subscription is just a moderate problem showing that subscribing is more accepted than in traditional library setup. Respondents likewise agreed that the online research repository is beneficial in terms of the predictable revenue of the school, building a long-term relationship, convenience, and minimizing workload. All stakeholders will benefit from these settings, from the creator of research, from the individual who is looking for a reference for research, for the school in maintaining and monitoring research—creating a single avenue where all stakeholders interact, thus providing a strong output on research.

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RECOMMENDATIONS

The repository and subscription model resolves the problems of availability of researches. With the current situation of the global pandemic, the system will converge all the stakeholders into a single avenue to address their needs. Universities and academic libraries are encouraged to utilize the system to cope and augment sharing of academic resources of research, thus minimizing the workload of the management in handling researches. When it comes to payment options for subscription it is recommended to use different payments APIs, such as payments REST API by PayPal, thus recommended to be integrated with the system. This will enable payments to be done online and may be able to attract more interested subscribers who do not have time to go to actual school or bank.

When it comes to security, it is recommended to use at least Two-Factor Authentication in retrieving passwords or accounts. This is to firmly avoid common system vulnerability, such as identity theft. Future researchers that are also conducting the related study may use this as a reference and guide for further studies in improving the related system.

REFERENCES

Bandi, I. (2021). Measuring Library Subscriptions to the availability of scholarly articles published by the academic and research community of the institution. Library Philosophy & Practice, 1–7.

Bangani, S. (2018). The History, Deployment, and Future of Institutional Repositories in Public Universities in South Africa. Journal of Academic Librarianship, 44(1), 39–51. https://doi.org/10.1016/j.acalib.2017.12.003

Barreau, D., Bouton, C., Renard, V., & Fournier, J.-P. (2018). Health sciences libraries' subscriptions to journals: expectations of general practice departments and collection-based analysis. Journal of the Medical Library Association, 106(2), 235–243. https://doi.org/10.5195/jmla.2018.282

Bell S., "Tipping Point for Design Thinking in Libraries (Finally. . . ?)," Designing Better Libraries, last modified September 30, 2016. http://dbl.lishost.org/blog/2016/09/30/tipping-point-for-design-thinking-in-libraries-finall y/

Bogucki J., Cloud Services for Digital Repositories, Vol. 57 / No. 5 ISSN 0024-2586, July 2021

Dawson, P. H., & Yang, S. Q. (2016). Institutional Repositories, Open Access, and Copyright: What Are the Practices and Implications? Science & Technology Libraries, 35(4), 279–294. https://doi.org/10.1080/0194262X.2016.1224994

Deseret, L.C. (April 2015). Universities seek alternatives to expensive textbooks. http://national.deseretnews.com/article/4212/universities-seek-alternatives-to-expensive-textbooks.html

Emblidge, D. (2015). A Publishing Studies Online Academic Database: In-progress Research Project. http://link.springer.com/article/10.1007/s12109-015-9410-y

Holley, R. P. (2018). Open Access: Current Overview and Future Prospects. Library Trends, 67(2), 214–240. https://doi.org/10.1353/lib.2018.0034

Hughes, B. (May 2014). Changing the Publishing Business Model: Consider the Whole Value Chain, Not Just Unit Price. http://www.bookbusinessmag.com/post/changing-publishing-business-model/

LoPresti, M. (June 2014). Publishing in the Cloud: Tips for Publishers on Making the Transition. http://www.econtentmag.com/Articles/Editorial/Feature/Publishing-in-the-Cloud-Tips-for-Publishers-on-Making-the-Transition-97166.htm

Mack, D. C. (2020). Open Access in the Academy: Developing a Library Program for Campus Engagement. Grey Journal (TGJ), 16(3), 181–185.

McNaught, K.. (2015). The Changing Publication Practices in Academia: Inherent Uses and Issues in Open Access and Online Publishing and the Rise of Fraudulent Publications. http://quod.lib.umich.edu/j/jep/3336451.0018.308?view=text;rgn=main Polit D., Beck C., Hungler B. (2001) Essentials of Nursing Research: Methods, Appraisal and Utilization, 5thedition Philadelphia, PA

Tapfuma, M. M., & Hoskins, R. G. (2019). Open science disrupting the status quo in academic libraries: A perspective of Zimbabwe. Journal of Academic Librarianship, 45(4), 406–412. https://doi.org/10.1016/j.acalib.2019.05.005

Tilley, E. (2004). The importance of online research dissemination. Retrieved from: http://espace.library.uq.edu.au/view/UQ:9475

Willinsky, J., & Rusk, M. (2019). If Research Libraries and Funders Finance Open Access: Moving beyond Subscriptions and APCs. College & Research Libraries, 80(3), 340–355.

John Wiley and Sons Inc. (2 C.E., December 2018). Wiley, the Austrian Academic Library Consortium (KEMÖ) and the Austrian Science Fund (FWF) Announce Combined Open Access and Subscription Agreement. Business Wire (English).

Worsley, A. J. (2019). The Views of Scholars on the Effects of Subscription Costs to E-Journals in an Academic Health & Life Sciences Library: A Case Study. Journal of Electronic Resources Librarianship, 31(4), 241–254.

The Open Group Architecture Framework. https://en.wikipedia.org/wiki/The_Open_Group_Architecture_Framework

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Formulation of Coenzyme Q10 Liquid Foundation With a Variations Linseed Oil as The Oil Phase

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Abstract

Antioxidant Coenzyme Q10 (CoQ10) has properties as a sunscreen that can protect the skin from the aging process accelerated by UVB rays. Linseed oil (LO) formulated in cosmetics aims to find out the characteristics of moisturizer liquid foundation with LO as oil phase. In this study, cosmetic formulations were carried out using a modified method of emulsification evaporation. In the moisturizer liquid foundation CoQ10, three other formulas were used with LO concentrations of 3%, 5%, 7% to determine the effect of vegetable oils used on cosmetic characteristics. Evaluation of physical properties includes organoleptic, pH, viscosity, spreadability, and adhesivity. Evaluation of the acceptance of preparations was carried out to 15 panelists. The results of the study showed that the use of LO influences the characteristics of moisturizer liquid foundation CoQ10. The increase in LO concentration increases viscosity value, adhesivity, and decreased pH and spreadability, but the results obtained still meet the criteria. The results of the hedonic test showed no significant difference from the three formulas of 0.911 (P>0.05). It was proven that the panelists preferred formula one, which has a texture that is not too thick.

Keywords: *CoQ10, Sunscreen, Linseed oil, Moisturizer Liquid Foundation*

INTRODUCTION

Along with the development of the times, the use of cosmetics has become a necessity for the community to encourage cosmetic manufacturers to increase competitiveness by producing cosmetics with many advantages, but it makes some people produce illegal cosmetics. Based on their usefulness, cosmetics are classified as products for skincare as well as to enhance the appearance of individuals or are known as decorative cosmetics (Anwar and Rizkamiarty, 2020).

Based on the results of BPOM supervision of the Republic of Indonesia, many illegal cosmetics with hydroquinone and mercury content are found. The content of hydroquinone in cosmetics used for a long time will increase the risk of developing skin cancer and can make the skin lose its ability to be protected from ultraviolet light (Pangaribuan, 2017). Skin that loses the ability to be protected from UV rays will make the skin wrinkle quickly, dry skin, and rough. Therefore the active ingredients in cosmetics are needed to protect the skin from the bad effects of UV rays. CoQ10 is an alternative to this problem.

In the field of cosmetics, CoQ10 is used to reduce the aging of the skin, but in a recent study conducted by (Wu et al., 2020), CoQ10 can be used as a sunscreen that effectively inhibits skin

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damage from UVB rays. The problems arising from the use of CoQ10 in topical preparations are the large molecular weight of 863.3 g/mol, high lipophilicity (P > 10), poor solubility of CoQ10 (0.7 μg/mL at 37°C), and resulting in low permeation and bioavailability (Ryu et al., 2020). Therefore is necessary to add an enhancer to improve the delivery of CoQ10. The enhancer used in the study was LO which was varied into three different concentrations to know the effect of the use of LO on the characteristics of the moisturizer liquid foundation CoQ10.

LITERATURE REVIEW

CoQ10 is a natural antioxidant compound that can protect the skin from the bad effects of UV rays. As a sunscreen in cosmetic preparations, CoQ10 can prevent the signs of aging accelerated by UV rays by the mechanism of increasing the skin's antioxidant capacity in the epidermis and preventing collagen degradation by suppressing the production of malondialdehyde and metalloproteinase-1 (Wu et al., 2020). The use of CoQ10 in cosmetics for daily use in addition to sunscreen can also restore the cellular antioxidant content of the epidermis that is lost due to environmental influences.

Research conducted by (Prahl et al., 2008) uses CoQ10 can restore cellular CoQ10 antioxidant levels lost from the skin. To improve the ability of CoQ10 in penetrating the skin, use enhancer linseed oil. Linseed oil contains a combination of omega-3,-6, and -9 fatty acids that play a role in increasing coQ10 delivery through the mechanism of interaction with keratin in the stratum corneum so that it will eventually increase the rate of release of CoQ10 (Tou et al., 2019).
Decorative cosmetics and treatments, namely moisturizers liquid foundation, is used to increase the aesthetic value of the physical appearance of individuals whose use should be easily applied to the skin, not make the skin dry or oily, not sticky, and produce a final natural appearance. The final result of the formulation of the manufacture of moisturizer liquid foundation will affect the physical characteristics of the preparation (Sivamani et al., 2016).

RESEARCH METHODOLOGY

Materials
Coenzyme Q10 (contributed from PT. Konimex, Indonesia), Linseed oil (purchased from Bali, Indonesia), BHT, Veegum, Titanium dioxide, Kaolin, Red iron oxides, Yellow iron oxides, Propilenglikol, Glycerin, Tween 80, Benzalkonium chloride, Citric acid, Stearic acid, Cetyl alcohol, Span 80, Vanilla.

Methods
Preparation of Moisturizer Liquid Foundation CoQ10
Moisturizer Liquid foundation preparations are made using a combination method of emulsification evaporation. Firstly, CoQ10 with PVP was mixed into 5 mL of 96% ethanol and then stirred using a magnetic stirrer at a speed of 500 rpm for 10 minutes. Secondly, oil phase materials

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are melted over water handlers at 70°C. Third, titanium dioxide is mixed with glycerin until homogeneous. Fourth, water phase materials are put together into the purified water that has been heated at 70° C while stirring over the water handler with the temperature maintained at 70° C. Moisturizer liquid foundation is made by mixing the oil phase into the water phase slowly while continuing stirring over the water handler for 5 minutes. The mixture of the two phases is then added titanium dioxide and glycerin then the mixture is stirred until it reaches a temperature of 40° C. After the previous mixture has been homogeneous, add CoQ10, then do constant stirring with the addition of the remaining purified water and the addition of Vanilla as much as 3 drops, stir until homogeneous then the moisturizer liquid foundation preparation is put into the appropriate container.

Evaluation of Moisturizer Liquid Foundation CoQ10
The evaluation of moisturizer liquid foundation CoQ10 includes organoleptic, pH, viscosity, spreadability, and adhesivity.
Investigation of Panelists Acceptance for Formulated Moisturizer Liquid Foundation CoQ10 by Hedonic Test
The acceptance for moisturizer liquid foundation CoQ10 conducted by the hedonic test was performed visually on 15 panelists. The test is conducted by asking panelists to apply a moisturizer liquid foundation CoQ10 with an oil phase of LO on the back of the skin of the hand. Panelists were then asked to choose the most preferred Moisturizer Liquid Foundation CoQ10.

FINDINGS AND DISCUSSION

The use of CoQ10 in this study is based on CoQ10's ability to protect the skin from UVB exposure so that the purpose of adding CoQ10 to the formulation of moisturizer liquid foundation is as a sunscreen with its mechanism of action is to prevent skin aging from UVB exposure by increasing the skin's antioxidant capacity and preventing collagen degradation by suppressing the production of malondialdehyde and metalloprotease-1 (Wu *et al*., 2020).

Preparations of moisturizer liquid foundation CoQ10 are made into three formulations with a difference in concentration in LO (see table 1). The first characteristic evaluation is organoleptic testing. The three moisturizer liquid foundation formulas CoQ10 have a distinctive vanilla smell because, in the manufacturing process, there is the addition of a fragrance that is Vanilla. From the aspect of color that is resulted in is the color ivory. In the addition of higher oil phase concentrations making the preparations increase thicken even more, observations of organoleptic test results are presented in (see table 2).

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Table 2. Moisturizer liquid foundation evaluation results organoleptic

Formula	Texture	Color	Odor
F1	Slightly viscous	Ivory	Vanilla
F2	Pretty viscous	Ivory	Vanilla
F3	Viscous	Ivory	Vanilla

Table 1. Formula of moisturizer liquid foundation CoQ10

Material	F I (% b/v)	F II (% b/v)	F III (%b/v)
CoQ10	0,5	0,5	0,5
Linseed oil	3	5	7
BHT	0,1	0,1	0,1
Veegum	2	2	2
Titanium dioxide	5	5	5
Kaolin	5	5	5
Red iron oxides	0,2	0,2	0,2
Yellow iron oxides	0,8	0,8	0,8
Propylene glycol	10	10	10
Glycerine	10	10	10
Tween 80	3,5	3,5	3,5
Benzalkonium Chloride	0,1	0,1	0,1
Citric acid	0,8	0,8	0,8
Stearic acid	3	3	3
Cetyl alcohol	4	4	4
Span 80	1,2	1,2	1,2
Vanilia	3 gtt	3 gtt	3 gtt
Purified water Ad	100	100	100

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In formulating a preparation for use on the skin, the pH of the preparation is one of the main factors that must be considered. A pH of a preparation that does not enter the pH range of the skin can irritate. Therefore the pH of the preparation must enter the physiological pH range of the skin, which is 4.5-7 (Siva and Afriadi, 2019). The pH measurements carried out in this study aimed to determine the acidity or numbness of the preparations made. Results of pH moisturizer liquid foundation CoQ10 test can be in table 3.

Table 3. Results of pH moisturizer liquid foundation CoQ10 evaluation

Formula	R1	R2	R3	$\bar{X} \pm$ SD
F1	6,14	6,22	6,15	6,17 ± 0,043
F2	6,14	6,10	6,13	6,12 ± 0,020
F3	6,16	6,01	5,95	6,04 + 0,108

The average pH measurement for F1 is 6.17, F2 is 6.12, and F3 is 6.04. The results obtained from pH in F1, F2, and F3 have met the standard criteria for physiological pH of the skin, which is 4.5-7. The results of pH measurements show that the higher the concentration of linseed oil used, the pH value will decrease to the more acidic. This is because the greater the concentration of vegetable oil added will affect the degree of acidity of the preparation due to the influence of high fatty acid content that is stearic acid on vegetable oil used.

Linseed oil has a fairly high fatty acid content, one of which is 12.34% saturated fatty acids consisting of palmitic acid and stearic acid (Pali and Mehta, 2014). The pH measurement decline data is analyzed using linear regression tests. The results of the linear regression test showed that a signification value of 0.042 ($P<0.05$), i.e., there was an influence between the use of variations in the concentration of linseed oil to decrease the pH moisturizer liquid foundation CoQ10.

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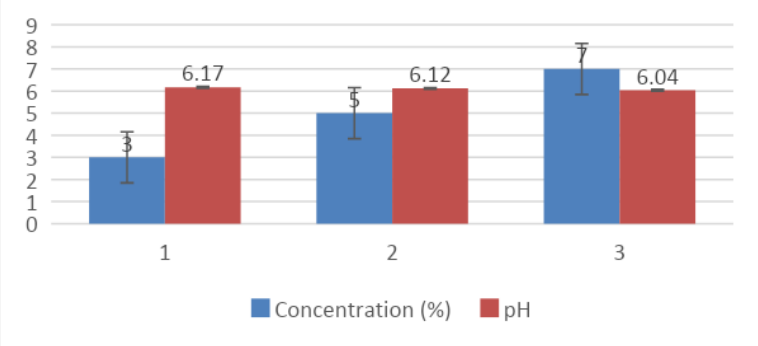

Figure 1. pH evaluation graph of moisturizer liquid foundation CoQ10

Viscosity testing aims to find out the viscosity of preparation to be able to flow. Viscosity testing aims to determine the effect of LO concentrations on the viscosity of the moisturizer liquid foundation CoQ10. Viscosity testing is done using a rion viscometer using spindle No. 1. moisturizer liquid foundation CoQ10 preparations are said to meet the quality requirements of topical preparations when the results of viscosity testing enter in the range of 2000-50.000 (cPas) (Rasydy *et al.,* 2021). The results of the viscosity test can be seen in table 4.

Table 4. Results of viscosity moisturizer liquid foundation CoQ10 evaluation

Formula	R1 (cPas)	R2 (cPas)	R3 (cPas)	$\bar{X} \pm$ SD
F1	4.800	4.800	4.800	4.800 cPas $\pm$ 0
F2	6.200	6.200	6.200	6.200 cPas $\pm$ 0
F3	8.700	8.700	8.700	8.700 cPas $\pm$ 0

Viscosity testing results showed that the greater the concentration of LO used, the viscosity of the preparation would increase. This is because LO has a saturated fatty acid content consisting of stearic acid and palmitic acid (Pali and Mehta, 2014). Based on research by Warnida *et al.* (2019), The increase in viscosity in the preparation is due to the presence of stearic acid content in vegetable oils.

The results of the increased viscosity of preparations were analyzed using linear regression tests. The results of the linear regression test showed that the signification value of 0.000

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(P<0.05), i.e., there was an influence between the use of variations in the concentration of linseed oil to increase the viscosity of the moisturizer liquid foundation CoQ10.

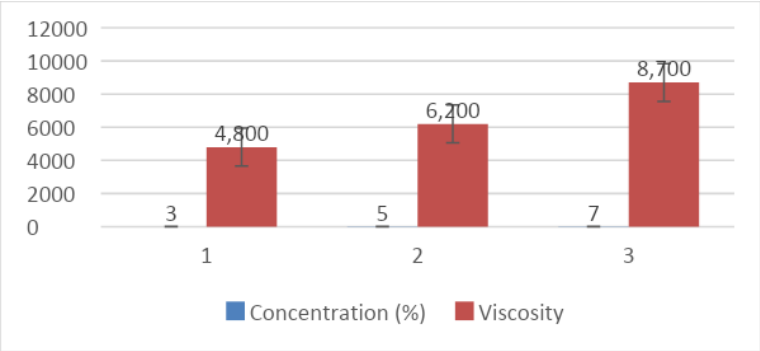

Figure 2. Viscosity evaluation graph of moisturizer liquid foundation CoQ10

The spreadability test aims to find out the ability of the preparation to be able to spread well when the preparation is applied to the skin. The spreadability test is a simulation of testing when the preparation is used on the skin which the higher the stock power of the stock it will make the active ingredients contained in it easily spread evenly on the skin (Rasydy *et al.,* 2021). The spreadability test results can be seen on table 6.

Table 6. Results of spreadability moisturizer liquid foundation CoQ10 evaluation

Formula	R1 (cm)	R2 (cm)		R3 (cm) $\bar{X} \pm$ SD
F1	6,33	7,10	6,40	6,61 cm ± 0,427
F2	6,30	6,65	6,53	6,49 cm ± 0,177
F3	6,25	5,85	5,90	6,00 cm ± 0,218

The results of the spreadability test are inversely proportional to the viscosity of the stock, which the higher the viscosity of the stock makes the distribution power value smaller. Furthermore, the stock diameter data of the preparation is analyzed using a linear regression test. The results of the linear regression test showed that the signification value obtained was 0.037 (P<0.05), which is an influence on the use of variations in the concentration of LO to the diameter of the spread of moisturizer liquid foundation CoQ10 (Figure 3)

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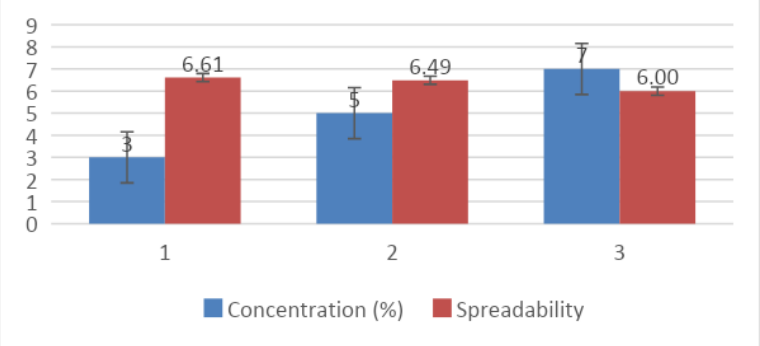

Figure 3. The spreadability evaluation graph of moisturizer liquid foundation CoQ10

The adhesivity test aims to find out the ability of a preparation to be attached to the skin. The adorable power test is carried out using round-scale glass. The criteria for testing the sticking power of topical preparations is no less than 4 seconds (Lumentut *et al.,* 2020). The results of the moisturizer liquid foundation CoQ10 sticking test can be seen in table 7.

Table 7. Results of adhesivity moisturizer liquid foundation CoQ10 evaluation

Formula	R1 (Second)	R2 (Second)	R3 (Second)	$\bar{X} \pm$ SD
F1	4,87	4,37	4,28	4,51 second ± 0,318
F2	5,66	5,23	5,34	5,41 second ± 0,223
F3	6,36	6,63	6,18	6,39 second ± 0,226

Based on the results of these tests, the adhesivity of the Moisturizer Liquid Foundation CoQ10 preparation has met the criteria of topical preparation. A preparation, if it can stick longer, will be better because it shows that the active ingredients absorbed into the skin will be more maximal. The ability of the dosage adhesivity is directly proportional to the viscosity of the preparation so that when the viscosity of the preparation is getting thicker, then the adhesivity of the preparation will be longer (Lumentut *et al.,* 2020).

Furthermore, the preparation adhesivity data is analyzed using a linear regression test. The results of the linear regression test showed that the signification value obtained from the adhesivity was 0.000 ($P<0.05$), which affects the use of variations in the concentration of LO against the increased adhesivity of the moisturizer liquid foundation CoQ10 (Figure 4).

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The hedonic or favorite test is a test used to find out the acceptance of liquid foundation moisturizer CoQ10. This test is used to assess the final result or quality of the product that has been produced. In hedonic tests involve humans assessing the quality of the product subjectively. Thus panelists are required to give their responses about the good or bad quality of the product honestly.

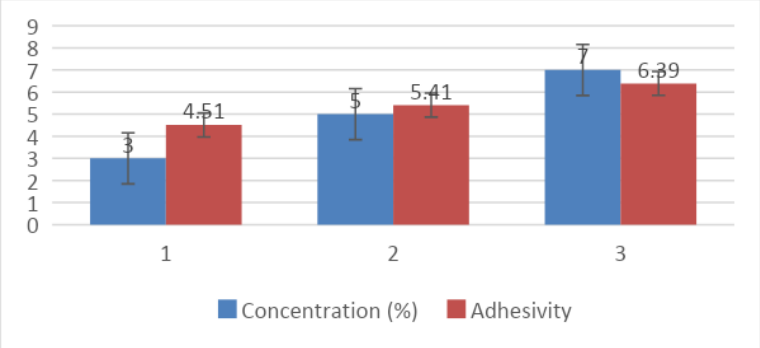

Figure 4. The adhesivity evaluation graph of moisturizer liquid foundation CoQ10

The hedonic test is done by applying moisturizer liquid foundation CoQ10 on the back of the panelist's hand, and then the panelist is asked to provide a personal response regarding the like or dislike of the preparation by assessing the texture of the preparation, aroma, color, and moisturizing ability of the moisturizer liquid foundation CoQ10. The inclusion criteria in this study are women aged 18-30 years. This is because at that age, it is a productive age where women often use decorative cosmetics, while for exclusion criteria are women with sensitive skin types. The total percentage of hedonic tests can be seen in figure 5.

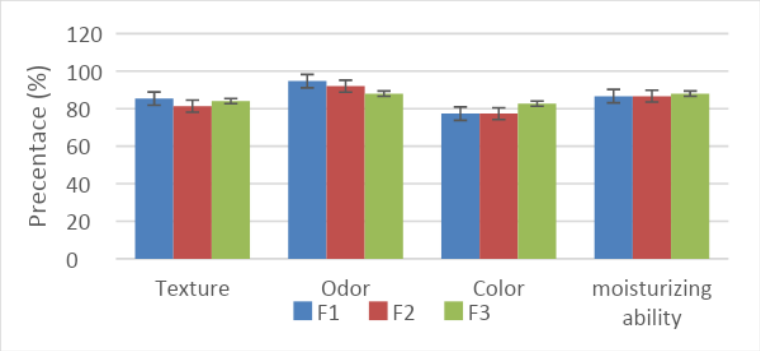

Figure 5. Graph of hedonic test results of moisturizer liquid foundation CoQ10

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The average percentage of hedonic test results in formula 1 is 86%, followed by formula 2, which is 84.22%, and formula 3 is 85.67%. Based on the data, it can be concluded that 15 panelists prefer formula 1 and formula 3 with a percentage of indicators for each texture, aroma, color, and moisturizing ability in formula one, which is 85.33%, 94.67%, 77.33%, and 86.67%. For formula 3 the percentage of 4 indicators of acceptance is 84%, 88%, 82.67%, and 88% while for formula 2 is 81.33%, 92%, 77.33%, and 86.67%. Based on the tests that have been carried out, the results show that the liquid moisturizer formula. The hedonic test data were then analyzed statistically using one-way ANOVA. The significance value obtained is 0.911 (P>0.05), indicating there is no difference from the third formula. Moisturizing liquid foundation CoQ10 with the use of variations in the concentration of linseed oil that the panelists most favored was formula 1 with a total percentage of 86%.

Figure 6. Hedonic cosmetic test moisturizer liquid foundation CoQ10

CONCLUSION

In conclusion, research conducted shows that the use of variations in the concentration of LO influences the characteristics of moisturizer liquid foundation CoQ10. The greater the concentration of LO added makes the preparations more viscous. In the hedonic test, 15 panelists preferred formula 1 because it has a more dilute texture than formula 2 or 3. In future studies, stability tests, irritation tests, and permeation tests are needed against moisturized liquid foundation CoQ10.

REFERENCES

Anwar, E. and Rizkamiarty, S. (2020) 'Formulation and evaluation of cosmetic foundation using epigallocatechin gallate as a sun protection', International Journal of Applied Pharmaceutics, 12(Special Issue 1), pp. 130–134. doi: 10.22159/ijap.2020.v12s1.FF029.

Lumentut, N., Edi, H. J. and Rumondor, E. M. (2020) 'Formulation and Physical Stability Preparation of Goroho Banana Peel Ethanol Extract (Musa acuminafe L.) Concentration of 12.5% as Sunscreen', Journal of MIPA, 9(2), p. 42. doi: 10.35799/jmuo.9.2.2020.28248.

Pali, V. and Mehta, N. (2014) 'Evaluation of Oil Content and Fatty Acid Compositions of Flax (Linum usitatissimum L.) Varieties of India', Journal of Agricultural Science, 6(9). doi: 10.5539/jas.v6n9p198.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Pangaribuan, L. (2017) 'Cosmetic Side Effects and Its Treatment for Women', Journal of Prosperous Healthy Families, 15(2), pp. 20–28. doi: 10.24114/jkss.v15i2.8771.
Prahl, S., Kueper, T., Bienorth, T., Wohrmann, Y., Munster, A., Furstenau, M., Schmidt, M., Schulze, C., Wittern, K.P., Wenck, H., Muhr, G.M., dan Blatt, T. (2008) Aging Skin Is Functionally anaerobic : Importance Of Coenzyme Q10 For Anti Aging Skin Care, BioFactors 32, 246-255.
Rasydy, L. O. A., Zaky, M. and Surtiana, R. (2021) 'Formulation and Physical Evaluation of Hand Body Lotion Preparations Of Ethanol Extract Miana Leaves (Pleactranthus scutellarioides (L.) R. Br.)',Pharmauho: Journal of Pharmacy, Science, and Health, 7 (1), v7il. 16320.
Ryu, K. A. et al. (2020) 'Topical delivery of coenzyme Q10-loaded microemulsion for skin regeneration', Pharmaceutics, 12(4). doi: 10.3390/pharmaceutics12040332.
Siva, J. and Afriadi, A. (2019) 'Gel Formulation of Strawberry Juice (Fragaria X ananassa Duchesne) as a Natural Moisturizer', World Journal of Pharmacy, 3(1), pp. 9–15. doi: 10.33085/jdf. v3i1. 4416.
Sivamani, R.K., Jagdeo, J., Elsner, P., dan Maibach, H.I. (2016) Cosmeceuticals And Active Cosmetics, Third Edition, CRC Press, United States.
Tou, K. A. S. et al. (2019) 'Influence of omega fatty acids on skin permeation of a coenzyme Q10 nanoemulsion cream formulation: characterization, in silico and ex vivo determination', Drug Developmentand Industrial Pharmacy,45(9),pp.1451–1458.doi: 10.1080/03639045.2019.1628042.
Warnida, H., Wahyuni, D. and Sukawaty, Y. (2019) 'Formulation and Evaluation of Tengkawang Fat-Based Vanishing Cream', 5(1), pp. 63–70.
Wu, H. et al. (2020) 'Coenzyme Q10Sunscreen Prevents Progression of Ultraviolet-Induced Skin Damage in Mice', BioMed Research International, 2020. doi: 10.1155/2020/9039843.

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Formulation Of Coenzyme Q10 Liquid Foundation With a Variations Virgin Coconut Oil as The Oil Phase

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Abstract

Coenzyme Q10 is a compound with potent antioxidants that can protect the skin from exposure to U.V. rays. Therefore researchers formulated a liquid foundation moisturizer coenzyme Q10 with VCO oil phase, which is able to provide many benefits. In addition, VCO is able to provide good physical characteristics to the preparation. The purpose of this study was to determine the effect of variations in the concentration of virgin coconut oil on the physical characteristics of the preparation and to determine the formula with the most preferred concentration of VCO by the public. The methodology in this research is evaporation emulsification with a 500 rpm magnetic stirrer for 10 minutes. Moisturizer liquid foundation is made by mixing the oil phase into the water phase above a water bath at a temperature of 70oC and adding white pigment to form an ivory color. The results showed that the higher the concentration of VCO, the lower the pH, viscosity, and adhesion of the preparation, and the greater the spreadability. In addition to testing the physical characteristics, the researchers also conducted a preference test, and the results obtained were that the respondents preferred formula 3 with a VCO concentration of 7%. Data were analyzed by descriptive statistics and linear regression with a 95% confidence level.

Keywords: *Moisturizer liquid foundation, coenzyme Q10, VCO*

INTRODUCTION

Coenzyme Q10 is an antioxidant compound that is synthesized endogenously through the mevalonate pathway in the human body. This compound is the only natural antioxidant that is fat-soluble and shows strong antioxidant activity (Bhagavan and Chopra, 2006). Coenzyme Q10 can counteract free radical damage and provide significant protection against UV-induced cell membrane damage. Coenzyme Q10 is able to avoid the occurrence of wrinkles by preventing collagen and helping the production of elastin (Korkmaz et al., 2013). However, in Indonesia, the production of coenzyme Q10 is still rare in topical dosage forms, so to take advantage of the antioxidant function of coenzyme Q10, researchers formulate coenzyme Q10 in liquid foundation moisturizer preparations.

The use of coenzyme Q10 in preparations has several drawbacks, namely coenzyme Q10 has a large molecular weight causing coenzyme Q10 cannot penetrate the stratum corneum, low water solubility causes an inappropriate appearance of the preparation, and high lipophilicity resulting in a lack of ability of coenzyme Q10 to penetrate the stratum corneum (Martinefski, 2016).

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This problem can be overcome by using VCO with a concentration of 5%, which is able to provide optimal release of coenzyme Q10 in the skin. In addition, the use of VCO as an oil phase can increase the solubility of coenzyme Q10 (Thanatuksorn et al., 2009). According to Mu'awanah et al. (2014), 0-7% VCO concentration as the oil phase is able to provide good physical characteristics to the preparation so as to provide a comfortable feeling when used on the skin.
Therefore, a concentration of 3, 5, 7% virgin coconut oil was used in the manufacture of coenzyme Q10 liquid foundation moisturizer.

LITERATURE REVIEW

Moisturizer liquid foundation containing coenzyme Q10 and VCO oil phase is a dosage form that can provide many benefits when used, although there are some shortcomings of coenzyme Q10 but can be overcome by using VCO. Research by Jing et al. (2015) stated that coenzyme Q10 with concentrations of 10 and 25 g/ml was able to reduce ROS production for 24 hours.
The results of research Thanatuksorn et al. (2009) use of VCO can increase the solubility of coenzyme Q10 because it contains medium-chain fatty acids such as lauric acid. VCO is proven to be able to increase the penetration of coenzyme Q10 based on research conducted by Lestari and Binarjo (2013). The results of research by Mu'awanah et al. (2014) stated that VCO could provide good physical characteristics of preparations seen from the results of physical tests of preparations, research by Purnamasari (2020) and Asmara (2008) explains that VCO affects physical tests of preparations such as pH, viscosity, and spreadability. . The higher the concentration of VCO, the lower the viscosity and pH of the preparation, but the greater the dispersion of the preparation.
In addition to the physical characteristics of the preparation, it is also necessary to do a hedonic test to find out whether the preparation made is liked by consumers or not. Aung and Than's (2017) research related to hedonic testing involved ten panelists with the results of panelists' preference for products reaching a medium-high level. Research by Suena et al. (2020) states that panelists prefer preparations with good physical characteristics or are not significantly different from products on the market.

RESEARCH METHODOLOGY

The formula for liquid foundation moisturizer coenzyme Q10 with VCO oil phase is as follows:

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Table I. Formulation of Coenzyme Q10 *moisturizer liquid foundation*

Materials	F I (%b/v)	F II (%b/v)	F III (%b/v)
Coenzyme Q10	0.5	0.5	0.5
VCO	3	5	7
BHT	0.1	0.1	0.1
Veegum	2	2	2
Titanium dioxide	5	5	5
Kaolin	5	5	5
Red iron oxide	0.2	0.2	0.2
Yellow iron oxide	0.8	0.8	0.8
Propanediol	10	10	10
Glycerin	10	10	10
Stearic acid	3	3	3
cetyl alcohol	4	4	4
Tween 80	3.5	3.5	3.5
Span 80	1.2	1.2	1.2
Benzalkonium chloride	0.1	0.1	0.1
citric acid	0.8	0.8	0.8
Vanilla	3 gtt	3 gtt	3 gtt
Purified water Ad	100	100	100

Information :

F1 = Liquid foundation moisturizer formula with 3% VCO concentration
F2 = Liquid foundation moisturizer formula with 5% VCO concentration
F3 = Liquid foundation moisturizer formula with 7% VCO concentration

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Method: evaporation emulsification by mixing coenzyme Q10 with PVP 100 mg then dissolved with 96% ethanol stirred with a 500 rpm magnetic stirrer for 10 minutes. Moisturizer liquid foundation is made by mixing the oil phase into the water phase above a 70oC water bath and then mixing it with the white pigment titanium dioxide, which has previously been mixed with glycerin. The oil phase consisted of VCO, span 80, BHT, stearic acid, and cetyl alcohol. The aqueous phase consisted of propylene glycol, benzalkonium chloride, tween 80, iron oxide, kaolin, veegum, and aquades.

After all the ingredients are mixed and form a liquid foundation moisturizer, 3 drops of vanilla fragrance are added. The preparations were tested for physical and hedonic characteristics.

Evaluation: physical characteristics of the preparation were evaluated, namely, organoleptic tests were analyzed descriptively, and pH, viscosity, dispersibility, and adhesion tests were analyzed by linear regression with a 95% confidence level.

Hedonic test: 15 panelists were asked to fill out a stock assessment questionnaire. The hedonic test data were analyzed descriptively.

FINDINGS AND DISCUSSION

Making coenzyme Q10 liquid foundation moisturizer with variations of virgin coconut oil
The formula used in this study refers to the research previously conducted by Aung and Than (2017). In this study, modifications were made to the formula, among others, by removing some of the ingredients from the previous formula and replacing them with new ingredients whose function was to support the purpose of making liquid foundation moisturizers. The new ingredient added is the active substance coenzyme Q10 which serves to add to the function of the preparation, namely protecting from exposure to free radicals (Armia, 2018). VCO is used as the oil phase here because it is proven to be able to provide good physical characteristics to the preparation (Mu'awanah et al., 2014), while in previous studies, the resulting preparation had poor characteristics. In this study, researchers added BHT antioxidants to prevent the autoxidation of VCO. If VCO is exposed to air, it will indirectly be oxidized, triggering the formation of other compounds such as acids, aldehydes, and short-chain ketones, which are volatile and cause a rancid odor.

Figure 1. The process of making Moisturizer Liquid Foundation Coenzyme Q10

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Evaluation of moisturizer liquid foundation coenzyme Q10

a. Organoleptic test

The organoleptic test aims to determine the physical appearance of the preparation, such as the color, scent, and texture of the preparation (Dimpudus et al., 2017). Based on the research results, the 3 formulas of liquid foundation moisturizers shown in Table II have the same physical appearance. In terms of texture, in general, it has a texture that is not significantly different. Likewise with color, at concentrations of 3%, 5%, and 7% tend to have the same color, namely ivory. This color is formed from a combination of red iron oxide and yellow iron oxide in a ratio of 1:4 mixed with the white pigment titanium dioxide (Mitsui, 2006). The scent of the preparations from the 3 formulas has the same characteristics, namely a combination of VCO and vanilla aroma, but the formula with a concentration of 7% has a more concentrated VCO scent than the formula with a concentration of 3% and 5%. This is because the highest concentration of VCO in formula 3 is 7%.

Table II. Table of Organoleptic Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	Texture	Color	Scent
F1	Thick	*Ivory*	Coconut Vanilla
F2	Thick	*Ivory*	Coconut Vanilla
F3	Thick	*Ivory*	Coconut Vanilla

b. pH test

The pH test was carried out to determine the level of acidity and alkalinity of the preparation. The goal is to make the pH of the preparation according to the pH of the skin, so that when applied to the skin there is no irritation due to an inappropriate pH. Skin pH ranges from 4.5-6.5.

Based on Table III, the pH of the F1 preparation was 6.03, F2 5.97 and F3 5.93. These results indicate that the higher the concentration of VCO, the lower the pH of the preparation. This is because VCO has a high fatty acid content, so that if the VCO used is higher, the amount of acid will also be higher, this causes the pH of the preparation to be more acidic or lower. The results of this study are in accordance with research conducted by Purnamasari (2020) and Mu'awanah et al (2014).

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Table III. Table of pH Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R 1	R 2	R 3	$\bar{X} \pm$ S.D.
F1	6.02	6.04	6.03	6.03 ± 0.01
F2	6.00	5.96	5.95	5.97 ± 0.03
F3	5.93	5.92	5.94	5.93 ± 0.01

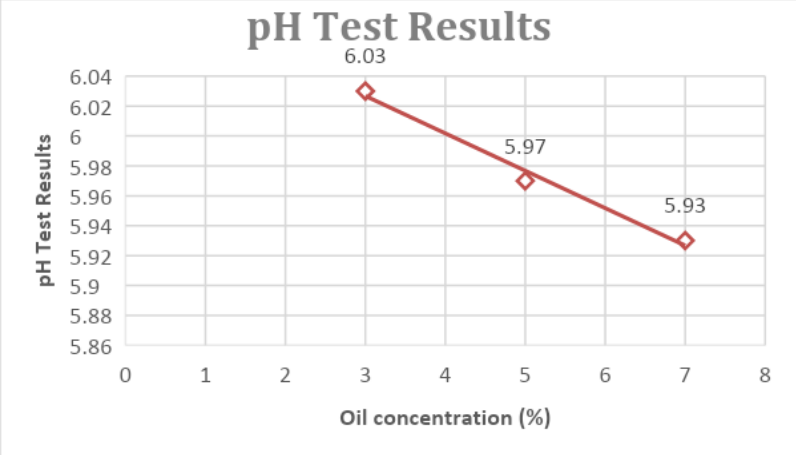

Figure 2. Graph of Relationship between VCO Concentration and pH

The results of the study were statistically analyzed, the results of the linear regression test the R2 value was 0.9868, meaning that 98.68% of the pH of the preparation was influenced by the concentration of VCO. The graph of the relationship between VCO concentration and pH can be seen in Figure 2.

c. Viscosity test

Viscosity test is carried out to determine the level of viscosity of the preparation, so that it feels comfortable when used on the skin. A good topical preparation has a viscosity between 2000-50000 cPas (Rasydy et al., 2021). Viscosity test results can be seen in Table IV.

Table IV. Table of Viscosity Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R 1 (cPas)	R 2 (cPas)	R 3 (cPas)	$\bar{X}$ (cPas) + SD
F1	6800	6700	6800	6766 ± 57.74
F2	6400	6500	6300	6400 ± 100
F3	6000	6200	6100	6100 + 100

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Table IV shows the viscosity of F1 6766 cPas, F2 6400 cPas, 6100 cPas, meaning that the viscosity of the preparations in the 3 formulas is theoretically appropriate. The results showed that the higher the concentration of VCO, the lower the viscosity. This is in accordance with the research of Mu'awanah et al. (2014). The decrease in viscosity due to the higher concentration of VCO is due to the coconut oil used made from coconut milk, namely VCO, which has a high water content compared to ordinary coconut oil. According to Surdianto et al. (2014), VCO contains 0.21% water, so the higher the VCO concentration, the higher the water content. Harun (2013) states that the lower the viscosity is if the water content contained in the material is higher.

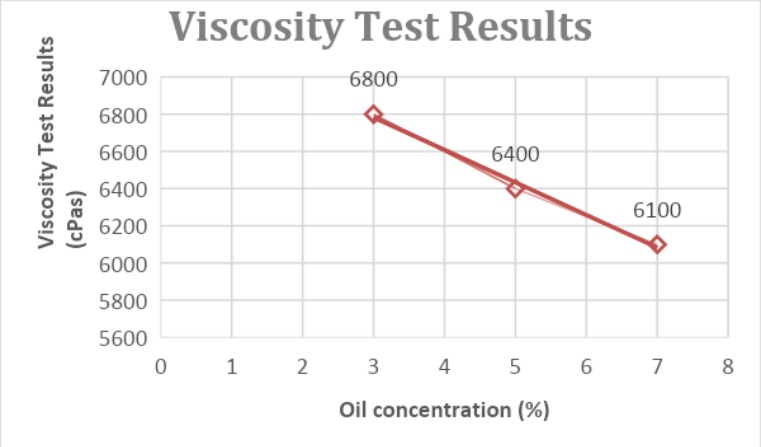

Figure 3. Graph of the Relationship between VCO Concentration and Viscosity

The results of the study were analyzed with linear regression statistics. The results of linear regression analysis were that there was an influence between variations in VCO concentration on viscosity. The magnitude of the effect shown from the value of R2, which can be seen in Figure 3 is 0.9932, meaning that 99.32% VCO concentration affects the viscosity of the preparation.

d. Spreadability test

The spreadability test was carried out to determine the even distribution of the preparation when applied to the skin. The greater the spreadability of the preparation, the more active substances are attached to the skin so that the pharmacological effect is maximized. Good dispersion of the preparation is between 5-7 cm (Swastika et al., 2013). Table 5 shows the dispersion of the preparations, respectively, namely 6.45 cm, 6.65 cm, and 6.80 cm. The research results are in accordance with the theory. The results showed

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that the higher the concentration of VCO, the greater the dispersion. This is in accordance with the research of Asmara (2008), which states that the dispersion is inversely proportional to the viscosity.

Table V. Table of Spreadability Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R 1 (cm)	R 2 (cm)	R 3 (cm)	$\bar{X}$ (cm) ± SD
F1	6.30	6.47	6.60	6.45 ± 0.15
F2	6.70	6.75	6.50	6.65 ± 0.13
F3	6.90	6.70	6.90	6.80 ± 0.12

The results of the study were analyzed by linear regression to determine the effect of VCO concentration on dispersion. The results of the linear regression test stated that 97.84% of the VCO concentration affected the dispersion of the preparation. The graph of the relationship between the two can be seen in Figure 4.

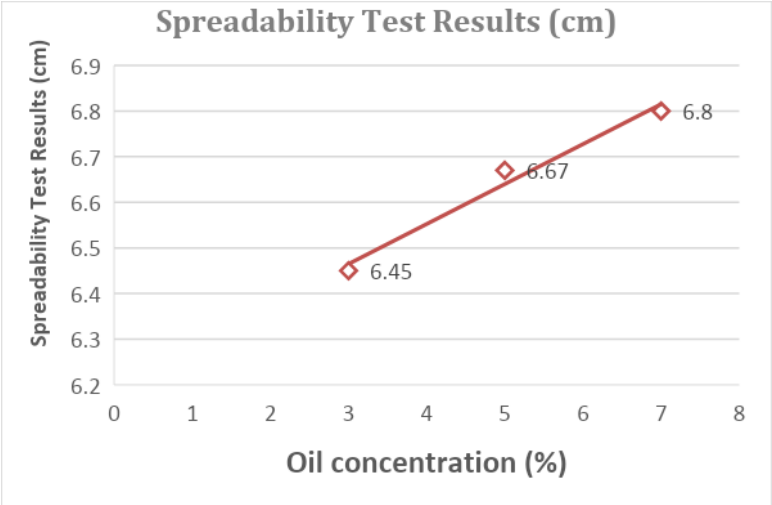

Figure 4. Graph of Relationship between VCO Concentration and Spreading Power

e. Adhesion test

The stickiness test aims to determine the length of time the preparation is in contact with the skin. Good adhesion is more than 4 seconds (Lumentut et al., 2020). The results of the adhesion test can be seen in Table VI. The value of F1 is 4.81 seconds, F2 is 4.39 seconds,

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and F3 is 4.13 seconds. According to Natalie (2017) adhesion is directly proportional to viscosity and inversely proportional to spreadability.

Table III. Table of Results of Adhesion Test for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R 1 (s)	R 2 (s)	R 3 (s)	$\bar{X}$ (s) ± SD
F1	4.87	4.98	4.57	4.81 ± 0.21
F2	4.65	4.33	4.20	4.39 ± 0.23
F3	4.11	4.01	4.26	4.13 ± 0.13

The results of the statistical test of linear regression of adhesiveness stated that 98.19% VCO concentration affected the adhesion of the preparation. The graph of the relationship between VCO concentration and adhesion is in Figure 5.

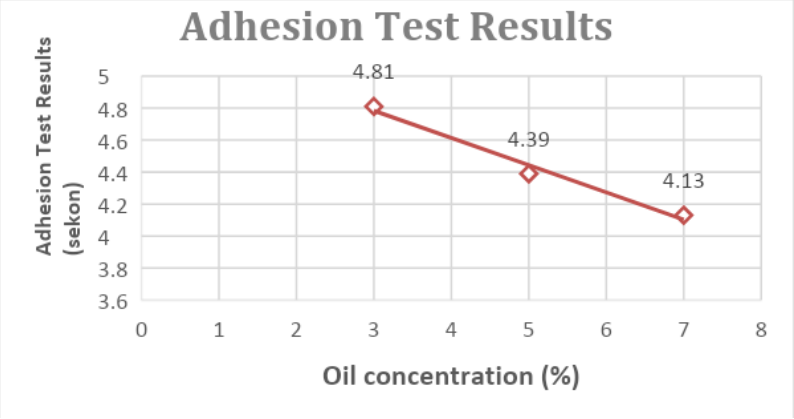

Figure 5. Graph of Relationship between VCO Concentration and Adhesiveness

f. Hedonic test

The hedonic test was carried out to determine the level of respondents' preference for the preparations made. Respondents were asked to provide an assessment of the preparations made according to the criteria determined by the researcher. Researchers used 15 respondents to assess the texture, aroma, color, and moisture of the preparations written in a questionnaire. The test results stated that respondents preferred formula 3 with a VCO concentration of 7% on the grounds of a strong aroma and texture, and humidity in accordance with preparations circulating in the market. This statement is in accordance with the research of Suena et al. (2020). The results of the descriptive statistical test showed a significance of >0.05, which means that there was no significant difference

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between the panelists' preference for F1, F2, and F3, or in general, the 3 formulas had the same attractiveness.

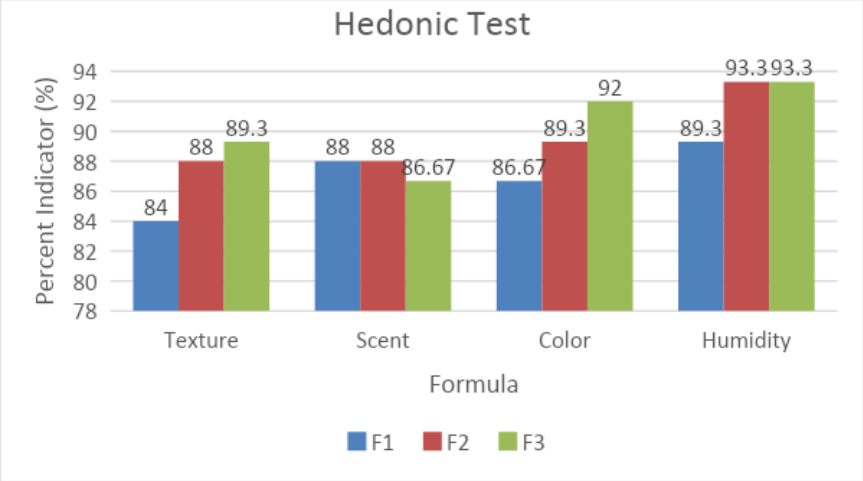

Figure 6. Hedonic Test Graph

CONCLUSION

Conclusion

The use of VCO (Virgin coconut oil) as the oil phase in the manufacture of a liquid foundation moisturizer has good characteristics, as evidenced by the results of the physical characteristics test that meet the theoretical requirements. Based on the linear regression test, the concentration of VCO affects the physical characteristics of the liquid foundation preparation of coenzyme Q10 moisturizer. The hedonic test (preferred test) shows that F3 is more preferred by the respondents, but based on descriptive statistical tests, there is no significant difference between respondents' assessments of the 3 formulas.

Suggestions

a. It is necessary to test the stability of the preparation to determine whether the preparation is stable in long-term storage.

b. It is necessary to test the particle size of coenzyme Q10 so that it can be seen the effect of VCO concentration on the particle size of the active substance of coenzyme Q10.

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REFERENCES

Armia, S. L. (2018). PHYSICAL CHARACTERISTICS AND pH OF LIPOSOME COENZIM Q 10 AND GLUTATHION AS ANTI AGING PHYSICAL CHARACTERIZATION AND pH OF LIPOSOME CONTAINING COENZYME Q 10 AND GLUTATHIONE AS. University of Surabaya Student Scientific Journal, 7(2), 866–885.

Asmara, S. D., 2008, Optimization of the Composition of Polysorbate 80 and Sorbitan Monooleate 80 as Emulsifying Agents in the Moisturizing Lotion Virgin Coconut Oil (VCO) Formula: Factorial Design Application, Thesis, Sanata Dharma University Yogyakarta, 55-58. Aung, T., & Than, S. S. (2017). Formulation of Foundation Makeup (Liquid) Using D-Optimal Mixture Design. 10(2).

Bhagavan, H. N., & Chopra, R. K. (2006). Coenzyme Q10: Absorption, tissue uptake, metabolism and pharmacokinetics. Free Radical Research, 40(5), 445–453. https://doi.org/10.1080/10715760600617843

Dimpudus, S. A., Yamlean, P. V. Y., & Yudistira, A. (2017). Formulation of Antiseptic Liquid Soap Preparations Ethanol Extract of Girlfriend Flower Water (Impatiens Balsamina L.) And Test Its Effectiveness Against Staphylococcus Aureus Bacteria In Vitro. Pharmacon, 6(3), 208–215. https://doi.org/10.35799/pha.6.2017.16885

Harun, Montolalu, and Suwetja, 2013, Physical Chemical Characteristics of Kappahycus alverezii Carrageenan at Different Harvesting Ages, Journal of Fishery Products Technology Media, 1, 10-11.

Jing, L., He, M. T., Chang, Y., Mehta, S. L., He, Q. P., Zhang, J. Z., & Li, P. A. (2015). Coenzyme Q10 protects astrocytes from ROS-Induced damage through inhibition of Mitochondria-Mediated cell death pathway. International Journal of Biological Sciences, 11(1), 59–66. https://doi.org/10.7150/ijbs.10174

Korkmaz, E., Gokce, E. H., & Ozer, O. (2013). Development and evaluation of coenzyme Q10 loaded solid lipid nanoparticle hydrogel for enhanced dermal delivery. Acta Pharmaceutica, 63(4), 517–529. https://doi.org/10.2478/acph-2013-0039

Lestari, M., & Binarjo, A. (2013). Cold Cream Propranolol Formulation For Transdermal Delivery With Emulsion Based Containing Vco (Virgin Coconut Oil). Pharmaciana, 3(2). https://doi.org/10.12928/pharmaciana.v3i2.429

Lucangioli, S., Tripodi, V. (2012). The Importance Of The Formulation In The Effectiveness Of Coenzyme Q10 Supplementation In Mitochondrial Disease. Therapy Der Pharmacia Sinica, 3(4), 406-407.

Lumentut, N., Edi, H. J., & Rumondor, E. M. (2020). Formulation and Physical Stability Test Preparation of Ethanol Extract Cream for Goroho Banana Peel (Musa acuminafe L.) Concentration of 12.5% As Sunscreen. MIPA Journal, 9(2), 42. https://doi.org/10.35799/jmuo.9.2.2020.28248

Martinefski, M., Samassa, P., Buontempo, F., Höcht, C., Lucangioli, S., & Tripodi, V. (2017). Relative bioavailability of coenzyme Q10 formulation for pediatric individualized therapy. Journal of Pharmacy and Pharmacology, 69(5), 567–573. https://doi.org/10.1111/jphp.12613

Mitsui, T. (2011). New Cosmetic Science. First Edition. Amsterdam: Elsevier Science. B.V., 342–345.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Mu'awanah, I., Setiaji, B., & Syoufian, A. (2014). Effect of Virgin Coconut Oil (VCO) Concentration on Cosmetic Emulsion Stability and Sun Protection Factor (SPF) Value. Bimipa, 24(1), 1–11.

Natalie, A., Mulyani, S., & Admadi, B. H. (2017). THE RELATIONSHIP OF STORAGE LONG WITH QUALITY CHARACTERISTICS IN SOME FORMULATIONS OF SURPRISE EXTRACT CREAM (Curcuma domestica Val.). Department of Agricultural Industrial Technology, Student of Agricultural Technology Unud, Faculty of Agricultural Industrial Technology, Lecturer, 5(4), 21–30. https://ojs.unud.ac.id/index.php/jtip/article/download/35543/21434 Purnamasari, R. (2020). FORMULATION OF PURE COCONUT OIL GEL OR VCO (VIRGIN COCONUT OIL) USED AS A FACE MOISTURIZER Gel Formulation of Pure Coconut Oil or VCO (Virgin Coconut Oil) Used as A Waste of Face. Luwu Raya Health Journal, 6(2), 37–43. Rasydy, L. O. A., Zaky, M., & Surtiana, R. (2021). Formulation and Physical Evaluation of the Preparation of Hand Body Lotion Ethanol Extract of Miana Leaves (Pleactranthus scutellarioides (L.) R. Br.). Pharmauho: Journal of Pharmacy, Science, And Health, 7(1), 33. https://doi.org/10.33772/pharmauho.v7i1.16320

Suena, N. M. D. S., Meriyani, H., & Antari, N. P. U. (2020). Physical Quality Test and Hedonic Test of Jatiluwih Red Rice Maserat Body Butter. Medicamento Scientific Journal, 6(1), 59–65. https://doi.org/10.36733/medicamento.v6i1.843

Surdianto, Y. N., Sutrisna and Rahcman. (2014). Making Virgin Coconut Oil (VCO) by centrifugation method. 1995, 426–435.

Swastika, A., Mufrod, and Purwanto. (2015). ANTIOXIDANT ACTIVITY OF TOMATO (Solanum lycopersicum L.) extract. Traditional Medicine Journal, 18(3), 132–140. https://doi.org/10.14499/mot-TradMedJ18iss3pp132-140

Thanatuksorn, P., Kawai, K., Hayakawa, M., Hayashi, M., & Kajiwara, K. (2009). Improvement of the oral bioavailability of coenzyme Q10 by emulsification with fats and emulsifiers used in the food industry. LWT - Food Science and Technology, 42(1), 385–390. https://doi.org/10.1016/j.lwt.2008.03.006

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Formulation Of Coenzyme Q10 Liquid Foundation With a Variations Olive Oil as The Oil Phase

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Abstract

Coenzyme Q10 contains antioxidants that can protect the skin from damage caused by harmful molecules, which are usually called free radicals. Moisturizer Liquid Foundation formulation with variations of olive oil as the oil phase can produce good physical stability of Moisturizer Liquid Foundation preparations during physical testing. The Moisturizer Liquid Foundation formulation was made using various concentrations of the olive oil phase, namely FI (3%), FII (5%), and FIII (7%). The Moisturizer Liquid Foundation formulation was made using the Emulsion evaporation method, and physical characteristics tests were carried out, including organoleptic tests, pH tests, viscosity tests, dispersibility tests, adhesion tests, and hedonic tests. The results showed that the organoleptic test of the three formulas had the same color and aroma, but the texture of the preparations was different due to variations in concentration. The higher the concentration of olive oil, the more viscosity will increase according to the data, namely F1:5200 Cpas, F2: 6400: Cpas, F3: 8400 Cpas. The higher the concentration of olive oil, the more acidic the pH value will be according to the data, namely F1: 6.17; F2: 6.11; and F3: 5.99. The results of the F1 dispersion test: 6,6; F2: 6.4; and F3: 6.2. The results of the F1 adhesion test: 6,11; F2: 6.25; and F3: 6.51. The most preferred hedonic test result is F2.

Keywords: *Coenzyme Q10, Moisturizer Liquid Foundation, Olive oil*

INTRODUCTION

The aging process on the skin is an unavoidable process, but the use of proper skin care will slow down and prevent the onset of skin aging. Basic skincare consists of 4 main basic functions, namely cleaning the skin, maintaining the skin, moisturizing the skin, and protecting the skin (Damayanti, 2017).

The use of various types of skincare does not provide satisfaction and efficiency in use, so women today want multifunctional cosmetic products. That is, it does not only function as decorative cosmetics but also as skincare products (Bowles et al., 2014).

One of the main types of decorative cosmetics is a foundation. Foundation has many types, one of which is Liquid foundation. Liquid foundation is used on top of moisturizer so that the pigments contained in the dye do not come into direct contact with the skin (Kustanti, 2008). And the use of antioxidants can protect cells from skin damage due to free radicals that cause the aging process

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of the skin (Andarina and Djauhari, 2017). So the formula for a suitable cosmetic product is a liquid foundation moisturizer with the addition of antioxidants.

Coenzyme Q10 is one of the non-enzymatic endogenous antioxidants, which is also known as Ubiquinone (Bhagavana, 2006). Coenzyme Q10 has drawbacks to be formulated in topical dosage forms, namely high lipophilicity (log P>10) so that Coenzyme Q10 is retained in the stratum corneum and causes a low release of Coenzyme Q10 in the skin (Lucangioli and Tripodi, 2012).
Seeing from the lack of Coenzyme Q10, it can be improved by using olive oil as the oil phase to increase the release of coenzyme Q10 (Deapsari, 2017). Olive oil with a concentration of 12% containing non-essential monounsaturated omega-9 fatty acids was used to increase the release of coenzyme Q10 into the skin (Tou et al., 2019). Research (Shoviantari et al., 2017), using olive oil with a concentration of 1.8% as the oil phase, resulted in the solubility and release of coenzyme Q10 in the skin after 6 hours. So that in this study, variations in the concentration of olive oil 3, 5, 7% were used for the formulation of coenzyme Q10 liquid foundation moisturizer.

LITERATURE REVIEW

Coenzyme Q10 is the only natural antioxidant that is lipid-soluble and has strong antioxidant activity. Coenzyme Q10, as an antioxidant, works by inhibiting lipid and protein peroxidase, and plays a role in scavenging free radicals, and works centrally in oxidative phosphorylation in mitochondria. Coenzyme Q10 has the disadvantage that it is unstable because it is very easily oxidized, so the activity of coenzyme Q10 decreases (Yamada Shao et al., 2015).
Moisturizer Liquid foundation is a type of foundation that is liquid and thick. This type of foundation is easy to spread and easily absorbs into the skin. A liquid foundation is the lightest foundation. The end result of using this foundation, makeup will look more natural. Liquid foundation is also suitable for use on normal, oily, and dry skin types (Fairuz, 2016).
To get a Moisturizer liquid foundation with good characteristics, it must be tested for its physical and chemical properties before proceeding with other tests. The tests that were carried out for the first time were physical evaluation or physical characteristics, physical characteristics testing of the liquid foundation moisturizer preparation, namely organoleptic test, pH test, viscosity test, spreadability test, adhesion test, and hedonic test.

RESEARCH METHODOLOGY

The materials used were then weighed with coenzyme Q10 with PVP 100 mg dissolved in 5 mL 96% ethanol, and then stirred using a magnetic stirrer at a speed of 500 rpm for 10 minutes. The oil phase ingredients were melted on a water bath at 70oC. Furthermore, titanium dioxide is mixed with glycerin until homogeneous. The water phase ingredients are combined into distilled water which has been heated at a temperature of 70oC while stirring on a water bath with the temperature being maintained at 70o. Then the oil phase was mixed into the water phase and stirred for 5 minutes. Then removed from the water bath and mixed with a mixture of glycerin and titanium dioxide. Then added Coenzyme Q10 and the rest of

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the aquadest and then carried out constant stirring with, add 3 drops of vanilla and stir again until homogeneous. Liquid foundation moisturizer preparations can be packaged and evaluated for characteristics.

Table I. Coenzyme Q1 moisturizer liquid foundation formula

Ingredients	F I (% b/v)	F II (% b/v)	F III (% b/v)
COQ10	0,5	0,5	0,5
Olive oil	3	5	7
BHT	0,1	0,1	0,1
Veegum	2	2	2
Titanium dioxide	5	5	5
Kaolin	5	5	5
Red iron oxides	0,2	0,2	0,2
Yellow iron oxides	0,8	0,8	0,8
Propylene glycol	10	10	10
Glycerine	10	10	10
Tween 80	3,5	3,5	3,5
Benzalkonium Chloride	0,1	0,1	0,1
Citric Acid	0,8	0,8	0,8
Stearic acid	3	3	3
Cetyl alcohol	4	4	4
Span 80	1,2	1,2	1,2
Vanilia	3 gtt	3 gtt	3 gtt
Purified Water Ad	100	100	100

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The data from the evaluation of the physical characteristics of the liquid foundation coenzyme Q10 moisturizer included organoleptic and hedonic tests descriptively, while the pH test, viscosity test, spreadability test, and adhesion test were statistically analyzed using linear regression with a 95% confidence level if there was a difference. meaning

FINDINGS AND DISCUSSION

The development of pharmaceutical preparations using coenzyme Q10 is formulated into a liquid foundation moisturizer with varying concentrations of olive oil as the oil phase to increase the solubility of coenzyme Q10 ingredients, which are difficult to dissolve in water (Bank et al., 2011). The concentration of olive oil was varied to determine the best liquid foundation moisturizer formula. The preparation of coenzyme Q10 liquid foundation moisturizer with various concentrations of olive oil that has been made is evaluated for physical characteristics by organoleptic tests, ph tests, viscosity tests, spreadability tests, adhesion tests, and hedonic tests. Olive oil concentrations from formulas 1, 2 and 3 were 3%, 5%, and 7% respectively. Organoleptic tests include shape, aroma, color, and texture. The following organoleptic test results are shown in the table below.

Figure I. Preparation of moisturizer liquid foundation from formulas F1, F2, and F3

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Tabel 2. Table of Organoleptic Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	Texture	color	Scent
F1	less thick	Ivory	Vanilla
F2	thick enough	Ivory	Vanilla
F3	thick	Ivory	Vanilla

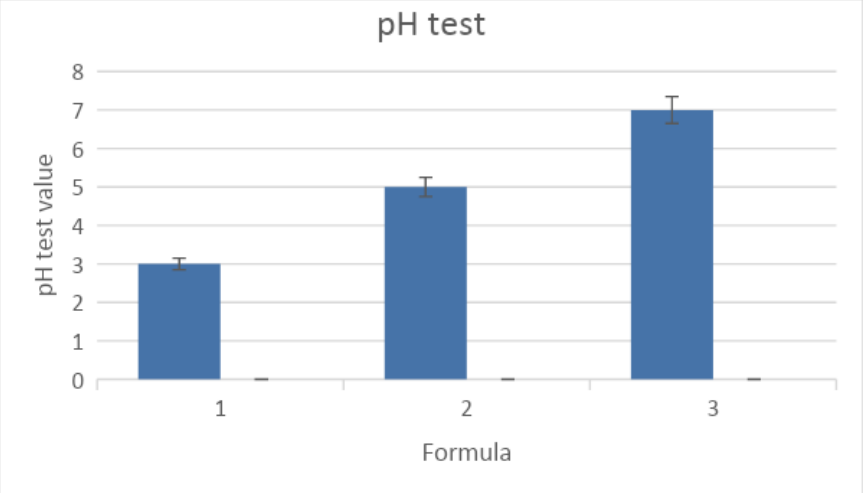

Figure II. Histogram average pH moisturizer liquid foundation from formula F1, F2, and F3 data is an average of 3 times of replication

There was no difference in the preparation of liquid foundation moisturizer after organoleptic testing for color and aroma, while the texture of the preparation had differences in each formula. Factors that influence the differences in the texture of these preparations are variations in the concentration of olive oil added to each formula. Concentrations of olive oil formulas 1 to 3 are 3%, 5%, and 7%. From the organoleptic test results, F3 showed a thicker consistency than F1 and F2 because the concentration of olive oil used was greater than F1 and F2, which was 7%. The vanilla aroma is due to the addition of vanilla essential oil. The following are the results of the pH test below.

Tabel 3. Table of pH Test Results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	**R1**	**R2**	**R3**	**x±SD**
F1	6.00	6.25	6.25	6.17±0.14
F2	6.00	6.16	6.18	6.11±0.09
F3	5.84	5.95	6.18	5.99±0.17

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The pH test of each formula can be seen in table 3. The results of the pH measurement of the moisturizer liquid foundation obtained from the three formulas are F1: 6.17; F2: 6.11; and F3: 5.99. This pH value meets the skin pH requirements of 4.5-6.5 (Tranggono, 2007). Then from these results, it can be seen that variations in olive oil concentration have an effect on the acquisition of pH values. Where based on the results obtained from the pH test, it is known that if the concentration of olive oil is increased, the pH of the liquid foundation moisturizer will be more acidic. This is influenced because olive oil is included in acidic ingredients (Anonymous, 2013). In addition, olive oil contains triacylglycerol, which is mostly in the form of monounsaturated fatty acids of the oleic type. Oleic acid content reaches 55-80% of the total fatty acids in acidic olive oil (Yunina, 2010). The statistical results of the pH value are 0.017 <0.05 (significant effect) Linear Regression. The following are the results of the viscosity test shown below.

Tabel 4. Table of Viscosity test results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R1 (cPas)	R2 (cPas)	R3 (cPas)	x±SD
F1	5100	5200	5400	5233±152.75
F2	6400	6400	6500	6433±57.73
F3	8300	8400	8500	8400±100

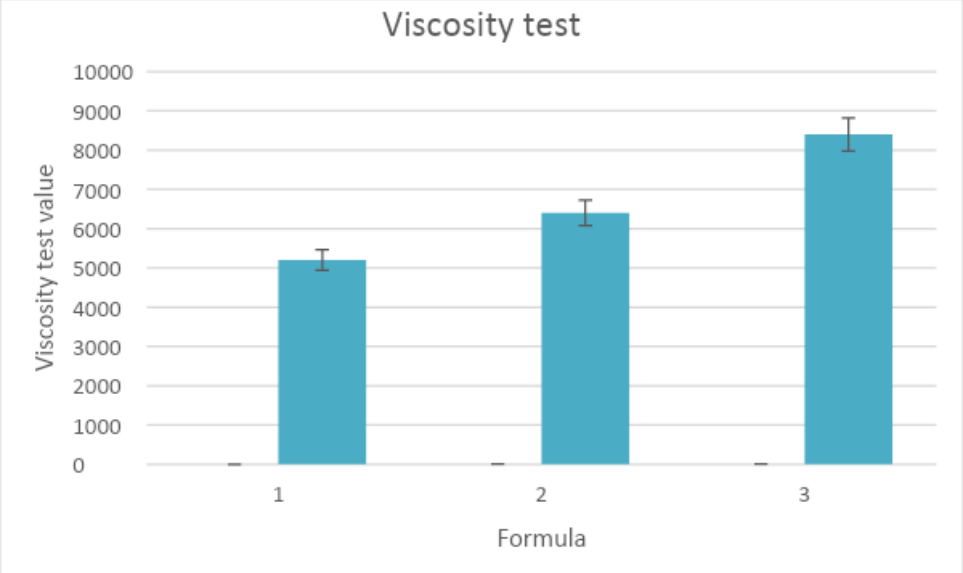

Figure II. The histogram of the average viscosity of the moisturizer liquid foundation from formulas F1, F2, and F3 data is an average of 3 times of replication

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Viscosity testing from the table above shows the viscosity value for F1 = 5233 cPs; F2 = 6433 cPs; F3 = 8400 cPs. This viscosity value meets the quality requirements of topical preparations, which have a viscosity value range of 2000-50,000 cPs (Rasydy et al., 2011). From the results above, it can be seen that variations in the concentration of olive oil cause an increase in the viscosity of liquid foundation moisturizer preparations because olive oil has a very large unsaturated fatty acid content of 86% (Boyle & Anderson, 2007). Viscosity is directly proportional to adhesion but inversely proportional to spreadability. The higher the viscosity value, the higher the stickiness and the less dispersion. In statistical testing, the significance value of 0.000 means that there is an influence between variations in the concentration of olive oil on the viscosity value. The following are the results of the viscosity test shown below.

Tabel 5. Table of Spreadability test results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R1 (cm)	R2 (cm)	R3 (cm)	x±SD
F1	6.8	6.7	6.7	6.7 cm±0.05
F2	6.4	6.6	6.0	6.3 cm±0.31
F3	6.5	6.0	5.9	6.1 cm±0.32

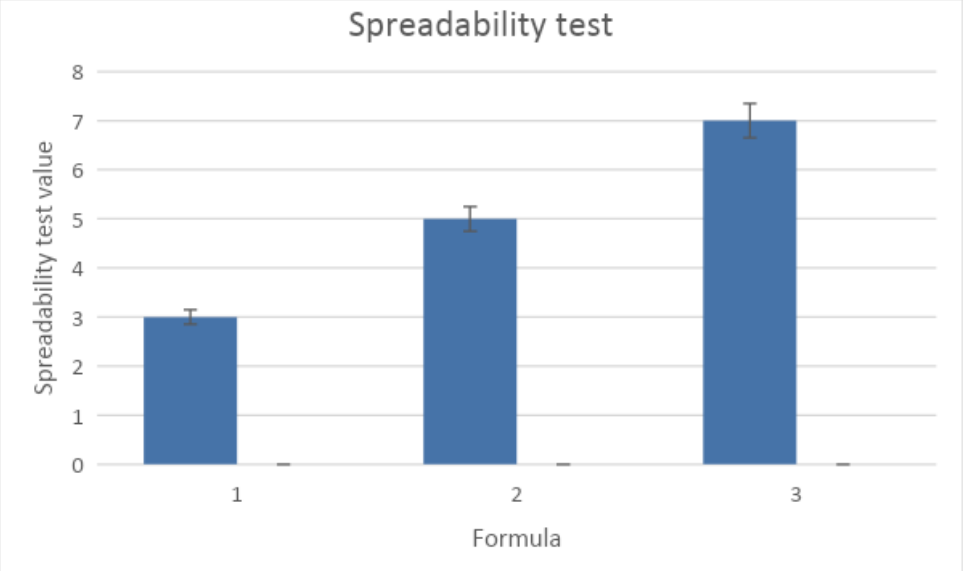

Figure III. Histogram of average dispersion power of moisturizer liquid foundation from formulas F1, F2, and F3 data is an average of 3 times of replication

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Testing the spreadability of the liquid foundation moisturizer obtained the results of F1: 6.7; F2: 6.3; F3: 6.1. This shows that variations in olive oil concentration cause a decrease in the value of dispersion, related to the viscosity test obtained previously, where the higher the viscosity, the smaller the diameter of the dispersion power of the preparation. In statistical testing, the significance value of 0.020 means that there is an influence between variations in olive oil concentration on the spreadability value. The following are the results of the viscosity test shown below. The spreadability test describes the spread of a liquid foundation moisturizer on the skin when used. To be able to determine the ability to spread liquid moisturizer foundation on the skin, it must meet the requirements for spreadability if the spreadability enters the measurement value range of 5-7 cm. The preparation of a liquid foundation moisturizer that has good spreadability will make it easier to apply to the skin. Factors that can affect the diameter of the spreadability of preparation, namely the amount of extract used in each formula, this statement refers to the fact that the lower the consistency of a liquid foundation moisturizer with a lower sticking time, the easier it is to spread the liquid foundation moisturizer. (Dominica, 2019). The statistical results of the pH value are 0.020 <0.05 (significant effect) Linear Regression. The following are the results of the viscosity test shown below. The following are the results of the viscosity test shown below.

Tabel 6. Table of Adhesion test results for Moisturizer Liquid Foundation Coenzyme Q10

Formula	R1 (Second)	R2 (Second)	R3 (Second)	x±SD
F1	5.87	6.00	6.45	6.1 second±0.30
F2	5.92	6.37	6.46	6.3 second±0.29
F3	5.83	6.84	6.85	6.5 second±0.59

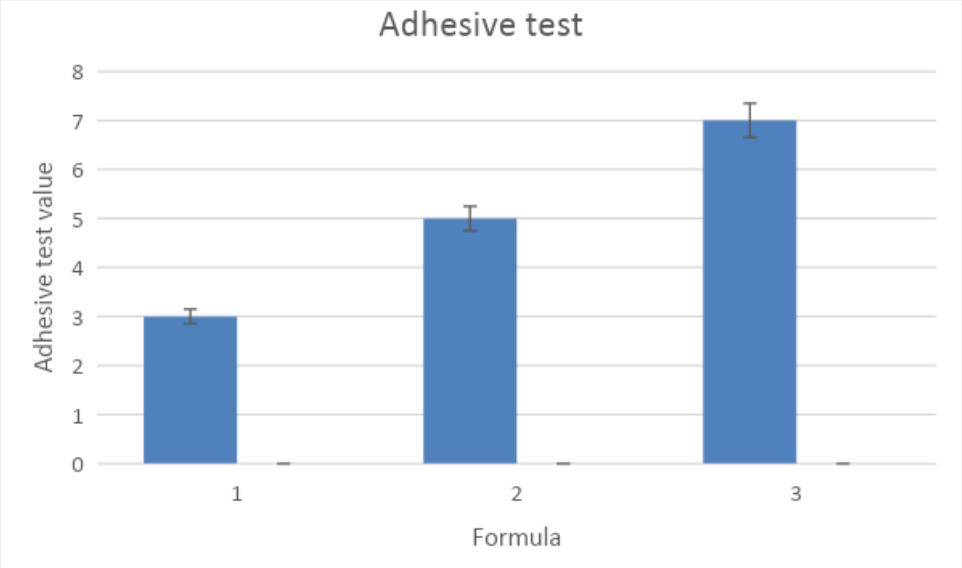

Figure IV. The histogram of the average test stickiness of the moisturizer liquid foundation from formulas F1, F2, and F3 data is an average of 3 times of replication

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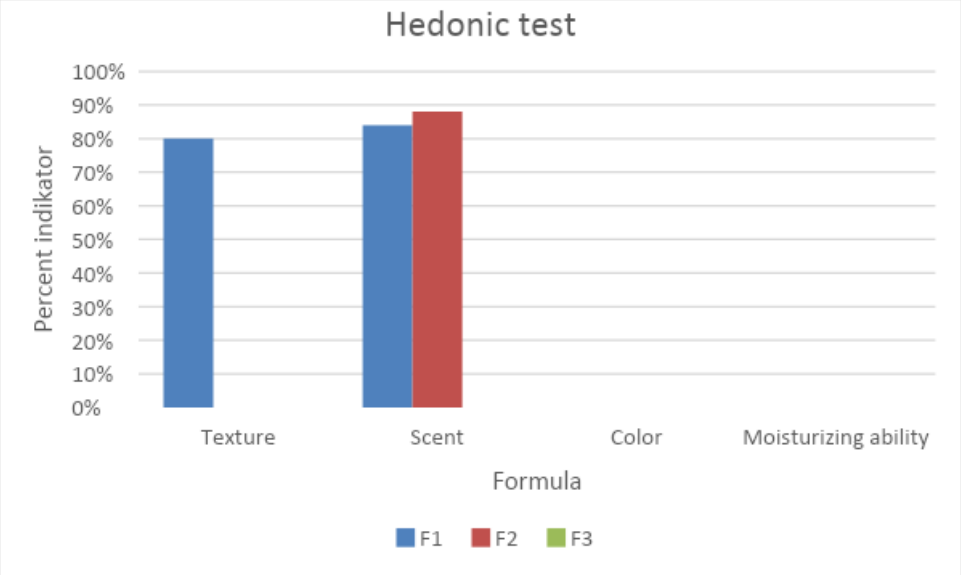

Figure V. Histogram of average hedonic test of moisturizer liquid foundation from formulas F1, F2, and F3 data is an average of 3 times of replication

An adhesion test is the ability of a preparation to adhere to the skin when used. Good preparations have high adhesion. The higher the adhesion, the better for liquid foundation preparations (Husnani et al., 2017). Testing the stickiness of the liquid foundation moisturizer obtained the results for F1: 6.1, F2: 6.3, and F3: 6.5. Adhesion for topical preparations for more than 4 seconds means that the test results are appropriate. The results of adhesion are directly proportional to the viscosity of the liquid foundation moisturizer preparation. The statistical results of the pH value are 0.015 <0.05 (significant effect) Linear Regression. The following are the results of the viscosity test shown below.

The hedonic test is one type of acceptance test for liquid foundation preparations. In the hedonic test, the panelists were asked to give their personal opinion about their likes or dislikes of the liquid foundation moisturizer that was made, and the panelists were invited to fill out the questionnaire. The purpose of the hedonic test is to obtain a formula for a liquid foundation moisturizer coenzyme Q10 with a variety of olive oil (Olive oil) which affects the texture, aroma, color, and ability to moisturize so that the most preferred liquid foundation moisturizer preparation is obtained from the panelists.

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The inclusion criteria set in this hedonic test are women aged 18-30 years. The selection of this category is based on an age assessment where at that age is a productive age for women who wear decorative cosmetics. The exclusive criteria set for the hedonic test for liquid foundation moisturizer preparations are having sensitive skin. The steps in hedonic testing are cleaning the back of the panelist's hand using a wet tissue so that it does not affect the panelist's assessment during the test, then a sample of the liquid foundation moisturizer is applied to the back of the panelist's hand.

Hedonic test (preferred test) was conducted on 15 women aged 18-30 years who were randomly selected with 4 categories, namely texture, aroma, color, and ability to moisturize. From the data obtained for the category of liking, the use of liquid foundation moisturizer preparations for the texture category the most in formula 1 with a concentration of 3% olive oil because the more olive oil concentration will affect the spreadability, adhesion, and viscosity of liquid foundation moisturizer preparations. For the Aroma category, formula 2 is preferred with a concentration of 5% olive oil. For the color category, formulas 1, 2, and 3 have the same score because all formulas use additional vanilla essence. For the ability to moisturize, formula 2 is preferred because it is influenced by the concentration of olive oil.

CONCLUSION

Moisturizer liquid foundation coenzyme Q10 with a variety of olive oil obtained organoleptic test results, which in terms of texture showed an increase in viscosity level had an ivory color and vanilla aroma. The increase in the concentration of olive oil affects the value of viscosity and the value of adhesion which is increasing, while for the spreadability test, it decreases. For the hedonic test, the most preferred formula is formula 3, wherefrom the level of texture that is considered the most preferred by the panelists.
Suggestions for research on liquid foundation moisturizer coenzyme Q10 with variations of olive oil need to do a stability test, irritation test, and penetration test in order to get the best preparation.

REFERENCES

Daley-bowles, T. et al. (2014) 'Foundation products have a measurable impact on moisturizing Foundation products have a measurable impact on hydration', (November).
Damayanti (2017) 'Skin Aging and Basic Skin Care in Elderly', Periodic on Skin and Sexual Health, 29(1), pp. 73–80.
Dominica, D. and Handayani, D. (2019) 'Formulation and Evaluation of Lotion Preparations from Longan (Dimocarpus Longan) Leaf Extract as Antioxidants', Indonesian Journal of Pharmacy and Pharmaceutical Sciences, 6(1), p. 1. doi:10.20473/jfiki.v6i12019.1-7.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Elaimi, A. et al. (2017) 'Skin Penetration of Coenzyme Q10 in Nanostructure Lipid Carriers Using Olive Oil and Cetyl Palmitate', International Journal of Pharmaceutical and Clinical Research, 9(2). doi: 10.25258/ijpcr.v9i2.8297.

Fairuz, D. N. (2016) Effect of species selection.

Husnani and Muazham, MF Al (2017) 'Optimization of Physical Parameters of Viscosity, Spreadability and Adherence to Sodium Base CMC and Carbopol 940 in Honey Gel Using Simplex Lattice Design Method', Journal of Pharmacy and Clinical Pharmacy, 1(1) , pp. 11–18.

Indonesia, U. et al. (2010) 'Microemulsion formulation..., Miranti Dewi, FMIPA UI, 2010'.

Indradewi Armadany, F. and Sirait, M. (no date) 'Antioxidant Peel-off Gel Mask Formulation from Tomato Extract (Solanum lycopersicum L. var. cucurbita)', Pharmaceutical Magazine, 1(2), pp. 29–32.

Lucangioli, S., Tripodi, V. (2012) 'The Importance Of The Formulation In The Effectiveness Of Coenzyme Q10 Supplementation In Mitochondrial Disease', Therapy Der Pharmacia Sinica, 3(4), pp. 406-407.

Mursyid, A. M. (2017) 'Evaluation of Physical Stability and Diffusion Profile of Gel (Olive Oil) Preparations', Indonesian Journal of Phytopharmaceuticals, 4(1), pp. 205–211. doi: 10.33096/jffi.v4i1.229

Murti, I. K. A. Y. et al. (2018) 'Optimization of Olive Oil Concentration on Physical Stability of Liquid Soap Preparation', Udayana Pharmaceutical Journal, 6(2), p. 15. doi:10.24843/jfu.2017.v06.i02.p03.

Rasydy,L.O.A.,Zaky,M.,&Surtiana,R.(2021). Formulation and Physical Evaluation of the Preparation of Hand Body Lotion Ethanol Extract of Miana Leaves (Pleactranthus scutellarioides(L.)R.Br.).Pharmauho: Journal of Pharmacy, Science ,And Health,7(1),33. https://doi.org/10.33772/pharmauho.v7i1.16320

Traits, P. et al. (2017) 'Measurement of Polarization Properties of Various Vegetable Oils Using Ir Lamps and He-Ne Lasers', MIPA Journal, 39(2), pp. 123–127.

Tou, K. A. S. et al. (2019) 'Influence of omega fatty acids on skin permeation of a coenzyme Q10 nanoemulsion cream formulation: characterization, in silico and ex vivo determination', Drug Development and Industrial Pharmacy, 45(9), pp. 1451–1458. doi: 10.1080/03639045.2019.1628042.

W., S., F., D. and TM, E. (2017) 'Penetration of Ubiquinone (Q10) Nanoemulsion Using Olive Oil Through Rat Skin', International Journal of Pharmaceutical and Clinical Research, 9(2), pp. 169–172. doi:10.25258/ijpcr.v9i2.8300.

Yamada, Y. et al. (2015) 'Mitochondrial delivery of Coenzyme Q10 via systemic administration using a MITO-Porter prevents ischemia/reperfusion injury in the mouse liver', Journal of Controlled Release, 213, pp. 86–95. doi: 10.1016/j.jconrel.2015.06.037.

Yuna. (2010) 'The Effect of Olive Oil on HDL Cholesterol Levels in White Rats (Rattus Norvegicus) Given a High Fat Diet', . Essay. Surabaya: Faculty of Veterinary Medicine Universitas Airlangga., Page 3-.

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Synergistic effect of *Euphorbia Milii* with Tannic Acid as a disinfectant against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Herbal disinfectant is the cheapest and most unique way to clean a surface. This study focused on the synergistic impact of *Euphorbia Milii* and Tannic acid as a disinfectant against microorganisms. The aqueous solvent extract of plant leaves was used mixed with tannic acid against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria tested by the disk diffusion method. Both bacterial species were isolated from the kitchen surface. Minimum Inhibitory Concentration (MIC) was recorded with an optical density at 600 nm using a UV-spectrophotometer, which showed inhibition of bacterial growth in a cultural broth mixed with extract of *Euphorbia Milii* and Tannic acid. According to the findings, the disinfectant showed a maximum zone of inhibition for *E. coli* (14 mm) and *S. aureus* (20 mm). The disinfectant activities of extract were tested and estimated using a time-kill analysis. Fourier transform infrared spectroscopy (FTIR) analysis was conducted to identify the chemical bond, giving information related to the active sites of chemical compounds present in disinfectants. Overall, this study reveals that *Euphorbia Milii* is an excellent candidate to formulate disinfection.

Keywords. *Disinfectant, Euphorbia, Minimum Inhibition Concentration, Spectrophotometer, Synergistic effect.*

INTRODUCTION

The bacterial spread of diseases occurs as a result of healthy infections fostered by the kitchen surface. It creates a crucial component during the disease's direct transmission. Disinfectants are chemicals or mixtures of substances that can be applied as antimicrobial agents on non-living things to eliminate germs in the environment (Padya and Doshi, 2017). There are two types of disinfectants on the market. chemically manufactured disinfectants and natural herbal disinfectants. Herbal disinfectants are now commercially accessible as a cost-effective and environmentally friendly option (Padya and Doshi, 2017 and Kaczmarek, 2010). Tannic acid is a biomaterial with biological and chemical characteristics, a natural tannin of phenolic acid molecules. Tannic acid has unique properties (Kaczmarek, 2010). *Euphorbia milii* is a flowering plant species in the Euphorbiaceae family. Researchers from all around the globe have studied the effects of different *Euphorbia* species on microbial infections (Saleem, 2019 and Singh, 2018 and Ismaila, 2017). Several types of *Euphorbia* have been used to treat infectious disorders, including warts, antimicrobials, and intestinal parasites. *Euphorbia milii latex* is known as the Crown of

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Thorns. This is the most common genus of the medicinal plant used globally. This plant is used for ornamental purpose and also contain excellent analgesic activity. This study will provide environmentally friendly and active disinfectants that kill these microbes and be used for domestic areas.

LITERATURE REVIEW

Basavegowda *et al*. 2020 investigated the antibacterial activity of several solvent extracts of E. *cotinifolia* leaves against certain human pathogenic microorganisms. They employed agar cup diffusion and minimum inhibitory concentrations with micro broth dilution (MIC). The inhibition zones measured against test microorganisms were 15.25-19.50 mm and 13.50-19.25 mm, respectively. Singh et al. (2018) investigated the antibacterial activity of Euphorbiaceae members against human infections using an ethanolic extract. They also assessed their phytochemical constituents. *Euphorbia milii, Euphorbia hirta, Euphorbia pulcherrima, Euphorbia tithymaloides, Euphorbia prostrata,* and *Emblica officinalis* are the species involved in their research. Narendra et al., 2015 investigated the antibacterial activities of hexane, ethyl acetate, acetone, and methanol extracts of *Euphorbia milii* (Euphorbia) flowers on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris* using the cup plate technique. Hexane, acetone, and methanol extracts reveal an open significant inhibitory zone for Staphylococcus aureus and *Bacillus subtilis* at 5g/ml concentrations.

RESEARCH METHODOLOGY

Preparation of disinfectant using Euphorbia milii extract and Tannic Acid
Euphorbia milii leaves were obtained from the USPCAS-W Garden of Mehran University, Jamshoro. The plants were harvested, cleaned with water, completely washed with distilled water, and dried. Dried leaves were pounded with a mortar and pestle and filtered through a muslin cloth. Tannic acid was measured and mixed with water.

Test organisms
As test organisms, two types of bacteria (*E. coli and S. aureus*) were used in this investigation. The bacterial strains were isolated from the USPCAS-W Kitchen and cultured on Muller-Hinton agar (MHA) for 24 hours at 37 °C incubation.

Antimicrobial properties of Euphorbia milii extract using the disk diffusion method
The disc diffusion technique was used to assess the antibacterial properties of *Euphorbia milii* leaves. Different concentrations of tannic acid mixed with plant leaves extract was administered to Whatman filter paper disks of 6 mm and dried. All tested disks were placed on MHA containing Petri plates having tested bacterial lawn. All Petri plates were incubated

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for 24 hours at 37°C. For each disk, the diameter of the inhibitory zone was measured and recorded.

Minimum Inhibitory Concentrations (MIC) of Euphorbia milii extract

The MIC of *Euphorbia milii* extract was also measured using a broth serial dilution technique, diluting in a series of test tubes containing Miller-Hinton broth and tested bacteria. The growth of bacteria was monitored using an optical density spectrophotometer set at 600 nm. MIC of *Euphorbia milii* was calculated in the presence and absence of Tannic acid was.

Time kill analysis

Time-killing analysis was carried out in broth culture medium using separate tubes containing 1 ml of the bacterial strain, 8 ml of Muller-Hinton broth, and 1 ml of *Euphorbia milii* extract mixed with tannic acid. The optical density was measured at various time intervals, particularly at 0, 15, 30, 45, 60, 75, and 90 minutes. Between optical density and time, a growth inhibition curve was plotted.

Fourier-transform infrared spectroscopy of Euphorbia milii extract

For the FTIR analysis, aqueous extracts of *Euphorbia milii* leaves were used. Ten mg of dried extract powder was encapsulated in a 100 mg KBr pellet to make clear sample tablets. The crushed plant sample was analyzed using an FTIR spectrometer (Shimadzu, IR Affinity 1, Japan) with a scanning range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}.

FINDINGS AND DISCUSSION

Antimicrobial effect of *Euphorbia milii* as a Disinfectant
The antibacterial activities of fresh *Euphorbia milii* leave extract were tested using the disc diffusion technique against *E. coli* and *S. aureus* bacteria at different concentrations. 6, 12.5, 25, 50, and 100 l. These microorganisms showed varying quantities of antibacterial capabilities for plant extract based on inhibitory zones. Figure 1 illustrates *E. coli* inhabiting zones of 10, 11, 11, 12, and 14 mm at doses of 6, 12.5, 25, 50, and 100 µl, respectively. *S. aureus* inhibited zones as wide as 16, 17, 17, 18, and 20 mm at a concentration of 6, 12.5, 25, 50, and 100 µl. As the concentration increased, the inhibition of microbes also increased.

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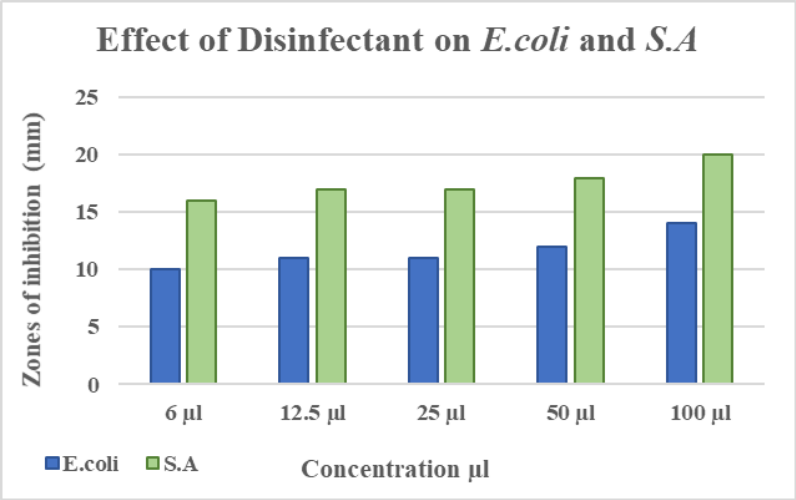

Figure 1. Antimicrobial effect of *Euphorbia milii disinfectant* on *E. coli* and *S. aureus*

Minimum Inhibitory Concentration of *Euphorbia milii* against Microbes

Figure 2 found that the concentration of *E. coli* at 125µl was increased while the growth, measured a 1.7OD, was decreased. The extract had a maximum growth on1.4 OD at 250 µl. It revealed a minimal reduction in growth at 0.2 and 0.1 OD at the concentration of 500 and 1000 µl, respectively.

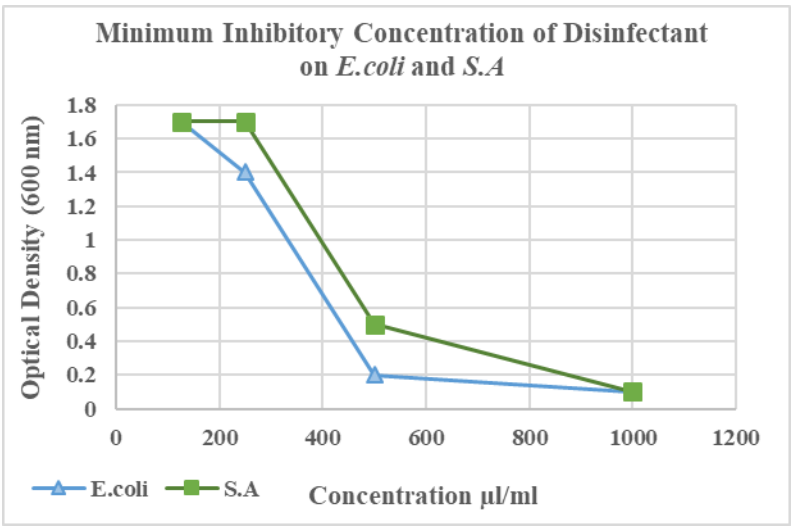

Figure. 2 MIC of *Euphorbia milii* against on *E. coli* and *S. aureus*

At 125 µl, the density of *S. aureus* began to rise, and the growth was observed slowing at 1.7 OD. A maximum increase in growth was measured at the concentration of 250 µl at 1.7 OD. In comparison, a minimum decrease in the growth of S. aureus was observed at the concentration of

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500 and 1000 µl of the extract with OD of 0.5 and 0.1, respectively. The growth rate of both of the microbes began to reduce as the concentration raised.

Time kill analysis

The growth of *E. coli* and S. aureus was significantly decreased from 0 to 15 minutes and stayed steady till 90 minutes, as observed in a time-kill study, shown in Figure 3. The result revealed that the mixture had good activity as a disinfectant.

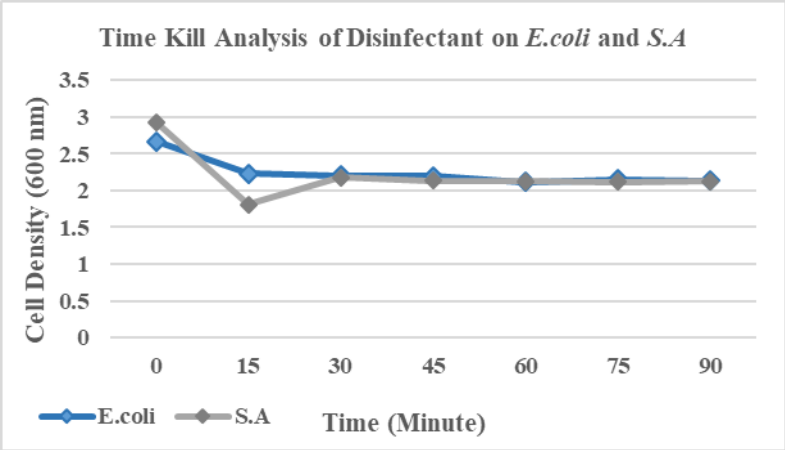

Figure. 3 Time kill analysis on *E. coli* and *S. aureus*

Fourier-transform infrared spectroscopy of *Euphorbia Milii* leaves extracts

The Fourier Transform Infrared Spectrophotometer (FTIR) was used to identify chemical bonds (functional groups) in compounds presented in Euphorbia milii extracts that displayed different characteristic peak values.

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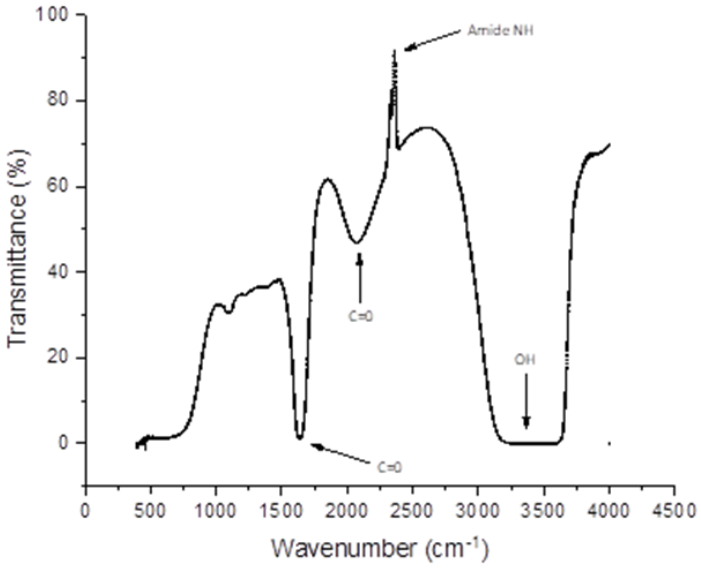

Figure. 4 FTIR of *Euphorbia Milii* leaves extracts

The FTIR spectrum of *Euphorbia Milii* leaves extracts showed the broadband at 3287 $cm^{-1,}$ identified as the OH group. The brand at peak 1223 cm^{-1} showed the presence of –C-O- bond.

CONCLUSION

The capacity of disinfectants to boost antibacterial characteristics is critical. In this study, we discovered that *Euphorbia Milii* leaves were effective against surface microorganisms at various doses in combination with tannic acid. The most potent inhibitory zones were determined, with varied doses suggesting the plant's most possible active against microbes. The lowest inhibitory concentration was also determined, indicating that various disinfectant concentrations had distinct effects on particular bacteria. To produce natural remedies for various general concerns and conquer many public issues, research on the phytochemicals found in these plants must be done.

REFERENCES

Abushady, H., El-Shatoury, E. and Abd-emerged, A.S., 2017. Antibacterial and antioxidant properties of some selected egyptian plants. Annals of Mechnikov's Institute, (1), pp.71-95.

Ashokkumar, R. and Ramaswamy, M., 2014. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. International journal of Current Microbiology and applied Sciences, 3(1), pp.395-406.

Chaman, S., Khan, F.Z., Khokhar, R., Maab, H., Qamar, S., Zahid, S., Ahmad, M. and Hussain, K., 2019. Cytotoxic and antiviral potentials of Euphorbia milii var. splendens leaf against Peste des petits ruminant virus. Tropical Journal Pharmacceutical Research,18(7), pp.1507-1511.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Chohan, T.A., Sarfraz, M., Rehman, K., Muhammad, T., Ghori, M.U., Khan, K.M., Afzal, I., Akash, M.S.H., Malik, A. and Chohan, T.A., 2020. Phytochemical profiling, antioxidant and antiproliferation potential of Euphorbia milii var.: Experimental analysis and in-silico validation. Saudi journal of biological sciences, 27(11), pp.3025-3034.

de Matos, R.A., da Silva Cordeiro, T., Samad, R.E., Vieira Jr, ND and Courrol, L.C., 2011 Green synthesis of stable silver nanoparticles using Euphorbia milii latex. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 389(1-3), 134-137.

Duffitt, A.D., Reber, R.T., Whipple, A. and Chauret, C., 2011. Gene expression during survival of Escherichia coli O157:H7 in soil and water. International journal of microbiology, 2011 , Article ID 340506. http://dx.doi.org/10.1155/2011/340506.

Ismaila, M., Owisa, A.I., Hettab, M., Mohammeda, R. Total phenolics and flavonoids content, antioxidant activity and GC/MS analyses of Euphorbia grandialata. Int. J. Appl. Pharm. Sci. Res. 7, 176–181, 2017.

Kaczmarek, B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview. Materials, 13(14), p.3224, 2020.

Kamba, AS and Hassan, L.G. Phytochemical Screening and Antimicrobial Activities of Euphorbia balasamifera Leaves Stems and Root against Some Pathogenic Microorganisms. African Journal of Pharmaceutical Sciences and Pharmacy, 1(1), pp.57-64, 2010.

Maneesha Singh and Sudha, Evaluation of Phytochemical and Antibacterial Activity of Euphorbiaceae Members Against Human Pathogens. Int J Recent Sci Res. 9(8), pp.28534-28538, 2018.

Mwine, J.T., Van Damme, P. Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. J. Med. Plants Res. 5, 652–662, 2011.

Pradyutha A. Ch. and Rao Uma Maheswara, 2015. Phytochemical screening and antimicrobial evaluation of Euphorbia milii leaf extracts. World Journal of Pharmaceutical Research, 4 (8), p.1626-1633.

Saleem, H., Zengin, G., Locatelli, M., Mollica, A., Ahmad, I., Mahomoodally, F.M., Abidin, SAZ and Ahemad, N. In vitro biological propensities and chemical profiling of Euphorbia milii Des Moul (Euphorbiaceae): A novel source for bioactive agents. Industrial Crops and Products, 130, pp.9-15, 2019.

Urja Pandya, Ankit Doshi & Nirmal S. Sahay. Development of herbal disinfectants formulation for mopping households and its antibacterial activity, Natural Product Research, 31:22, 2665-2668, 2017.

Zeghad, F., Djilani, S.E., Djilani, A. and Dicko, A., 2016. Antimicrobial and antioxidant activities of three Euphorbia species. Turkish Journal Pharmaceutical Sciences, 13(1).

Zheng, F., Luo, Y., Wei, X. and Wang, B., 2009. Non-terpenoid constituents from the seeds of Euphorbia lathyris. Journal of Tropical and Subtropical Botany,17(3), pp.298-301.

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In-vitro antimicrobial activity of *Lactuca Sativa* Leaves against Isolated Clarithromycin-resistant Superbugs

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Abstract

Antibiotics were one of the modern advancements in the 20th century, but they have been less active and have become more alarming due to antibiotic resistance. Antimicrobial resistance among pathogenic microorganisms is rapidly increasing, posing a danger to human health. However, the most essential biologically bioactive components are sourced by plants and are industrially used to produce drugs against several antibiotic-resistant bacteria. Antimicrobial agents based on plants possess fewer side effects and have immense potential than available drugs in clinics to combat superbugs. This study investigated bioactive components of Lactuca sativa (Lettuce) that were energetic in our research against Clarithromycin-resistant bacteria. *Lactuca sativa* had a substantially stronger antimicrobial effect on gram-negative bacteria than it did on gram-positive. Using a UV-visible spectrophotometer at 600nm, distinct behaviors of isolated bacteria were detected at varied optical densities; the highest activity was reported at 1 ml/50ml. Various phytochemicals were detected qualitatively, including carbohydrates, proteins, saponins, flavonoids, alkaloids, terpenoids, phenolic compounds, and tannins. Anthraquinones and glycosides were not discovered in lettuce. A quantitative investigation was conducted to detect unique phenolic compounds using High-Pressure Liquid Chromatography (HPLC) with varied peaks. Gallic acid, syringic acid, sinapic acid, and vanillin were identified as phenolic components by HPLC. However, further study on the analysis of isolated phytochemicals is required to identify novel antibiotics and their rapid and plant-based control and the proper management of antibiotic resistance spread and its risk to human health.

Keywords. *Lactuca sativa, lettuce, clarithromycin-resistance, bioactive components, phytochemicals*

INTRODUCTION

Antibiotic resistance refers to a microbe's capacity to thrive and suppress antibiotic activities, and it is a significant cause of a variety of illnesses and fatalities across the world (Kleina, E. Y., et al. 2018). In the past, infectious diseases were a major cause of death. However, developments in medicine and public health over the twentieth century helped reduce the burden of infectious diseases considerably. On the other hand, infectious diseases are on the rise once more, particularly those that can no longer be treated with previously available drugs. Infectious bacteria may acquire resistance to antibiotics, and many have developed resistance to both regularly used and newly developed medicines throughout time. On a global level, antibiotic resistance has become a huge threat to people's health. (Chokshi, A., & et al., 2019). Antibiotics were developed to give a simple and effective therapy for bacterial illnesses, and they have had a

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tremendous impact on human health and longevity since then. Antibiotic resistance (ABR) poses a concern to several organisms: Many pathogenic bacteria have developed resistance to the most common antibiotic classes, and multidrug-resistant bacteria have resulted in illnesses that are untreatable. ABR already has significant health and economic consequences, and by 2050, the worldwide yearly cost of ABR might reach 10 million fatalities and US$100 trillion (MacLean, R.C. and San Millan, A., 2019). Bacterial resistant strains (with immune genes) enter the water through faecal material, eventually distributing their genes (superbugs) to water-indigenous microorganisms (Baquero F. et al., 2008).

Antibiotic-resistant bacteria can be treated using a range of commercially available drugs. The fast growth of multidrug-resistant microorganisms, on the other hand, is jeopardising the therapeutic viability of many present medicines. Many known bacterial infections have been treated with natural items throughout human history (Naveed, R. et al., 2013). Bacterial resistant strains (with immune genes) enter the water through faecal material. Eventually, *Lactuca sativa* is a well-known universal plant because of its use in salads, soups, and vegetable curries. It also has excellent therapeutic effects. It is high in carotene, vitamin C, and vitamin E. Given the presence of these beneficial phytochemical constituents, it is assumed that the plant could have antioxidant potential (Hajare, A.G., et al., 2013). Despite this, there is a lot of room to explore plant potential to resist these human adversary superbugs, and the scientific community is seizing the issue of world health. In this study, it was tried to isolate Clarithromycin-resistant and -sensitive bacteria. To find the components present in *Lactuca sativa* active against those isolated bacteria.

LITERATURE REVIEW

Misuse and abuse of antibacterial medications, both in human health and agriculture, is one of the key driving causes behind the development of antibacterial drug resistance (Gajdács, M. and Albericio, F., 2019). Antibiotic resistance develops due to a variety of factors, including inappropriate antibiotic use, over-prescription, and patients who do not finish their antibiotic medication; antibiotic misuse in livestock and fish farming; poor infection control in healthcare settings; lack of hygiene and sanitation; unavailability of newly discovered antibiotics; and genetic mutations. Wastewater treatment accounts for just 1% of the industry in Pakistan, and sewage discharge without proper treatment is a major source of surface and groundwater contamination (Jabeen et al., 2015). Antimicrobial stewardship includes making judgments such as choosing the most appropriate antimicrobial(s) for the patient with the least amount of side effects, ensuring minimum influence on local resistance levels, and maintaining their availability and efficacy in the future (Dyar, O.J., & et al., 2017). Another growing component of antimicrobial stewardship is quick diagnostic procedures in clinical microbiology laboratories (diagnostic stewardship) to help in medication therapy selection (Morgan, D.J., Malani, P., and Diekema, D.J., 2017). Water habitats are relatively conducive for resistance gene transfer. Antibiotics and their resistance genes may be bypassing water treatment facilities, tipping the balances in favor of microorganisms gaining antibiotic resistance. Such resistance genes can be discovered in bacteriophages with wandering genetic

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sources, allowing for the unrestricted transmission of resistant features from one bacterium to another (Roopan, S.M. et al., 2016). A new antibiotic must be absorbed by body tissues in a consistent and timely manner. Antibiotics are most effective when it comes to destroying germs (Vineetha, N. et al., 2015). The efficiency of antibiotics in use throughout the world is gradually deteriorating - the medications we use are less effective. If the user does not take any action, the day will come when we will be unable to treat such bacterial infections with any medication. The scenario necessitates extensive research into novel, less costly, more secure, and dependable antimicrobial therapies. Natural ingredients are a tried-and-true option, and in recent decades, various plant research has been done to uncover novel anti-infection compounds (Sood, A. et al., 2012). According to WHO, almost 80% of the world population is entirely reliant on traditional medicine, which is blended for various ointments with plant chemical derivatives and raw extracts (Dar, P. et al., 2014). Traditional pants have made significant contributions to the advancement of modern medicine, particularly antimicrobials, which cure infectious disorders. To combat diseases, innovative antimicrobial agents with correct chemical structures and distinct mechanisms of action must be developed. The plant disinfectant has a broad restorative potential for a variety of infectious pathogens (Naveed, R. et al., 2013).

Hundreds of "superbugs" resistant to antibiotics have made headlines during the last quarter-century. Antimicrobial substances are also abundant in medicinal plants (Bebell, L. M., & Muiru, A. N. 2014). Traditional remedies are utilized by 80% of the people in Pakistan's rural areas (Javid et al., 2015). Although resistance has evolved to every antibiotic used in clinical practice, most efforts to treat AMR are focused on identifying new medicines. As a result, discovering new strategies to control the development of drug-resistant illnesses is a top concern for public health (Ragheb, M.N., & et al., 2019). Lettuce is one of the world's most commonly eaten leaf vegetables. Lettuce is abundant in water (95%) and fibers, as well as vitamins (A, B1, B2, B3, B9, C, and E), beta-carotene, phenolic compounds, minerals, and carotenoids. (Kim et al. 2016; Shams et al. 2019). Lettuce is one of the most often consumed raw edible plants and is high in phytonutrients, including vitamins, carotenoids, fiber, and phenols (Shahidi and Ambigaipalan 2015; Sönmez et al. 2017; Shams, M. et al., 2019). In vitro and in vivo studies have demonstrated that eating lettuce offers health benefits, including decreasing cholesterol (Lee et al. 2009), inflammatory activities (Pepe et al. 2015), diabetes (Cheng et al. 2014), and other disorders (Pelegrino, M.T., & et. Al., 2020). Non-nutritive bioactive plant compounds with disease-fighting or prevention properties are known as phytochemicals. These bioactive components are responsible for plant extracts' antibacterial activity in vitro (Bhat, R.S. and Al-Daihan, S., 2014).

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RESEARCH METHODOLOGY

Isolation and Characterization of Isolated bacteria:

Clarithromycin-resistant and -sensitive microorganisms (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Shigella flexneri*) were isolated. They characterized using culture and biochemical identification of microorganisms from samples taken from tap water.

Sample preparation:

Lactuca sativa was bought from a local market in Latifabad, Sindh, and cleaned with tap water before being cleansed with distilled water. One gram of plant yielded 200-300 µl of crude extract. Clarithromycin and plant extract were each diluted in distilled water separately. The test substances were then put onto the 6mm antibiogram plane discs.

Susceptibility Testing:

To test the susceptibility of *Lactuca sativa*, a disc diffusion technique was used. Antibiotic-loaded discs served as the control group, whereas *Lactuca sativa* extract-loaded discs served as the treatment group. The measurements were obtained in terms of zones of growth inhibitions. The turbidity levels were used to determine the MIC of extract for all bacterial strains, which had been quantified with a UV-Visible Spectrophotometer at 600nm.

Hight-throughput Phytochemical Screening:

High-throughput phytochemical screening of extract was conducted to detect carbohydrates, amino acids, saponins, phenolic compounds, tannins, and proteins, qualitatively (Santhi, K. and Sengottuvel, R., 2016; Bhandary, S.K. et al., 2012). Anthraquinones, flavonoids, glycosides, alkaloids, and terpenoids were also discovered (Krishnapriya, T.V., and Suganthi, A., 2017; Hajare, A.G.,2013; Santhi, K. and Sengottuvel, R., 2016; Doss, A. 2009).

Quantitative analysis of Phenolic Compounds through HPLC:

The quantitative analysis of lettuce extract was performed at the National Centre of Excellence in Analytical Chemistry. Free and bound phenolic acids from *Lactuca sativa* extract was separated using Spectra System SCM 1000 (Thermo Finnigan, California, USA) liquid chromatograph equipped with a diode array detector system. U.V. analysis was performed at 270, 310, and 325 nm using the DAD package. Individual phenolic acid structures were validated by relating their U.V. spectra and retention time (tR) to the

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applicable parameters. Based on the peak area of their associated standard curves, the concentrations of the chemicals were figured (Siddiqui, M.S. et al., 2017).

FINDINGS AND DISCUSSION

Antibacterial Screening of *Lactuca Sativa* Extract

As stated in table 1, a crude extract of *Lactuca sativa* at a concentration of 10µl/50µl demonstrated the lower and higher efficiency against clarithromycin-resistant (R25) *S.flexneri* (10.6±0.6mm) and (R100) *S.typhi* (11.3±3.3mm), respectively.

Table 1. Antibacterial Screening of *Lactuca sativa* against Clarithromycin-resistant and -sensitive pathogens

Microbial strain	***Concentration with Zone of inhibion* ((Mean±SE)) *of Lactuca sativa* Extract**			
	5µl	**10µl**	**20µl**	**30µl**
***E.coli* (S)[1]**	0	10.1±1.1	12.0±2.3	12.61±1.7
***E.coli* (R50)[2]**	0	0	17.31±0.6	19.32±0.6
***S. typhi* (S)**	8.61±0.6	9.32±0.6	11.33±0.6	14.21±0.6
***S. typhi* (R100)[3]**	8.62±0.6	11.31±3.3	13.32±2.4	14.61±1.7
***S. flexneri* (S)**	8.60±0.6	9.30±1.3	10.1±1.1	12.21±2
***S. flexneri* (R25)[4]**	9.34±0.6	10.63±0.6	12.61±0.6	14.21±2
***S. aureus* (S)**	8.1±0	8.61±0.6	10.11±0	10.63±1.7
***S. aureus* (R25)**	0	0	10.12±0	10.61±0.6

[1](S)= Sensitive to Clarithromycin, [2]R (50) = Resistant at 50 µl, [3](R100)= Resistant at 100 µl, [4](R25)=Resistant at 25 µl (p-value < 0.5)

The crude extract concentration of 20µl/50µl revealed that (R25) S.flexneri (12.6±0.6mm) had the lowest antibacterial activity, whereas (R50) E.coli (17.3±0.6mm) and (R25) S.aureus (10 ±0mm) had the highest antibacterial activity. The significant efficacy of *Lactuca sativa* extract was reported against resistant bacterial strains at a concentration of 30µl/50µl.

MIC of *Lactuca sativa* Extract against Resistant Microbes

To estimate the MIC of *Lactuca sativa* extract, optical density was measured at 600nm. Various growth inhibition behaviours were detected in the presence of extract. The maximum MIC was reported against gram-negative bacteria, as illustrated in figures 1A, B, C, and D. *Lactuca sativa* had the highest MIC at 500µl/ml against clarithromycin (S) *E.coli* at 0 and 24 hours. While testing against (R50) *E.coli*, good MIC was obtained at the concentration of 500µl/ml at 0 hours, as shown in figure 1 A. *Lactuca sativa* extract had the greatest MIC against

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(S) S.typhi at 500lµl/ml at 0 hours, excellent activity at 125µl/ml, and the best MIC activity at 500µl/ml during 24 hours. Similarly, for resistant S.typhi, the MIC was 125µl/ml at 0 hours and 125 µl /ml after24 hours, as illustrated in figure 1 B. At 0 hours, Lactuca sativa extract had the greatest MIC against (S) S. flexneri at a dosage of 250µl/ml. For (R25) S. flexneri, the extract had the highest activity at a concentration of 250µl/ml at 0 hours. It had the best MIC at 24 hours at 125µl/ml, as illustrated infigure 1C. *Lactuca sativa* extract had a good MIC at a concentration of 250µl/ml against (S25) *S. aureus* at 0 hours and 125µl/ml after 24 hours, indicating inhibition of bacterial growth. As demonstrated in figure 1D, the most significant MIC against (R) *S.aureus* was observed at 500µl/ml at 0 h and 125l/ml at 24 hours.

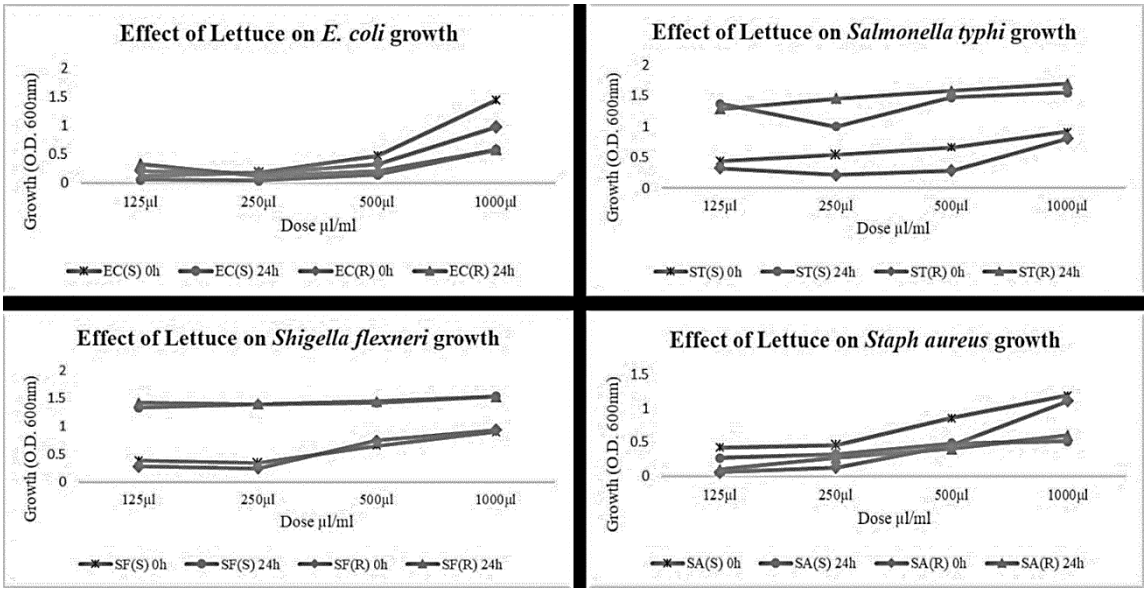

Figure 1. Effect of different concentrations of Lettuce aqueous extract on isolated resistant and sensitive microbes: A) effect on the growth of *E.coli*, B) effect on the growth of *Salmonella typhi*, C) effect on the growth of *Shigella flexneri*, and D) effect on the growth of *Staph aureus*

Qualitative Phytochemical Analysis of *Lactuca Sativa* Extract

The phytochemical contents of *Lactuca Sativa* were studied qualitatively. *Lactuca sativa* extract included carbohydrates, proteins, saponins, flavonoids, alkaloids, terpenoids, phenolic chemicals, and tannins. Anthraquinones and glycosides, on the other hand, were not found in *Lactuca Sativa* extract.

HPLC of *Lactuca Sativa*

The phenolic compounds present in *Lactuca Sativa* that may be accountable as bioactive components in *Lactuca Sativa* (Lettuce) extract against the Clarithromycin-resistant bacteria were quantified using High-Pressure Liquid Chromatography (HPLC).

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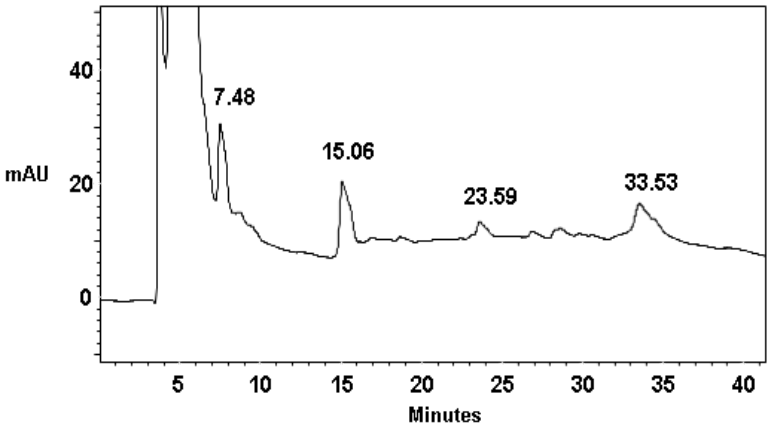

Figure 2. HPLC separated fractions from Lettuce water extract.

Figure 2 depicted four distinct phenolic compounds as bioactive components at varying retention times and in varying amounts. Gallic acid was discovered at 42.1677 µg/ml at a retention time of 7.48, while syringic acid was detected at 49.7982 µg/ml at a retention time of 15.06. Sinapic acid was 12.4573µg/ml after 23.59 retention periods. Vanillin had a retention time of 33.53 and a concentration of about 68.0183µg/ml.

CONCLUSION

In this study, Clarithromycin-resistant bacteria were isolated and identified form drinking water; the *E.coli* colonies were found resistant at 50µg/mL, *S.typhi* at 100µg/mL, and *S.flexneri* and *S.aureus* at 25µg/mL of extract concentration. Microbial inhibitory activity against resistant *E.coli* was the highest in *Lactuca Sativa* (Lettuce), whereas it was the lowest against sensitive and resistant *S.aureus*. The extract of *Lactuca Sativa* included alkaloids, phenolic compounds, tannins, and sapssssonins, all of which are important antibacterial ingredients and carbohydrates and proteins, which are required macromolecules with nutritional value. The phenolic compound detected in *Lactuca sativa* (Lettuce) were gallic acid, syringic acid, sinapic acid, and vanillin. Further study by comparing with other vegetable extracts should be carried out to develop natural-based drugs to combat such superbugs.

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REFERENCES

Chokshi, A., Sifri, Z., Cennimo, D. and Horng, H., 2019. Global contributors to antibiotic resistance. *Journal of global infectious diseases*, *11*(1), p.36.

MacLean, R.C. and San Millan, A., 2019. The evolution of antibiotic resistance. *Science*, *365*(6458), pp.1082-1083.

Gajdács, M. and Albericio, F., 2019. Antibiotic resistance: from the bench to patients.

Balouiri, M., Sadiki, M. and Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), pp.71-79.

Baquero, F., Martínez, J.L. and Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. *Current opinion in biotechnology*, *19*(3), pp.260-265.

Bebell, L.M. and Muiru, A.N., 2014. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide?. *Global heart*, *9*(3), pp.347-358.

Bergeron, S., Boopathy, R., Nathaniel, R., Corbin, A. and LaFleur, G., 2015. Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. *International Biodeterioration & Biodegradation*, *102*, pp.370-374.

Bhandary, S.K., Bhat, V.S., Sharmila, K.P. and Bekal, M.P., 2012. Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. *Journal of Health and Allied Sciences NU*, 2(04), pp.34-38.

Bhat, R.S. and Al-Daihan, S., 2014. Phytochemical constituents and antibacterial activity of some green leafy vegetables. *Asian Pacific journal of tropical biomedicine*, *4*(3), pp.189-193.

Cheng, D. M., Pogrebnyak, N., Kuhn, P., Poulev, A., Waterman, C., Rojas-Silva, P., Johnson, W. D., & Raskin, I. (2014). Polyphenol-rich rutgers scarlet lettuce improves glucose metabolism and liver lipid accumulation in diet-induced obese C57BL/6 mice. Nutrition, 30(7–8), S52–S58. https://doi.org/10.1016/j.nut.2014.02.022.

Dar, P., Ahmed, D., Waqas, U., Saeed, R. and Chaudhery, R., 2014. Comparative analysis of antimicrobial potential of peel and mesocarp of Lagenaria siceraria fruit extracts in various solvents against clinically important pathogens. *Online*, *3*, pp.100-105.

Doss, A., 2009. Preliminary phytochemical screening of some Indian medicinal plants. *Ancient science of life*, *29*(2), p.12.

Dyar, O.J., Huttner, B., Schouten, J. and Pulcini, C., 2017. What is antimicrobial stewardship. Clinical microbiology and infection, 23(11), pp.793-798.

Gajdács, M. and Albericio, F., 2019. Antibiotic resistance: from the bench to patients.

Hajare, A.G., Choudhary, M.D. and Gupta, N.S., 2013. Plant review: Phytochemical constituents and their important characterization of Butea monosperma (palash). *International Journal of Application or Innovation in Engineering & Management*, *5*, pp.1-4.

Jabeen, A., Huang, X. and Aamir, M., 2015. The challenges of water pollution, the threat to public health, flaws of water laws, and policies in Pakistan. *Journal of Water Resource and Protection*, *7*(17), p.1516.

Javid, T., Adnan, M., Tariq, A., Akhtar, B., Ullah, R., and Abd El-Salam, N.M., 2015. Antimicrobial activity of three medicinal plants (Artemisia indica, Medicago falcate, and Tecoma stans). African Journal of Traditional, Complementary, and Alternative Medicines, 12(3), pp.91-96. http://dx.doi.org/10.4314/ajtcam.v12i

ISSN 2828-1500 (Online) | 2828-1985(Print)

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis, 49, 19–34. https://doi.org/10.1016/j.jfca.2016.03.004.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis, 49, 19–34. https://doi.org/10.1016/j.jfca.2016.03.004.

Kleina, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H. and Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences*, *115*(15), pp.E3463-E3470.

Krishnapriya, T.V., and Suganthi, A., 2017. Biochemical and phytochemical analysis of Colocasia esculenta (L.) Schott tubers. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, *2*(3), pp.21-25.

Morgan, D.J., Malani, P. and Diekema, D.J., 2017. Diagnostic stewardship—leveraging the laboratory to improve antimicrobial use. *Jama*, *318*(7), pp.607-608.

Naveed, R., Hussain, I., Tawab, A., Tariq, M., Rahman, M., Hameed, S., Mahmood, M.S., Siddique, A.B. and Iqbal, M., 2013. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against Salmonella and other multi-drug resistant bacteria. *BMC complementary and alternative medicine*, *13*(1), pp.1-10.

Pepe, G., Sommella, E., Manfra, M., De Nisco, M., Tenore, G. C., Scopa, A., Sofo, A., Marzocco, S., Novellino, T., & Campiglia, P. (2015). Evaluation of anti-inflammatory activity and fast UHPLC–DAD–IT-TOF profiling of polyphenolic compounds extracted from green lettuce (Lactuca sativa L.; var. Maravilla de Verano). Food Chemistry, 167, 153–161. https://doi.org/10.1016/j.foodchem.2014.06.105.

Pelegrino, M.T., Kohatsu, M.Y., Seabra, A.B., Monteiro, L.R., Gomes, D.G., Oliveira, H.C., Rolim, W.R., de Jesus, T.A., Batista, B.L. and Lange, C.N., 2020. Effects of copper oxide nanoparticles on growth of lettuce (Lactuca sativa L.) seedlings and possible implications of nitric oxide in their antioxidative defense. *Environmental monitoring and assessment*, *192*(4), pp.1-14.

Ragheb, M.N., Thomason, M.K., Hsu, C., Nugent, P., Gage, J., Samadpour, A.N., Kariisa, A., Merrikh, C.N., Miller, S.I., Sherman, D.R. and Merrikh, H., 2019. Inhibiting the evolution of antibiotic resistance. *Molecular cell*, *73*(1), pp.157-165.

Roopan, S.M., Rajeswari, V.D., Kalpana, V.N. and Elango, G., 2016. Biotechnology and pharmacological evaluation of Indian vegetable crop Lagenaria siceraria: an overview. *Applied microbiology and biotechnology*, *100*(3), pp.1153-1162.

Shahidi F, Ambigaipalan P (2015) Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—a review. J Funct Foods 18(Part B):820–897

Santhi, K. and Sengottuvel, R., 2016. Qualitative and quantitative phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*, *5*(1), pp.633-640.

Shams, M., Ekinci, M., Turan, M., Dursun, A., Kul, R. and Yildirim, E., 2019. Growth, nutrient uptake and enzyme activity response of Lettuce (Lactuca sativa L.) to excess copper. *Environmental Sustainability*, *2*(1), pp.67-73.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Siddiqui, M.S., Memon, A.A., Memon, S. and Baloch, S.G., 2017. Cuscuta reflexa as a rich source of bioactive phenolic compounds. *Journal of Herbs, Spices & Medicinal Plants*, *23*(2), pp.157-168.

Sönmez I, Kalkan H, Demir H, Külcü R, Yaldiz O, Kaplan M (2017) Mineral composition and quality parameters of greenhouse grown lettuce (Lactuca sativa L.) depending on fertilization with agricultural waste composts. Acta Sci Polonorum Hortorum 16:85–95

Sood, A., Kaur, P. and Gupta, R., 2012. Phytochemical screening and antimicrobial assay of various seeds extract of Cucurbitaceae family.

Vineetha, N., Vignesh, R.A., and Sridhar, D., 2015. Preparation, standardization of antibiotic discs, and study of resistance pattern for first-line antibiotics in isolates from clinical samples—i*nternational journal of applied research*, *1*(11), pp.624-631.

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Formulation and Stability Determination of Anti-Acne Cream Containing Black Cumin Seed Oil and Kaolin Clay

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Abstract

Acne is the most common skin problem that could occur to any individual. *Nigella sativa* seed oil and kaolin as natural antimicrobial agents have been utilized in anti-acne cream formulated in this study. This study aimed at the development of anti-acne cream with antimicrobial properties using a crude extract of black cumin (Nigella sativa L.). Anti-acne creams had been formulated from cream-based agents with various percentages of crude black cumin seed extract and 1.0% (w/w) of mineral clay (Kaolin). Physical properties and stability of anti-acne cream at various storage conditions, including incubation at and freeze-thaw at 4, 40, and 45 °C for 28 days. The results showed that developed anti-acne cream containing a crude black cumin seed extract in 0.1 and 1.5% (w/w) had good physical stability. Therefore, the suitable formulation was then tested for anti-Propionibacterium acnes (*P. acnes*) susceptibility by the broth dilution method. From these results, it was found that 1.0 % (w/w) of crude black cumin seed extract had the ability to inhibit P.acnes with MIC (minimal inhibition concentration) of 15.6 mg/mL.

Keywords: *Nigella sativa, Kaolin clay, Acne, Propionibacterium acnes*

INTRODUCTION

Acne is the most common skin problem caused by chronic inflammation of a sebaceous follicle. It is usually observed in 80% of young adults and adolescents. The symptom may vary from mild to severe conditions. It can appear on the face, scalp, neck, chest, back, upper arms, and shoulders, where the sebaceous glands are widely present. This is one of the skin diseases that may affect not only the appearance but also result in discomfort and emotional stress. Many findings revealed the association of microbial infection with this disease. The common microbes found in pustular contents of acne are *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis,* and *Candida Albicans* (Achermann et al., 2014). *P. acnes* were found to be the main organism involved in acne development. It triggers the production of various inflammatory mediators and turns sebaceous triglycerides into fatty acids, which result in neutrophil recruitment. The removal and reduction of *P. acne* is the main strategy to overcome this skin condition (Varma et al., 2014). The general treatments recommended for acne vulgaris are topical application and oral medication. The topical cream containing antimicrobial agents has been applied. Both natural and synthetic sources of antimicrobial agents have been added to cosmetic

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formulations to inhibit the growth of bacteria as well as the inflammation caused by them. However, the microbes become resistant to the existing antibiotics most of the time (Nawarathne et al., 2019). Thus, the utilization of natural extracts as antimicrobial agents is one of the major concerns. In addition, the demands for a cosmetic product containing natural products are rapidly growing. Natural clay is of natural product which has been applied since prehistory for would healing, reducing skin irritation. It is reported to use for skin inflammation as well as preservation of Egyptian mummies (Robertson et al., 1982). *Nigella sativa* seed oil is also reported as a potential antimicrobial agent against a variety of pathogens (Ali et al., 2001; Najah et al., 2012). The presence of Thymoquinone and other phytochemical compounds in its oil is also attributed to its antioxidant and anti-inflammatory properties, which are found to be very beneficial when applied to the skin. The *Nigella sativa* seed oil will not only be capable of inhibiting the growth of *P. ance,* but it will also provide a gentle skin with the inflammation from acne scar is recovered. Therefore, kaolin clay and black cumin seed oil are incorporated in a cream formulation. The physical properties and stability of anti-acne cream at various storage conditions are performed. The antimicrobial property of anti-acne cream against *P.ance* is evaluated.

LITERATURE REVIEW

Acne vulgaris

Acne vulgaris is one of the skin conditions that occur when chronic inflammation of the sebaceous follicle takes place. The formation of comedones, papules, pustules, inflamed nodules, superficial pus, field cysts, and deep scaring is a common characteristic of this skin condition. These skin conditions can be occurred due to many factors, including the release of inflammatory mediators into the skin, comedones development, the alteration of sebum production, as well as follicular colonization of *P. acnes* (Achermann *et al*., 2014). *P. acnes* is a gram-positive, anaerobic, and lipophilic bacterium known as a commensal bacterium colonize and inhabitant of human skin with *Staphylococcus, Corynebacterium, Streptococcus, and Pseudomonas spp*. It sometimes becomes an opportunistic pathogen when the anaerobic and lipid-rich condition within the pilosebaceous unit is attractive for its growth, especially when hair follicles are blocked by some dead skin cells, oil, and other bacteria. *P. acnes* may activate TLR-2 on macrophages which result in the production of IL-12 and IL-8 and recruitment of neutrophil. *P. acnes* are reported to produce biofilm formation, which triggers the chronic skin condition of acne vulgaris (Achermann et al., 2014).

Nigella sativa

Nigella sativa Linn. is one of the aromatic plants belonging to the Ranunculaceae family. The plant can grow from 20 -25 cm tall. The color of flowers varies from white to pale blue with 5-10 petals. The bearing seeds are black with 2-3 mm long (Sudhir *et al*., 2016). Numerous phytochemical compounds were reported in many studies. The phytochemicals found in *Nigella sativa* are Thymoquinone, p-cymene, longifolene carvacrol, limonene, alpha-pinene,

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thymol, 4-terpineol, t-anethole benzene, alpha-thujene, alpha-hederin, nigellone, nigellimine, nigericin. These bioactive compounds claimed to display the properties of anticancer, antidiabetic, antimicrobial, anti-inflammatory, antioxidant and wound healing activities (Gharby et al., 2015; Harzallah et al., 2011; Kooti et al., 2016; Sudhir et al., 2016). The antimicrobial properties of black cumin have been well addressed in many studies. In vitro antimicrobial studies indicated the efficacy of Thymoquinone and *Nigella sativa* seed oil against various types of bacteria, which caused an oral infection known as dental caries (Harzallah et al., 2011). A clinical study by Rafati *et al.* (2014) revealed that the *Nigella sativa* seed extract is effective against *s. aurous,* which can be comparable to the standard drug, mupirocin, when treated to neonates that encounter staphylococcal skin infections (Rafati et al., 2014). Many studies also reported that Nigella sativa seed was found to be more effective to gram-positive bacteria than gram-negative bacteria (Ali et al., 2001; Najah et al., 2012). Interestingly, the topical gel formulation containing *Nigella sativa* seed extract indicated a good antimicrobial property against acne-causing bacterial species (Nawarathne et al., 2019). The antimicrobial property of *Nigella sativa* seed is attributed to the Thymoquinone present in the *Nigella sativa* seed.

RESEARCH METHODOLOGY

Plant material
Black cumin seeds were collected from the local market of a local supplier (Bangkok, Thailand).

Extraction of black cumin seed oil
The blended of powdered black cumin seed 25 gram with ethanol 250 mL, at 4 °C for overnight. The obtained mixtures were then filtered, and the solvent was evaporated under a vacuum. The obtained oil and extract were refrigerated (4 °C) until further analysis. The oil yield can be calculated by using the following formula.

$$\% \text{ yield of Black cumin seed oil} = \frac{\text{Weight after evaporation}}{\text{Weight before evaporation}} \times 100$$

Formulation of anti-acne cream using black cumin seed oil
Acne cream formulation was two formulations of O/W cream with different compositions that were prepared with non-comedogenic ingredients (Table 1). Black cumin seed oil extracts were incorporated in the cream base in variable quantities of 0.1-10% (i.e. 0.1, 0.2, and 0.5 %). The formula with the most suitable rheological and physical properties (color, odor, and creaming) was selected for in vitro testing.

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Table I Ingredients of the cream base

Ingredients	Amount
Cetyl Alcohol	0.1-5 %
Stearic acid (Enenorst)	0.1-5 %
PEG-150 Distearate	1-10 %
TSF 405	1-10 %
SF 1540	1-10 %
Finsolve TN	1-10 %
Glycerin (Colagate)	1-10 %
1,3Butylene Glycol	1-10 %
EDTA-Base	0.05-1 %
Propylene Gylcol	0.1-10 %
Kaolin	0.1-10 %
Bentonite	0.1-10 %
HydroxyethylAcrylate/SodiumAcryloyldimethyl Taurate Copolymer and Isohexadecane and Polysorbate 60	0.1-10 %
Allantoin	0.1-10 %
Salicylic acid	0.01-5 %
Aloe Vera Liquid	0.1-10 %
Black Cumin Extract	0.1-10 %
Phenoxyethanol and Methyl Paraben and Ethyl Paraben and Propyl Paraben and Butyl Paraben and Isobutyl Paraben	0.01-5 %
DI Water	to 100%

All the ingredients of phases A, B, C, D, and E were taken in separate beakers. Phase A and B were kept in a water bath till the temperature reached 75-80 °C, phase C was heated at 60 °C. Part A was mixed with a homogenizer at 5000-6000 rpm, then add Part B, Part C, respectively. After the temperature down to 45 °C, part D, E was added and mixed until all solution was homogeneous.

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Then the cream was allowed to cool down to room temperature and transferred to a suitable container.

Physical stability testing
The stability studies were carried out in all formulations at different temperature conditions (room temperature, heating-cooling cycle, and Cooling cycle) for 28 days. All the evaluation parameters, i.e., pH, viscosity, and phase separation studied at different time intervals of 4 weeks.

In vitro test of antimicrobial activity
Antibacterial/antifungal susceptibility was determined using the agar-well diffusing method according to the methodology established by the Faculty of Pharmacy, Mahidol University, Thailand. In this method, the activity of a compound is indicated by a clear zone around the 'cup,' and a hole is cut in the agar and filled with preparation under test. The zones of inhibition were measured. This method has the advantage of being reproducible and accurate comparisons between compounds can be made, and hence this method is selected for the present study. Anti-acne creams were evaluated for their antimicrobial activity against pure cultures of Pseudomonas aeruginosa (MTCC 1688), Staphylococcus aureus (MTCC737), Candida albicans (MTCC 227), and Propionibacterium acnes (MTCC 1951).

FINDING AND DISCUSSION

The stability studies were characterized in terms of viscosity and pH in different temperature conditions (room temperature, heating-cooling cycle, and cooling cycle) for 28 days. As shown in Fig. 1, the results showed that the viscosity stability of the formulation containing 1% black cumin extract was increased after the third week in all temperature conditions. In addition, the higher temperature resulted in higher viscosities. This may be caused by water molecules being repelled when high temperature, allowing intermolecular interaction of ingredient and polymeric bases in formulation (Abbasi et al., 2010; Sharif et al., 2017).

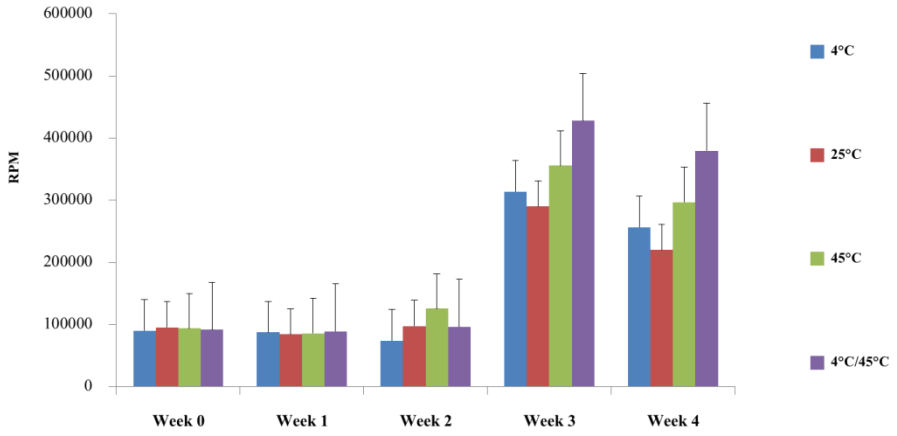

Figure 1. Viscosity of formulation in different temperatures during 4 weeks.

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Physical stability test results showed the level of acidity of the obtained formulation was stable at room temperatures and low temperatures during storage for 4 weeks, as shown in Fig. 2. However, the high temperature might induce the oxidation of active ingredients, leading to a decreased level of pH (Misar et al., 2020).

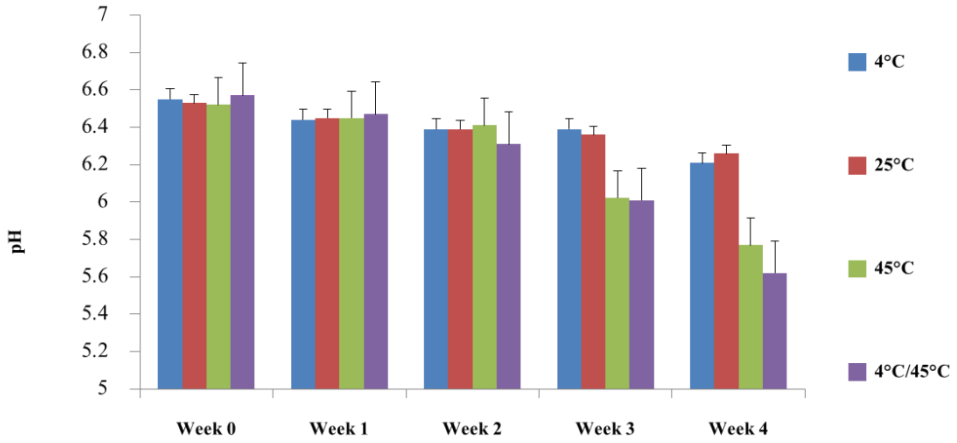

Figure 2. Stability test of pH in different temperatures from the first week to the fourth week

The results showed that 1% of Black cumin seed extract has minimal inhibition concentration of P.acnes at 15.6 µg/ml similarly as Ampicillin (positive control), while 1.5% of Black cumin seed extract has minimal inhibition concentration at 31.3 µg/ml, as shown in Figure 3(McGinley et al., 1980).

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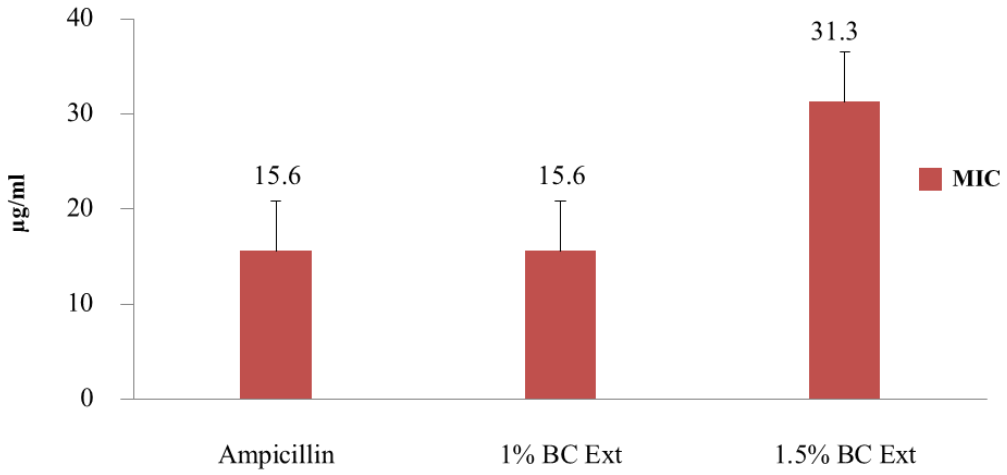

Figure 3. Comparison of different percentages of Black cumin Extract with Ampicillin (positive control) versus Minimal inhibition concentration of *P.acnes*

CONCLUSION AND FURTHER RESEARCH

From the experiments, 1% of the Black cumin extract formula has suitable physical stability; pH, viscosity, color, odor, emulsion dispersion instability compared with another formula (data not shown). This formula showed great minimal inhibition concentration similarity to Ampicillin as the positive control. The increasing amount of crude black cumin seed extract to 1.5 % (w/w) caused oily feeling and color instability which were clearly observed by naked eyes.

REFERENCES

Abbasi, M. A., Kausar, A., Aziz ur, R., Saleem, H., Jahangir, S. M., Siddiqui, S. Z., & Ahmad, V. U. (2010). Preparation of new formulations of anti-acne creams and their efficacy. *african journal of pharmacy and pharmacology, 4*(6), 298-303.

Chaieb, K., Kouidhi, B., Jrah, H., Mahdouani, K., & Bakhrouf, A. (2011). Antibacterial activity of Thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. *BMC Complementary and Alternative Medicine, 11*(1), 29.

Eady, E. A., & Ingham, E. (1994). Propionibacterium acnes - friend or foe? *Reviews in Medical Microbiology, 5*(3), 163-173.

Holland, K. T., Ingham, E., & Cunliffe, W. J. (1981). A review, the microbiology of acne. *J Appl Bacteriol, 51*(2), 195-215.

ISSN 2828-1500 (Online) | 2828-1985(Print)

McGinley, K. J., Webster, G. F., Ruggieri, M. R., & Leyden, J. J. (1980). Regional variations in density of cutaneous propionibacteria: correlation of Propionibacterium acnes populations with sebaceous secretion. *J Clin Microbiol, 12*(5), 672-675.
Misar, K. S., Kulkarni, S. B., & Gurnule, W. B. (2020). Formulation and evaluation of anti-acne cream by using Clove oil. *Materials Today: Proceedings, 29*, 1251-1258.
Robertson, A., Dodgson, J., Gormley, I. P., & Collings, P. (1982). An investigation of the adsorption of oxides of nitrogen on respirable mineral dusts and the effects on their cytotoxicity. *Ann Occup Hyg, 26*(1-4), 607-624.
Achermann Y, Goldstein EJ, Coenye T, Shirtliff ME, Shirtliffa ME: Propionibacterium acnes: From commensal to opportunistic biofilm-associated implant pathogen. *Clin Microbiol Rev* 2014;27:419-440.
Ali NA, Julich WD, Kusnick C and Lindequist U, Screening of Yemeni medicinal plants for antibacterial
and cytotoxic activities, J Ethnopharmacol, 2001, 74(2), 173-179.
Gharby, S., Harhar, H., Guillaume, D., Roudani, A., Boulbaroud, S., Ibrahimi, M., Charrouf, Z., 2015. Chemical investigation of Nigella sativa L. seed oil produced in Morocco. Journal of the Saudi Society of Agricultural Sciences 14(2): 172-177
Harzallah, H.J., Kouidhi, B., Flamini, G., Bakhrouf, A.,Mahjoub, T. 2011. Chemical composition, antimicrobial potential against cariogenic bacteria and cytotoxic activity of Tunisian Nigella sativa essential oil and Thymoquinone. Food Chemistry 129:1469-1474
Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N. Asadi-Samani, M., Ashtary-Larky, D. 2016. Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa) Chinese Journal of Natural Medicines 14(10): 0732-0745
Najah A. Mohammed, Effect of Nigella sativa L. extracts against Streptococcusmutans and Streptococcus mitis in Vitro, J Bagh College Dentistry Vol. 24(3), 2012 Effect of Nigella sativa Basic Sciences 154 -157.
Nakatsuji T, Kao MC, Fang JY, et al. Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 2009;129:2480-8

Nawarathne, N. W., Wijesekera, K., Wijayaratne, W. M. D. G. B., Napagoda, M. 2019. Development of Novel Topical Cosmeceutical Formulations from *Nigella sativa* L. with Antimicrobial Activity against Acne-Causing Microorganisms", *The Scientific World Journal*, vol. 2019
Rafati S, Niakan M, Naseri M. Anti-microbial effect of Nigella sativa seed extract against staphylococcal skin Infection. Med J Islam Repub Iran 2014 (8 June). Vol. 28:42

ISSN 2828-1500 (Online) | 2828-1985(Print)

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Feasibility Study of Slaughterhouses as A Source of Halal Meat Processed Meat-Based Food in Bandung City

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Abstract

The good food for consumption by a human is the food halal and tayyib. Halal is free from haram raw material, and tayyib is free from chemical or biological contamination, etc. Thayyib food, in other words as safe food based on food safety standards. Meanwhile, meat-based food, food safety standards start from the Slaughterhouse. The Slaughterhouse that registered as a large government slaughterhouse and a center for slaughtering livestock to be applied to the community is one of the benchmarks for standardizing meat safety. The purpose of this study was to determine the feasibility of slaughterhouses in the city of Bandung. The results of the study show that there are two slaughterhouses that are registered as government slaughterhouses and are a source of halal meat and have national standards. This study uses a type of field observation, research conducted in real life. The conclusion Government Slaughterhouses, namely Cirangrang and Ciroyom slaughterhouses, are clarified as proper slaughterhouses and have operating permits from the local government. The enumerators and slaughterhouse employees have received training and are regularly monitored by the local government. So that the Slaughterhouse can be assumed as a slaughterhouse that has appropriate standardization based on food safety. Slaughterhouse has stable sales and has consumers who become regular customers. Several meat brokers and traders in wholesale and traditional markets source their meat for sale from these abattoirs. So based on the results of observations, the source of food used by snack producers circulating in the city of Bandung should not be contaminated by pork and appropriate based on the source of the meat.

Keywords: *Halal food, food safety, meat-base food, Slaughterhouse*

INTRODUCTION

Consuming halal food for Muslim society is an obligation. To make it easier for people to choose halal food, to buy products that already have halal certification. Halal certification is issued by an agency appointed by the government to produce an audit process on food manufacturers to obtain a halal label certification. Based on Law no. 33 of 2014 and regulation of the Religion No. 26 of 2019 concerning the Implementation of Halal Product Guarantee (*Penyelanggaraan jaminan Produk Halal*) in Indonesia, it is necessary to have laboratory testing to prove the halalness of the product.

Halal food is food that is allowed to be eaten. There are 3 criteria for understanding halal food. 1) Halal based on the substance and its content, 2) halal based on how to get it, and 3) halal based on the processing. In addition, food that is included in the halal food category is *Tayyib* food. *Tayyib* means that the food is good and safe. Safe food is that does not contain chemical contamination, biological and other objects that can harm humans (Adawiyah et al., 2020). The food either for

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consumption or sale must comply with food safety standards. One form of food that has a high level of awareness regarding food safety is processed food derived from meat.

Processed foods made from meat can be in the form of sausages, meatballs, minced meat, corned beef, and nuggets (Septiani, 2019). One type of food that has a high level of vigilance or has a critical point regarding halal food is processed food that comes from meat. These meat-based snacks can be in the form of sausages, meatballs, minced meat, corned beef, and nuggets.

This study, there are two criteria to be analyzed, namely; Halal food is based on its substance and content, both halal foods are based on their processing. Analysis of halal food based on its substances and contents can be seen from the composition of the basic ingredients used. While the analysis of halal food based on its processing can be seen from the processing process starting from the slaughtering process in accordance with Islamic law. The slaughter process according to Islamic law can be reviewed starting from the Slaughterhouse. A slaughterhouse is a building or location where animals or livestock are slaughtered.

Slaughterhouse is one of the important things in meat hygiene to obtain safe, healthy, whole, and halal meat quality. In abattoirs, animals are slaughtered, and there is a change (conversion) from otor (live animals) to meat, and contamination of microorganisms can occur on the meat, especially at the evisceration stage (removal of offal). The handling of meat in slaughterhouses that are not good and not hygienic will have an impact on the halalness, quality, and safety of the meat produced. (Mahardika, 2020) reported that the Slaughterhouse located in the district of Ponorogo has been proven to be a halal slaughterhouse and in accordance with Islamic law. However, it was found that several abattoirs were not able to support the availability of beef in the market because they did not have the proper equipment, so that there needed to be an increase and investment of equipment for RPH from the government (Aqidawati & Sutopo, 2017). (Tawaf et al., 2013) reported that based on the physical condition of the Slaughterhouse owned by the government of West Java, there are 2 proper slaughterhouses, 3 inadequate slaughterhouses, 6 inadequate slaughterhouses, and very inappropriate slaughterhouses. Based on the results of some of these findings, it is necessary to conduct a feasibility study for special abattoirs in the city of Bandung. Nusran et al., (2019) reported the management of the supply chain process for meat products in Slaughterhouse by analytical hierarchy process (AHP) method, but it still did not apply to specific Slaughterhouse yet.

This study was conducted to determine the feasibility of slaughterhouses in the city of Bandung as a meat production unit in the city of Bandung and to determine the distribution of government-owned slaughterhouses in the city of Bandung. Based on that background, analyzing and identifying the feasibility at slaughterhouses as the first location that supports halal and safety in meat-based foods is urgent.

LITERATURE REVIEW

Definition of Halal

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Halal can be defined as something that is permitted under the rules. The word halal is derived from the phrase "*Halalan Toyyiban*" in Al-Quran. The term *Halalan Toyyiban* has been mentioned in the Quran 13 times (Jais, 2014). Halal is an Arabic word that literally means lawful and permitted (Laluddin et al., 2019).

For a Muslim, eating halal food is contrary to the lust of the devil, who wants humans to fall into haram. So avoiding the haram is an attempt to defeat the temptations of Satan. In Surah Al-Baqarah, Allah says: "O mankind, eat what is halal and good from what is on the earth, and do not follow the steps of the syaitan; Because verily the devil is a real enemy to you" (QS. Al-Baqarah: 168). Getting used to eating halal food and tayyib will bring not only to health but also to goodness. Basically, everything that exists on this earth is lawful except what is prohibited in the Qur'an and Hadith.

Halalan Toyyiban, or the concept of halal, clean and good, is a principle that is emphasized in Islamic teachings. Islamic teachings include the whole of life that spreads the balance between the life of this world and the hereafter—the concept of food in Islam as a tool for worship and acceptance from Allah in total. A Muslim is ordered to consume only halal, clean, good, and sufficient food. In consuming food, it should not be excessive, but the most important thing is that it must be halal. One of the other concepts in Halalan Toyyiban is that the food and the source of it must be halal. This is in accordance with the objectives of Sharia which tries to protect religion, mind, property, and descendants (Jais, 2014) .

One of the determinants of halal food is the substance contained in the food. To determine the halalness of a product, it can be done by detecting pork DNA in several types of food (Widayat et al., 2019). Currently, many development methods have been reported for the detection of pork DNA as one of the activities for halal certification and identification of efforts to prevent the spread of haram food (Cai et al., 2017);(Septiani, 2019);(Cahyanto et al., 2020);(Safitri & Wardani, 2015);(Fibriana et al., 2012);(Rohman et al., 2020).

Halal supply chain

Halal food can be grouped based on three criteria. The first criterion is halal food based on the substance and content of the food; the second criterion is based on how to obtain it, and the third criterion is based on the processing process.

1. Halal food is based on the substance and content of food and drink

Halal food and drinks, according to their substance, are foods that have a halal status for consumption. Halal food has been determined halal in the holy book Al-Quran and al-Hadith. Example: beef, chicken, goat, fruits such as apples, kurma, etc.

2. Halal food based on how to get it

Apart from the food that has been forbidden, all foodstuffs and drinks found on earth are allowed to be consumed. Food will be halal if it is obtained in a halal way. On the other hand, if food is obtained in an unlawful way, then the food will become haram.

3. Halal food based on its processing

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Any food that is halal in its content can turn into haram if the management process is not carried out according to Islamic law. For example, beef that does not go through a slaughter process according to Islamic law.

Halal assurance system in Indonesia

The system called Halal Assurance System (SJH) (Departemen Agama, 2003) consist of several components:

1. Halal management standards and halal system
2. Haram analysis critical control point (HRACCP)
3. Halal database

RESEARCH METHODOLOGY

Research Method

This study uses a type of field observation, research conducted in the real life. Field observation is a method to find out specifically and realistically about what is happening at one time in people's lives. The research was carried out in several stages: among others, taking and field observations as well as taking samples from several food products, this activity was carried out in the field, visits and interviews at slaughterhouses in the city of Bandung.

Research Location

The research location is an abattoir that has been officially registered as a government slaughterhouse (RPHU) in the city of Bandung-Indonesia.

Data dan It sources

The data used are facts that can be drawn into a conclusion within the framework of the problem being worked on. The data needed in this study are:

Profile data of Slaughterhouse that have been registered as Public Slaughterhouse in Bandung City
Distribution data from Public Slaughterhouse to meat sellers and producers

Sources of data used are primary and secondary data. Primary data are the results of interviews with RPHU employees, while secondary data are books and literature related to this problem.

Data Collection and Data Analysis Techniques

the type and sources of data obtained from:

1. Primary data, obtained by conducting direct observation and interviews with the employees of Slaughterhouse
2. Secondary data, obtained from several pieces of literature and related to the problems in this study

The primary data is a questionnaire from a number of slaughterhouses in Bandung, namely government slaughterhouses.

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Qualitative research uses inductive analysis, that is, starting from empirical facts. Research findings in the field are then shaped into theories. Laws developed from the field (Sugiyono, 2013 in RPH thesis).

FINDING AND DISCUSSION

Halal Food

Halal food is food that is free from anything or haram substances. The majority of Indonesian people embrace Islam, so it is appropriate to provide the needs for food ingredients that are produced and consumed, guaranteed to be halal in raw material, and legal. Indonesian people are used to consuming beef which is one of the most preferred types of cooking ingredients because it contains a lot of iron, protein, and nutritional needs that are needed by our bodies. Meatballs or meat-based foods are some of the favorite foods of the Indonesian people. In addition to meatballs, sempolan, sausages, or other types of meatballs are the most popular choices for consumption by all people in all walks of life. However, the higher competition in the business of selling meat-based foods is a challenge for producers of meat-based processed foods. To cut production costs, a mixture of meat other than beef is an option to lower production costs. However, if mixed meat is haram and prohibited, then the Muslim community is prohibited from consuming it.

In the Qur'an Surah Al-an'am: 118, which means: Than eat (halal) animals that are called by Allah's name when slaughtering them, if you believe in His verses. Based on the verse of the Qur'an, food can be said to be halal if it is not mixed with haram food. When the Halal products have been mixed with haram products, then the quality is changed to become haram. And also, when the Products that clearly use basic halal ingredients can also become haram if one of the packaging processes or the preparation process for these basic ingredients is not in accordance with quality standards. In meat-based foods, one process that needs to be considered is the source of the meat. Beef or other livestock meat can be haram if: 1) the slaughtering process is not in accordance with the shari'ah; 2) the condition of the location of the facilities and infrastructure for slaughtering animals does not meet the requirements. Currently, the government in order to implement a program under the name "ASUH" (*in Indonesian terms, Aman, sehat, utuh dan halal*) Safe, Healthy, Whole and Halal are one of the priority programs in the context of organizing a healthy and halal slaughterhouse for the wider community to consume. Halal slaughter process and cattle slaughtering techniques at RPH are thought to affect the evaluation of livestock productivity in order to obtain products of "*ASUH*" from Slaughterhouse.

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Standard Method of Slaughter

Animals must be treated properly to avoid pressure before they are slaughtered, do not harm animals, do not cause permanent damage to the body/organs, and are examined by supervisors. The stunning method must be regularly validated and verified by LPPOM MUI. Mechanical cutting carried out in a factory or abattoir must follow a number of Islamic rules, such as stating *Bismillahi Allahu Akbar* or *Bismillahi Rahmanirr Rahim* (Mahardika, 2020).

Indonesian Council of Ulama (MUI) on their Fatwa about Standard Halal Shipping Certification, which has several legal bases based on the Qur'an, hadith, opinions of scholars, and meeting decisions they mention:

- Animals to be slaughtered are sunnah for facing the Qibla
- Slaughter is carried out with the intention of slaughtering and mentioning the name of Allah
- Slaughter is done by draining the blood through cutting the food duct (esophagus), respiratory tract/throat (trachea), and two blood vessels (jugular vein and arterial) carotids
- Slaughter is done once and separately fast
- Ensuring blood flow and/or animal movement as a sign of animal life (hayah mustaqirrah)
- Slaughter is carried out as much as possible manually, without being preceded by stunning, etc
- Stunning to simplify the process, it is legal to slaughter animals.

Based on observation and interviews with the employee of Slaughterhouse, the standardization of these activities is usually carried out.

In this first phase of research, surveys and direct observations were carried out to the Bandung City Food Security Service as the regulator of Slaughterhouse licensing (RPH) in Bandung City. The Bandung city government has two abattoirs in the Ciroyom and Cirangrang areas, Kopo. There are three abattoirs in Ciroyom, which consist of abattoirs for cattle 1, cattle 2, and pigs. In the city of Bandung, there is a private Slaughterhouse in the Regol area, but the operating permit has expired. In the city of Bandung, there are only two chicken slaughterhouses that already have operating permits from the Bandung City Food and Agriculture Security Agency, namely Sentra Proteina Prima in Holis and Jaya Giri on Jalan Cipaganti. However, Slaughterhouse Jaya Giri is no longer operating due to declining sales and an unsupportive location. The operating license that has been granted by Bandung City Food and Agriculture Security Agency can be proven by NKV (Veterinary Control Number). So, the Slaughterhouse located throughout the city of Bandung does not have a permit from DISPANGTAN except for the RPH Sentra Proteina Prima and Jaya Giri. The obstacles experienced were caused by the administrative requirements

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that were not met in the operation of the Slaughterhouse, namely in terms of location and sanitation. Therefore, the government often provides Halal-certified training for slaughterers (chicken). In this training, participants are guided from preparation for slaughtering animals to processing the resulting waste.

Criteria for Slaughterhouse

Slaughterhouse is a building or complex of buildings with certain designs and conditions used as a place for slaughtering animals for general public consumption (Ministerial regulation of Indonesia Republic No. 13/*Permentan*/OT. 140/I/2010). Another definition of abattoir is a building complex with special design and construction that meets certain technical and hygienic requirements and is used as a place for slaughtering animals other than poultry for public consumption (SNI 01-6159-1999). According to Manual Kesmavet (1993:74) Slaughterhouse is a building or building complex with a certain design that is used as a place for slaughtering animals other than poultry for public consumption.

Tabel 1. The Slaughterhouse in Bandung City

NO	**NAME**	**ADDRESS**	**Number of slaughtering per day**	**Number of Slaughter in 1 month (head)**
1.	RPH-R Ciroyom	Jl. Arjuna No.45	± 25	± 300 head
2.	RPHR Cirangrang	Jl.. Cirangrang No. 487 KM.6	± 13	± 400 head

The city of Bandung has two slaughterhouses that have been certified and monitored by the government and owned by the government (Table 1). The two of slaughterhouses is located on Jl. Arjuna No.45 and Jl. Cirangrang No. 487 KM.6 Bandung City. Both of these slaughterhouses are special slaughterhouses for cattle, with the number of slaughtering in 1 day can reach 13-25 heads/day.

Slaughterhouse procedures and standardization

A slaughterhouse is a large slaughterhouse. According to Ministerial Regulation No.13/Permentan/Ot.140/1/2010, Slaughterhouse is a building or complex of buildings with certain designs and conditions used as a place for slaughtering animals for consumption by the general public. Slaughterhouse is a community service unit that provides safe, healthy, whole, and halal meat (*ASUH*) and functions as a means to carry out: the proper slaughter of animals (in accordance with the requirements of the veterinary public health, animal welfare, and religious Sharia).

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Animal health check before slaughter (ante-mortem inspection), an inspection of the carcass, and offal (post-mortem inspection) to prevent transmission of zoonotic diseases to humans. And monitoring and surveillance of animal and zoonotic diseases found during antemortem and post-mortem inspections for the prevention, control, and eradication of infectious and zoonotic animal diseases in areas of animal origin. According to Ministerial regulation No.13/Permentan/Ot.140/1/2010, location requirements, supporting facilities, layout, design, and construction of slaughterhouse buildings and equipment have been regulated and become general requirements for slaughterhouses in carrying out their activities.

Referring to the results of the abattoir survey based on Indonesian national standards, it can be said that the two slaughterhouses in the city of Bandung are worthy as sources and distributors of meat for public consumption. (Table 2.a.b).

Table 2.a. The identity of the Central Slaughterhouse in Cirangrang

I.	**The identity of the Central Slaughterhouse**	
1	Districts	Babakan Ciparay
2	Village	Cirangrang
3	Name	RPHR Cirangrang
4	Address	Jl. Kopo Cirangrang No.487 KM.6
5	Respondent Number	1
6	Officer Name/Keumaster	Pak Moch. Taufik; Pak Yayan
7	Phone number	-
8	Status	Government slaughterhouse
9	Cattle Carcass Waste Facility	Electric Scales
II.	**Butcher Identity**	
1	Name	Asep
2	Religion	Islam

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Table 2.b. The identity of the Central Slaughterhouse in Ciroyom

I.	**The identity of the Central Slaughterhouse**	
1	Districts	Cicendo
2	Village	Husen Sastranegara
3	Name	RPH-R Ciroyom
4	Address	Jl. Arjuna no. 45
5	Respondent Number	2
6	Officer Name/Keumaster	drh. Setiati Gita P (*veterinarian*)
7	Phone number	-
8	Status	Government slaughterhouse
9	Fasilitas Timbahan Karkas Ternak	Dutch era scales; Cap electric scales. 1 ton
II.	**Butcher Identity**	
1	Name	Kasmin
2	Religion	Islam

The evaluation of the feasibility of the Slaughterhouse is based on the Indonesian national standard, the technical standard of the Slaughterhouse and Indonesian national standard for the quality of carcass and beef.

Meat distribution Slaughterhouse

The quality of beef in traditional markets is affected by the slaughtering process at the Slaughterhouse and handling methods during distribution from abattoir to market. As a means of community service (public service) in the provision of meat that is safe, healthy, whole, and halal (*ASUH*) (table 3).

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Table 3. Merchant Distribution
TRADERS DISTRIBUTION REPORT
The Slaughterhouse of CIROYOM

NO	SHIPPING HOURS	AMOUNT OF PURCHASE	TYPE OF LIVESTOCK	DISTRIBUTION OF TRADERS/PORTERS
1	15.00-finished	± 20 head	Cow	Pasar ciroyom
				Pasar baru
				Pasar caringin
				Cimahi
				Padalarang
				Gede bage
				Pasar astana anyar
2	14.00-finished	± 13 head	Cow	Pasar caringin
				Pasar ciroyom
				Kab. Bandung
				Pasar gede bage

Based on (table 3), Ciroyom slaughterhouse is sufficient to become a distributor center for traditional markets in Bandung City. Communities and street vendors Mostly shop at traditional markets for daily necessities and food production. For this reason, it can be stated that meat-based processed foods using meat from these markets are considered safe for consumption by the public and halal for trading as long as there is no fraud from the traders themselves.

Through this research, it is hoped that it can provide information about the importance of knowing the source of the basic ingredients used by producers as well as quick and accurate solutions in sorting halal food, as well as finding patent methods that will be used routinely in the identification and quantification of pork in meat-based processed food products.

CONCLUSION AND FURTHER RESEARCH

1. Government Slaughterhouses, namely Cirangrang and Ciroyom RPHs, are clarified as proper RPHs and have operating permits from the local government. The enumerators and RPH employees have received training and are regularly monitored by the local government so that the RPH can be assumed as an RPH that has appropriate standardization based on food safety.
2. Slaughterhouse has stable sales and has consumers who become regular customers. Several meat brokers and traders in wholesale and traditional markets source their meat for sale from these abattoirs. So based on the results of observations, the source of food

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used by snack producers circulating in the city of Bandung should not be contaminated by pork and appropriate based on the source of the meat.

This research was carried out in several stages as described in the Methods section, in the first year conducting surveys and field observations and taking samples from several food products, this activity was carried out in the field, visits to the Bandung City Food Security Service and a number of registered slaughterhouses at the government. For the next year, detection system development for halal detection contamination polymerase chain reaction based method will be implemented.

REFERENCES

Adawiyah, A., Nurjanah, D., Suryani, Y., Cahyanto, T., Kurniawan, I. D., Kulsum, Y., Shofwaturrohmani, F., & Darniwa, A. V. (2020). Analisis Kandungan Zat Kimia Berbahaya dan Bakteri Patogen pada Jajanan Berbahan Olahan Daging di Sekolah Dasar Negeri Kota Bandung. *Indonesian Journal of Halal Science*, *1*(2), 45–53.

Aqidawati, E. F., & Sutopo, W. (2017). Kajian Tekno Ekonomi Perbaikan Rumah Potong Hewan untuk Mendukung Penyediaan Daging Sapi di Pasar Tradisional yang Aman, Sehat, Utuh dan Halal: Studi Kasus. *Prosiding Seminar Dan Konferensi Nasional IDEC, Surakarta*, 8–9.

Cahyanto, T., Suryani, Y., Adawiyah, A., Kulsum, Y., & Kurniawan, I. (2020). *Detection of Pork Contamination on Meat-Based Foods at Public Elementary School In Bandung*. *January*, 1–8. https://doi.org/10.4108/eai.1-10-2019.2291685

Cai, Y., He, Y., Lv, R., Chen, H., Wang, Q., & Pan, L. (2017). Detection and quantification of beef and pork materials in meat products by duplex droplet digital PCR. *PLoS One*, *12*(8), e0181949.

Departemen Agama, R. I. (2003). *Pedoman Produksi Halal*. Jakarta: Proyek Pembinaan Pangan Halal Ditjend Bimas Islam dan ….

Fibriana, F., Widianti, T., & Retnoningsih, A. (2012). Deteksi Daging Babi Pada Produk Bakso di Pusat Kota Salatiga Menggunakan Teknik Polymerase Chain Reaction. Biosaintifika: Journal of Biology & Biology Education, 4(2).

Jais, A. S. (2014). Halal in Mainstream Education: Where are We Now and What The Future Holds for Halal Education. International Seminar On Global Education II, February 2014, 1179–1191. https://doi.org/10.13140/RG.2.1.4788.5041

Laluddin, H., Haneef, S. S. S., Saad, N. M., & Khalid, H. (2019). The Scope, Opportunities and Challenges of Halal Industry: Some Reflections. International Journal of Economics, Management and Accounting, 27(2), 397–421.

Mahardika, D. W. I. (2020). Analisis Fatwa MUI No. 12 Tahun 2009 Tentang Standart Sertifikasi Halal Terhadap Pemotongan Sapi di RPH Rita Jaya Beef Desa Pijeran Kecamatan Siman Kabupten Ponorogo. IAIN Ponorogo.

Nusran, M., Alam, R., Triana, D., Parakkasi, I., & Abdullah, T. (2019). Management of Supply Chain Process for Meat Products. Indonesian Journal of Halal Research (IJHAR), 1(1), 18–25.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Rohman, A., Pebriyanti, N. W., Sismindari, Windarsih, A., Ramadhani, D., Larasati, R., & Yulisa, H. (2020). Real-time polymerase chain reaction for identification of dog meat in adulterated beef meatball using specific primer targeting on cytochrome-b for halal authentication. *International Journal of Food Properties*, *23*(1), 2231–2241. https://doi.org/10.1080/10942912.2020.1844748

Safitri, K., & Wardani, A. (2015). Deteksi Cemaran Babi pada Produk Sosis Sapi di Kota Malang dengan Metode Polymerase Chain Reaction. *J Pangan Dan Agroindustri*, *3*, 1006–1014.

Septiani, T. (2019). Detection of Porcine DNA in Processed Beef Products Using Real Time–Polymerase Chain Reaction. *Indonesian Journal of Halal Research*, *1*(2), 31–34.

Tawaf, R., Rachmawan, O., & Daud, A. R. (n.d.). *Kelayakan Fisik dan Teknis Prosedure Pemotongan di RPH Pemerintah Jawa Barat*.

Widayat, W., Winarni Agustini, T., Suzery, M., Ni'matullah Al-Baarri, A., & Rahmi Putri, S. (2019). Real Time-Polymerase Chain Reaction (RT-PCR) sebagai Alat Deteksi DNA Babi dalam Beberapa Produk Non-Pangan. In Indonesia Journal of Halal (Vol. 2, Issue 1, p. 26). https://doi.org/10.14710/halal.v2i1.5361

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Effect of Amino Acids and Taste Components on Fermented Fish Sauce (Budu) from Thailand

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Abstract

Budu is one of the most popular fermented fish products in Thailand's southern area due to its distinctive flavor. It is being manufactured in large quantities for usage in cuisine as seasonings and sauces. The objective of this study was to determine the effect of amino acids on the distinctive taste components of Budu in southern Thailand. The amino acids in Budu were determined using GC-MS after fish was fermented for 6–12 months as recommended by the manufacturer. Lysine, glutamic acid, and aspartic acid are the three most abundant amino acids, with 1600, 1,540, and 1,260 mg/100g, respectively. Additionally, it was revealed that the umami taste was formed by a group of amino acids (glutamic acid and aspartic acid) followed by sweetness and bitterness. Sensory analysis discovered salty tastes, followed by umami, sour, sweet, and bitter. Four Budu samples generate a salty and umami flavor. Salt is mixed with cleaned fresh fish and fermented to enable native enzymes to auto-digest the protein and produce amino acid-rich products. Fish enzymatic fermentation produces short chain peptides and amino acids that contribute to the umami flavor and taste. Additionally, the fermentation process creates a high glutamic acid concentration, as well as other amino acids and nucleotides that add to the umami flavor of the products. The study findings will be information that is particularly benefit to consumer and manufacturers to promote Budu products in the country's region.

Keywords: *Budu, Taste, Amino acid, Southern, Thailand*

INTRODUCTION

In Thailand's southern border cuisine, Budu is a fish sauce that is often used as a flavoring component and has a high salt content due to the fermentation process (Sukjuntra, 2018). The production of Budu contains small saltwater fish, mainly anchovies, which are combined with salt at 25–30% (w/w) and then spontaneously fermented under anaerobic conditions for 6–12 months. This food has a salty and unique flavor and is brown or dark brown in color (Mohamed et al., 2012). The manufacturing procedures utilized to create these items vary by nation, since each culture has its own traditional processes. (Mohamed & Mustafa, 2021). As a result of the fermenting process, Budu develops a distinct flavor. Hydrolysis of fish proteins is initiated by enzymes found in fish and

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certain halotolerant and halophile bacteria, which subsequently create amino acids, peptides, and ammonia, making it an excellent source of protein (Lee et al., 2016; Wu et al., 2017). Moreover, the high salt content of Budu sauces is able to inhibit the spread of harmful microorganisms, resulting in their signature salty umami and aroma (Mohamed et al., 2012). Free fatty acids are released during the process, which is called lipolysis, and play a critical role in determining taste qualities (Chen et al., 2017). In Thailand, the fermentation of Budu was considered an indigenous method because it required minimal practical guidance on a small industrial scale, but it required a lengthy fermentation time to guarantee that the fish combination was completely solubilized. Furthermore, no control measures are used during this spontaneous process, which is often linked with inconsistent product quality and poor production yield. Moreover, the unique taste of Budu depends on the type of fish, the ratio of fish to salt, including fermentation time and temperature, as well as specific techniques in each production site (Sukjuntra, 2018; Tsai et al., 2006). Relationships between the amino acid composition and taste analysis for determining basic flavors in each location of the Budu process are important for the quality improvement of Budu production. Therefore, the aim of this study is to identify the amino acid compounds that contribute to the special tastes in four locations of the Budu factory by using gas chromatography-mass spectrometry (GC-MS). These findings may be used to make predictions on the taste that will increase the quality of Budu in Thailand.

LITERATURE REVIEW

Salting is an easy and economical technique of food preservation that avoids spoiling and allows for fermentation of food products. In Southeast Asia, the process of salting fish is often employed to create fermented fish seasonings. Among a variety of fermented products, fish sauces are the most popular seasonings containing umami components and are normally required in this region's cuisine. Nuoc mam in Vietnam, Tuk trey in Cambodia, Patis in the Philippines, Bakasang in Indonesia, Ngan-pya-ye in Burma, Budu in Malaysia and Nam pla in Thailand are some of the names for the sauce used in the region (Yoshida, 1998). In Thailand, Nam pla is the fish sauce and is used in practically all Thai cuisine. Pla ra and Budu is a traditional fermented fish food common in northeastern and southern regions, respectively (Saisithi et al., 1966). Budu is a colloidal substance that stands between fish sauce and fermented fish paste, while prala is fermented fish paste that contains rice bran as a carbohydrate source. The main ingredients of Nam pla and Budu are the same as seawater fish (*Stolephorus* sp.), whereas Pla ra is freshwater fish (*Trichogaster* sp*., Crossocheilus* sp.). The traditional process of producing fish sauce varies by location. The ratio of salt to fish, the fermentation temperature, the kind of fish, and minor components all have a significant impact on the composition and nutritional quality of fish sauce (Lopetcharat et al., 2001). In fermentation stage, hydrolysis breaks down fish proteins into peptides and amino acids. Small peptides, amino acids, ammonia, and trimethylamine contribute to fish sauce's unique scent and taste (Dougan & Howard, 1975). Budu is a high-protein food that provides a variety of necessary amino acids (Mohamed et al., 2012).

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RESEARCH METHODOLOGY

Sample collection

Four Budu samples were purchased and collected from prevalent sites in three southern provinces, namely Yala, Pattani, and Narathiwat. All samples were kept in a chiller (6 °C) until they were needed for analysis. Information about the production process was collected by interviewing the manufacturer.

Sample preparation

Budu puree (cloudy) and Budu sauce (clear) were mixed thoroughly and 200 ml of methanol was added. Mixed samples were stirred by a magnetic stirrer for 45 min and then centrifuged at 8,000 rpm for 15 min (Refrigerated Centrifuge 5920R, Eppendorf, Germany). The supernatant was filtered, and the sediment was re-extracted until the color of the Budu water became lighter. Extracted samples were then filtered to make it concentrated by using rotary evaporator (Rota vapor R-3, Buchi, Flawil, Switzerland). The extracts were weighed and freeze dried (LABCONCO/FreeZone 2.5, Missouri, U.S.A.) to give the extracts of Budu powder.

Analysis of amino acid

From Budu powder samples (0.4 g) obtained from freeze drying, 2 g of liquid sample were put into a 100 ml extraction bottle. Then, the sample was added to 10 ml of 6 M HCl and mixed, and the bottle was tightly closed (for Typtophan, add 10 mL of 4.2M NaOH). The samples in the bottle were autoclaved at 121–123 ^{O}C for 3 hrs and then diluted with 2M NaOH in a 20 ml volumetric flask. The sample was passed through filter paper no. 42 by a syringe filter with a 0.45 µm and performed derivatization with BSTFA with 1%TMCS. The sample was performed on a GC/MS (7000D Triple Quad, Agilent, Santa Clara, CA, USA) using helium as the carrier gas. The column used was a HP-5MS 5% phenyl, 95% dimethyl-polysiloxane (30 m, 0.25 mm, 0.25 m). The injector with a split ratio of 3:1, a split flow of 3 ml/min, and a 2 µl. The initial temperature for the column temperature program was set at 15 ºC/min from 100 ºC to 300 ºC, and the MS Temp was set at 230 °C (Source) and 150 °C.

Sensory analysis

The panelists were trained and followed the process (Meyer et al., 2016; Scharbert & Hofmann, 2005). There are four males and eight females, aged 25–40 years, who have been trained as tasters to be proficient in taste. The scoring test is one method for training gourmets. The sensory test allows the tester to taste the freeze-dried Budu sample by dissolving the sample in water (Sprinkle, Thailand). The panelists were asked to rate the taste profile on a five-point scale for sweetness, saltiness, sourness, bitterness, umami. Four Budu samples (A, B, C and D) were presented to each panelist in random order.

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FINDINGS AND DISCUSSION

Production survey

The Budu production process was found in the households and small industries of southern Thailand (Jariya Sukjuntra, 2009). The research team selected and collected samples from four different sources (3 districts of 2 provinces): Narathiwat Province (sample A), Pattani Province (sample B, C and D) as shown in Fig. 1. Budu is made from anchovies, which may be combined with tiny quantities of roundbelly sardine. The fish were washed, marinated with salt in different ratios (fish and salt; 3:1 and 4:1) and fermented in a closed container for a fermentation period of 6–12 months. After Budu fermentation is complete, it is separated and packaged into three kinds of products: Budu sauce (clear sauce), Budu puree (cloudy sauce), and mixed Budu (clear-cloudy; 50:50). This product is turbid because to the presence of heavy sediments and appears darker than Nam pla (Chotechuang, 2013). The production process was shown in Fig.2. Numerous ingredients contribute to the particular taste of Budu, the bulk of which are small fish based on anchovies. Other species, such as roundbelly sardines, are sometimes fermented concurrently (Beddows, 1998). Other factors affecting production are the ratio of fish to salt. with a ratio of 3:1 (B and C) and 4:1 (A and D) while the containers used for fermentation are both glazed water jars and a large plastic bucket. Table 1 contains information about Budu's four manufacturing locations and manufacturing processes in Southern Thailand. Due to the cultural and dietary differences amongst Thai consumers, Budu processing is significantly distinct from Nam pla preparation. Nam pla manufacture begins with washing of fresh fish to eliminate contaminants and limit of microorganisms in the raw materials (Setyahadi, 2014). Generally, cleaned fish is combined with salt in a 2:1 or 3:1 ratio (w/w). The salt-mixed fish is then transferred to a fermentation tank covered with a bamboo mat for fermentation period 12-18 months (Lopetcharat et al., 2001). Nam pla has a distinct rate of fermentation and end product synthesis than Budu (Saisithi et al., 1966).

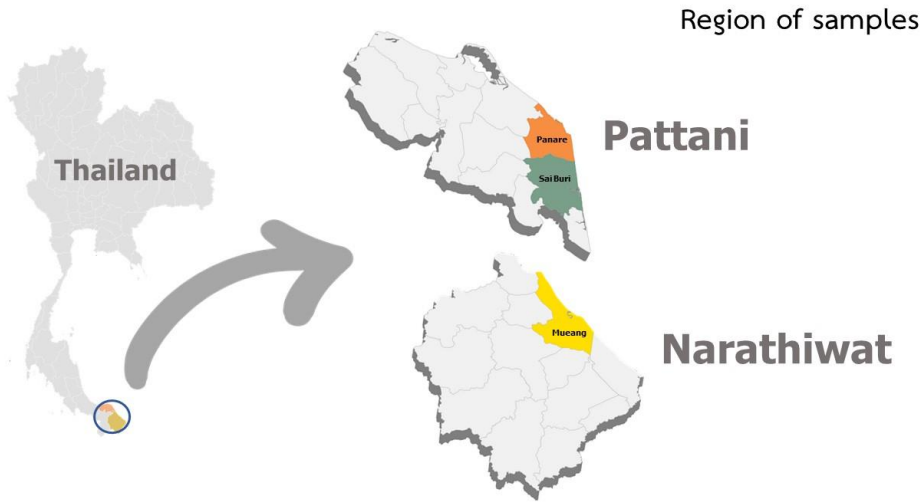

Fig. 1 A survey of data sources and collecting Budu samples in 4 different sources (3 districts of 2 provinces)

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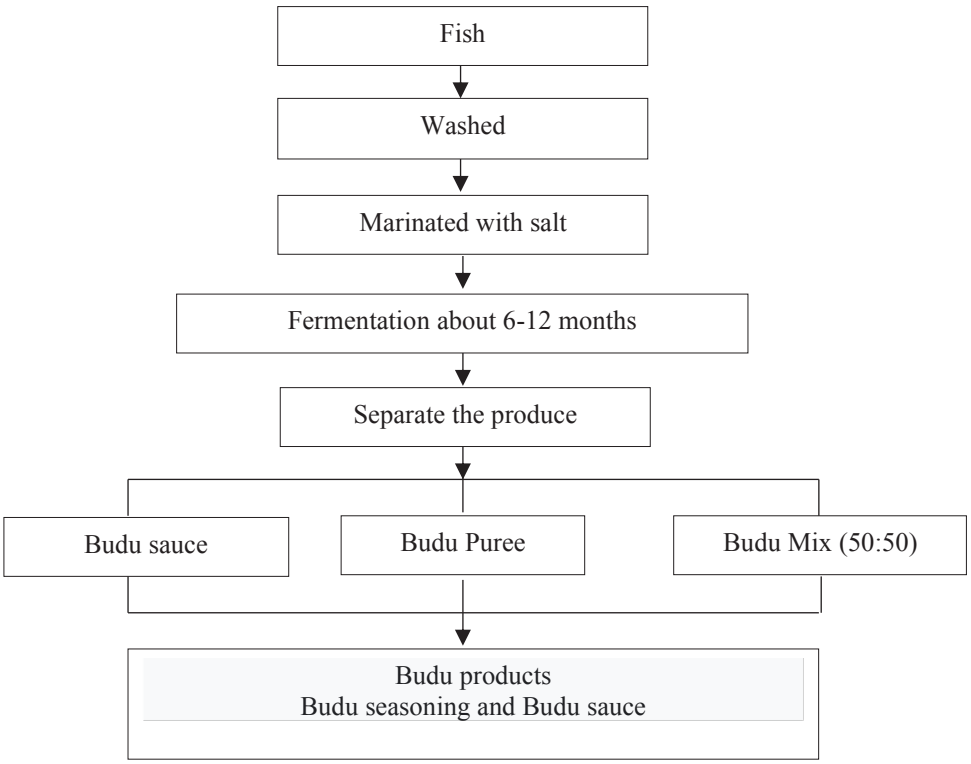

Fig. 2 Budu production process from the southern border provinces of Thailand

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Table 1 The information of 4 production sites and production processes of Budu in Southern Thailand

Brand code	A	B	C	D
District	Muang	Saiburi	Panare	Saiburi
Province	Narathiwat Province	Pattani Province	Pattani Province	Pattani Province
Type of establishment	SMEs	Factory	SMEs	Factory
Kind of fish	Anchovies/ Roundbelly sardine	Anchovies/ Roundbelly sardine	Anchovies/ Roundbelly sardine	Anchovies/ Roundbelly sardine
Kind of salt	Table salt (Pattani)	Table salt (Bangkok)	Table salt (Phetchaburi)	Table salt (Phetchaburi)
Ratio (Fish: Salt)	4:1	3:1	3:1	4:1
Fermentation duration	6 months	12 months	12 months	12 months
Fermentation container	Glazed water jar with dragon patterns (1 meter deep)	Large plastic bucket (500 liters)	Glazed water jar with dragon patterns (1 meter deep)	Glazed water jar with dragon patterns (1 meter deep) or Cement Pond (3 meters deep)

Relationship between amino acid and taste

The results of quantitative testing of amino acids in four Budu samples were found to indicate that the samples with the highest amino acid content were sample B (12,796.9 mg/100g) in Saiburi district, Pattani province. Lysine, glutamic acid, and aspartic acid are the three most abundant amino acids in Budu, with levels of 1,600, 1,540, and 1,260 mg/100g, respectively (Table 2). They account for about 35% of the amino acid content. These findings supported the amino acid profiles of fish sauce previously published (Park et al., 2001). Typically, Budu is used in cuisine as a seasoning. Importantly, Budu is strong in lysine and contains all necessary amino acids. By means of grouping the flavored amino acids and calculating the values of Dose-over-Threshold (DoT), it was found that the group of amino acids that give umami flavor is L-glutamic acid in the range of 77.25–95.17. The most common sweet and bitter amino acids are L-alanine and L-leucine (range of 6.15-7.66 and 4.29-6.36, respectively) (Table 3). Similar to Nam pla, glutamic acid is the most prevalent amino acid in Budu. These findings imply that the flavor active components, like in Nam pla, are related to the umami taste compound. In Southeast Asia, the most popular umami-containing condiments are fish sauces. Fish and salt are the two main components in fish sauce, although the proportions vary by country (Lopetcharat & Park, 2002). The degraded products, amino acids, nucleotides, and salt give a distinctive umami flavor and aroma (Otsuka, 1998).

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Table 2 The amount of amino acid in the Budu samples A, B, C and D, respectively

Analyte	Result(mg/100g)			
	A	B	C	D
Aspartic Acid	970.0	1,260.0	972.0	1,060.0
Glutamic Acid	1,350.0	1,540.0	1,250.0	1,310.0
Glycine	614.0	704.0	612.0	661.0
Histidine	270.0	362.0	219.0	370.0
Isoleucine	423.0	555.0	414.0	384.0
L-Alanine	688.0	819.0	658.0	663.0
L-Arginine	650.0	960.0	593.0	588.0
Leucine	733.0	918.0	714.0	619.0
Lysine	1,450.0	1,600.0	1,320.0	1,490.0
Methionine	293.0	381.0	288.0	264.0
Phenylalanine	406.0	533.0	256.0	377.0
Proline	401.0	650.0	358.0	440.0
Serine	312.0	584.0	376.0	447.0
Threonine	619.0	765.0	533.0	600.0
Tryptophan	79.7	86.9	89.5	86.3
Tyrosine	192.0	323.0	204.0	210.0
Valine	599.0	756.0	576.0	586.0
Total amino acid	10,049.7	12,796.9	9,432.5	10,155.3

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Table 3 Taste Qualities, Taste Thresholds (TC), Concentrations in Budu, and Dose-over-Threshold (DoT) Factors of Taste-Active Compounds in Budu sample A, B, C and D, respectively

Taste compound	TC (µmol/kg)	Dose-over-threshold (DoT) in			
		A	B	C	D
Group I: Sweet-Tasting Compounds					
Glycine	25000	3.27	3.75	3.26	3.52
L-Alanine	12000	6.43	7.66	6.15	6.20
L-Methionine	5000	3.93	5.11	3.86	3.54
L-Proline	25000	1.39	2.26	1.24	1.53
L-Serine	25000	1.19	2.22	1.43	1.70
L-Threonine	35000	1.48	1.84	1.28	1.44
Group II: Umami-Tasting Compounds					
L-Aspartic Acid	4000	18.22	23.66	18.26	19.91
L-Glutamic Acid	1100	83.43	95.17	77.25	80.96
Group III: Bitter-Tasting Compounds					
L-Histidine	45000	0.39	0.52	0.31	0.53
L-Isoleucine	10000	3.22	4.23	3.16	2.93
L-Arginine	75000	0.50	0.73	0.45	0.45
L-Leucine	11000	5.08	6.36	4.95	4.29
L-Lysine	80000	1.24	1.37	1.13	1.27
L-Phenylalanine	45000	0.55	0.72	0.34	0.51
Tryptophan	4000	0.98	1.06	1.10	1.06
Tyrosine	4000	2.65	4.46	2.81	2.90
L-Valine	30000	1.71	2.15	1.64	1.67
Total		**135.66**	**163.27**	**128.62**	**134.41**

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Note: If the value is greater than 1, it means that it has contributed or attributed that flavor.

Sensory analysis

The sensory profiles of materials determined by twelve panelists using the quantitative descriptive analysis (QDA) test have five characteristics presented in Fig. 3. The fermentation raised the salty, umami, sour, sweet, and bitter scores. The salty score was the highest of all samples, followed by umami, sour, sweet, and bitter, respectively. Sample D presented the saltiest taste (2.56 score) and sample B presented the most umami taste (2.37 score). The sweet, sour, and bitter tastes showed the highest scores in sample C. Each Budu presented a different taste and number of amino acids depending on the production factors.

Fig. 3 Sensory analysis

CONCLUSION

This study, Budu is made in the southern part of Thailand produced high amount of glutamic acid depending on the treatment of raw materials prior to fermentationGlutamic acid and its salts are very important in flavor of fish sauce. Budu has a high concentration of essential amino acids, including lysine. This data suggests that Budu may be a beneficial source of protein. There are also other factors that affect the taste, such as microorganisms in the fermentation process and other compounds. This research needs more study to contribute to the development of the business by increasing the value of Budu products so they can be exported to the global market.

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REFERENCES

Beddows, C. G. (1998). Fermented fish and fish products. In *Microbiology of fermented foods* (pp. 416-440). Springer.

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience & Biobehavioral Reviews*, *23*(3), 423-438.

Chen, Q., Kong, B., Han, Q., Xia, X., & Xu, L. (2017). The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. *LWT*, *77*, 389-396.

Chotechuang, N. (2013). Taste active components in Thai foods: a review of Thai traditional seasonings. *Nutrition & Food Sciences*.

Dougan, J., & Howard, G. E. (1975). Some flavouring constituents of fermented fish sauces. *Journal of the Science of Food and Agriculture*, *26*(7), 887-894.

Lee, H.-W., Choi, Y.-J., Hwang, I. M., Hong, S. W., & Lee, M.-A. (2016). Relationship between chemical characteristics and bacterial community of a Korean salted-fermented anchovy sauce, Myeolchi-Aekjeot. *LWT*, *73*, 251-258.

Lopetcharat, K., Choi, Y. J., Park, J. W., & Daeschel, M. A. (2001). Fish sauce products and manufacturing: a review. *Food Reviews International*, *17*(1), 65-88.

Lopetcharat, K., & Park, J. W. (2002). Characteristics of fish sauce made from Pacific whiting and surimi by-products during fermentation stage. *Journal of Food Science*, *67*(2), 511-516. Meyer, S., Dunkel, A., & Hofmann, T. (2016). Sensomics-assisted elucidation of the tastant code of cooked crustaceans and taste reconstruction experiments. *Journal of agricultural and food chemistry*, *64*(5), 1164-1175.

Mohamed, H. N., Man, Y. C., Mustafa, S., & Manap, Y. A. (2012). Tentative identification of volatile flavor compounds in commercial Budu, a Malaysian fish sauce, using GC-MS. *Molecules*, *17*(5), 5062-5080.

Mohamed, H. N., & Mustafa, S. (2021). Fermented Fish Products: A Review on the Manufacturing Process, Technological Aspect, Sensory, Nutritional Qualities and Metabolite Profiles. *International Journal of Synergy in Engineering and Technology*, *2*(1), 16-35.

Otsuka, S. (1998). Umami in Japan, Korea, and Southeast Asia. *Food Reviews International*, *14*(2-3), 247-256.

Park, J.-N., Fukumoto, Y., Fujita, E., Tanaka, T., Washio, T., Otsuka, S., Shimizu, T., Watanabe, K., & Abe, H. (2001). Chemical composition of fish sauces produced in Southeast and East Asian countries. *Journal of Food Composition and Analysis*, *14*(2), 113-125.

Saisithi, P., KASEMSARN, R. O., Liston, J., & Dollar, A. M. (1966). Microbiology and chemistry of fermented fish. *Journal of Food Science*, *31*(1), 105-110.

Scharbert, S., & Hofmann, T. (2005). Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. Journal of agricultural and food chemistry, 53(13), 5377-5384.

Setyahadi, S. (2014). Utilization of fish waste for the making of fish sauce. In *Seafood processing by-products* (pp. 171-181). Springer.
Sukjuntra, J. (2018). Shelf Life Extension of Pasteurized Budu Mix. *Burapha Science Journal*, *23*(2), 902-916.
Tsai, Y.-H., Lin, C.-Y., Chien, L.-T., Lee, T.-M., Wei, C.-I., & Hwang, D.-F. (2006). Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food chemistry*, *98*(1), 64-70.
Wu, R., Chen, L., Liu, D., Huang, J., Zhang, J., Xiao, X., Lei, M., Chen, Y., & He, H. (2017). Preparation of antioxidant peptides from salmon byproducts with bacterial extracellular proteases. *Marine drugs*, *15*(1), 4.
Yoshida, Y. (1998). Umami taste and traditional seasonings. *Food Reviews International*, *14*(2-3), 213-246.

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Simultaneous identification of four meat species (cattle, chicken, fish, and pig) using next generation sequencing (NGS)

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Abstract

Meat adulteration has become a serious problem in global which directly affects to food consumers and producers. Therefore, it requires a tool to authenticate meat species to ensure safety of food products. Next generation sequencing (NGS) coupled with ribosomal RNA mitochondrial DNA gene can be used to analyze mixture of meat species in multiple meat samples. Therefore, this study aims to utilize NGS coupled with rRNA gene to identify 4 meat species (cattle, chicken, fish, and pig). Three primer sets (12S-Ki, 16S-KH, and 16S-Ki) were used to amplify DNA from the four meat species. All primer sets could be successfully amplified DNA fragments which corresponded to their size expectation. 16S-KH showed better detection effect in all species comparing with others. While the 12S-Ki and 16S-Ki could not be used to amplify in fish and chicken species. This may occur due to mismatches between sequences of primers and annealed regions of these species. Library construction of all PCR amplicons were prepared and sequenced by NGS. Amplicons amplified by 12S-Ki (fish) and 16SKi (chicken and fish) could not be mapped to the database because no PCR amplicons could not be amplified. NGS coupled with 16S-KH was then evaluated for precision test. The experimental precision was directly investigated comparing the results obtained from libraries that derives from DNA of four meat species which separately amplified for 3 different runs. As expected, the number and proportion of mapped reads between three different runs were also concordant. The percentage of mapped reads ranged from 14.05% to 31.04%, 15.14% to 31.98%, and 14.21% to 33.05% (1st, 2nd, and 3rd run, respectively). This demonstrated that NGS coupled with rRNA mtDNA gene could be reliably implemented as a routine testing. This developed technique can be applied to control and monitor meat adulterations in halal meat production and industry.

Keywords: *Next Generation Sequencing, Ion Torrent PGM, Halal species, meat species identification, ribosomal RNA*

INTRODUCTION

Meat adulteration has become a serious predicament in global. The adulterations could be occurred by accidental contamination or mislabelling. The cheaper meat is often used to substitute in raw meat and meat products such as beef meatballs substituted with rat meat in

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Indonesia (Suryawan et al., 2020), mutton adulterated by rat meat in China (Fang & Zhang, 2016), and chicken substituted with pork in China (Yin et al., 2020). Recently, fraudulent labelling of meat product was found in Thailand. Pork dyed with cow blood was sold as beef in the fresh markets (Denyingyhot et al., 2022). This directly affected to halal entrepreneurs, SMEs and restaurants which operate their business for using raw meats as ingredients. In addition, this raises concerns about food safety, religions, and ethics. Therefore, it requires a tool to identify meat species for food authentication.

Next generation sequencing (NGS) is an advanced high throughput sequencing technology which provides a massively parallel analysis in multiple samples. It has a high potential to use as tool for routine testing of meat inspection and authentication. Recently, many disclosed studies have reported in seafood (Giusti et al., 2017; Piredda et al., 2022), dairy products (Ribani et al., 2018), poultry meat (Dobrovolny et al., 2019), and pork (Akbar et al., 2021). Therefore, this emerging technology will provide the reliable tool for identifying the mixed meat species in meat product without any previous knowledge required. It will help the food producers to assure the authenticity of meat species with precise labeling on the products.

LITERATURE REVIEW

Thailand has a potential to serve halal food market in global with high volumes of halal meat production. However, obstacle in the meat production is adulteration of cheaper meat which prohibited by Islamic law. Halal meat product is commonly used beef and chicken which slaughtered following Islamic law and practices for consumption. Besides, fish is traditional allowed to use in Halal consumption and industrial usage. Pork is the most popular use in meat products. It is considered as the cheapest meat and widely used as a substitute for expensive meat such as beef (Denyingyhot et al., 2022). However, it is prohibited for Muslim consumer. Therefore, routine inspection of the prohibited meat adulteration is required.

Species identification is essential to determine mixed species in food and meat products. Many different methods have been used to identify the species of meat. Amongst, DNA techniques have been effectively developed and used in the meat species identification. (Amaral, 2021) Most of them employ Polymerase Chain Reaction (PCR) to amplify specific DNA fragments which further analyzed by different methods such as DNA barcoding (Kappel et al., 2017; Pan et al., 2020), real time PCR (Mahama et al., 2020), multiplex high-resolution melting analysis (Denyingyhot et al., 2021), and DNA strip (Denyinghot et al., 2022). Commonly, ribosomal RNA mitochondrial DNA gene is used as targeting region for identifying the species of meat. There are hundreds to thousands of copies of mtDNA in each cell so it will lower risk to fail with degraded templates (Yang et al., 2014; Liu et al., 2021; Bertolini et

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al., 2015). The traditional workflow analysis is to amplify the complete specific gene sequence by PCR and then use the conventional Sanger sequencing method for gene sequence analysis. However, this method can only sequence a single DNA fragment at the time. Therefore, it can not be applied for detecting mixtures of unknown species in meat products (Kappel et al., 2017).

Next generation sequencing (NGS) technology can be used to analyze meat products containing different species mixtures by parallel sequencing from a sample (Jagadeesan et al., 2019). In the last decade, NGS has transformed from being solely a research tool to becoming routinely applied in many fields including diagnostics, outbreak investigations, antimicrobial resistance, forensics, and food microbiology (Allard et al., 2018; Goodwin et al., 2016; Quainoo et al., 2017). The technology is developing at a rapid pace, with continuous improvement in quality and cost reduction and is having a major influence on food authenticity. The NGS coupled with 12S and 16S rRNA mitochondrial DNA gene have been successfully employed for identifying species of meat products. The species mixture of common meat (pig, cattle, sheep, chicken, turkey, duck, horse, rat, and rabbit) can be mixed and detected (Karlsson et al., 2007; Kitano et al., 2007; Giusti, 2017; Bertolini et al., 2015; Liu et al., 2021). Therefore, this proves that NGS coupled with rRNA mitochondrial DNA gene can be used as a tool for species identification. However, there is no reported study in fish species with these primer sets. Thus, this study aims to utilize NGS coupled with ribosomal RNA gene to identify 4 meat species including Halal meat species (cattle, chicken, and fish) and non-Halal meat species (pig).

RESEARCH METHODOLOGY

Sample

A total of four meat samples; cattle *(Bos indicus),* chicken (*Gallus gallus*), fish *(Chitala ornate)*, pig *(Sus scrofa)*, was obtained from purchased from supermarket in Bangkok during June 2020. They were transferred to the refrigerator to the laboratory and stored in -20˚C until use.

DNA extraction

DNA was extracted from 80-100 mg of meat sample using the Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. The extracted DNA purity and quality was measured by NanoDrop™ 2000/2000c spectrophotometers (Thermo Fischer Scientific, MA, US). Calculating DNA concentration and absorbance ratio at both 260/280 and 230/260 nm. Degradation of DNA of each species was checked by 1% agarose gel electrophoresis.

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PCR amplification

PCR amplification with the 12S rRNA and 16S rRNA universal primer pairs were performed in a volume of 50 µl containing 50 ng of the extracted DNA, 2x PCRBIO HS Tag Mix Red (PCR Biosystems Ltd., UK), 0.6 µM concentration of each universal primer (Table 1.). PCR was amplified on an Applied Biosystems Veriti™ 96 well Thermal Cycler (Thermo Fisher Scientific) with the following cycling program: denaturation at 95°C for 1 min; 32 cycles at 94°C for 15 s, 59°C, 63°C, and 62°C (12S-Ki, 16S-KH, and 16S-Ki, respectively) for 15 s, and 72°C for 30 s; final extension at 72°C for 7 min. The presences of PCR products were confirmed by 2% agarose gel electrophoresis.

Table 1. The sequences of universal primer.

Gene	Primer name	Primer Sequence (5' – 3')	Expected size (bp)	References
12S-Ki	Forward	CCCAAACTGGGATTAGATACCC	215-222	Karlsson et al., 2007
	Reverse	GTTTGCTGAAGATGGCGGTA		
16S-KH	Forward	GACGAGAAGACCCTATGGAGC	112-130	Karlsson et al., 2007
	Reverse	TCCGAGGTCGCCCCAACC		
16S-Ki	Forward	GCCTGTTTACCAAAAACATCAC	243-249	Kitano et al., 2007
	Reverse	CTCCATAGGGTCTTCTCGTCTT		

Library preparation, quantification, emulsion PCR, and sequencing

Preparation of barcoded libraries; A specific barcoded library was prepared for the amplicon obtained from each meat samples using the Ion Plus Fragment Library Kit (IPFL kit-Thermo Fisher Scientific), that allowed amplicons' end-repair and ligation to Ion-compatible adapters. Amplicon's end-repair and purification were performed according to the manufacturer's instructions. The samples were purified with Agencourt AMPure XP Kit for DNA purification on a DynaMag™-2 magnet magnetic rack (Thermo Fisher Scientific) following the procedure proposed by the manufacturer. The barcoding was done using Ion Xpress™ Barcode Adapters (Thermo Fisher Scientific). The same Ion Xpress™ P1 Adapter was ligated to the amplicons obtained from all the meat samples whereas a unique Ion Xpress™ Barcode Adapter for each sample was used. The samples were then purified with Agencourt AMPure XP Kit for DNA purification on a DynaMag™-2 magnet magnetic rack following the procedure proposed by the manufacturer. Libraries were amplified on an Applied Biosystems Veriti™ 96 well Thermal Cycler (Thermo Fisher Scientific) according to the manufacturer's instructions and quantified by a using the Qubit dsDNA HS Assay Kit to determine the molar concentration of each barcoded library, and then diluted as proposed by the Ion PGM™ Hi-Q™ sequencing Kit (Thermo Fisher Scientific). Emulsion

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PCR was performed following the manufacturer's protocol for the Ion OneTouch 2 Instrument (Life Technologies) to produce the required Ion Sphere Particles (ISPs). For sequencing chip, a pool of 30 µl containing equal amounts of each sample was prepared. The libraries were sequenced on the Ion Torrent PGM platform with an Ion PGM™ Hi-Q Sequencing 200 Kit. A sample was deemed to have a successful sequencing run if at least 50,000 reads with a quality score of Q20 (one misaligned base per 100 bases) were obtained, a minimum coverage of 500x was required.

Data analysis

The Ion Torrent PGM reads were compared to the GenBank nucleotide database. Ion Torrent PGM reads were collected by the Ion Torrent Suite software, which also sorted the data according to the barcodes. The software also scores the quality of the reads by assignment of Q20 scores according to the Ion Torrent's quality scoring computation. The percentage of genus identity and species identity value required at 97% and 99%, respectively.

FINDING AND DISCUSSION

To verify the usable of ribosomal RNA (12S and 16S rRNA) mitochondrial DNA gene, DNA of four meat species (cattle, chicken, fish, and pig) were used as templates for PCR amplification using adopted primer sets. Three adopted primer pairs (12S-Ki, 16S-KH, and 16S-Ki) were selected from the literature to amplify mtDNA regions (12S and 16S) containing species-specific information. All primer sets could be amplified DNA fragments of the four species according to their size expectation (Table 2). For 12S-Ki, agarose gel electrophoresis showed that three species (cattle, chicken, and pig) could generate PCR amplicons except one (fish). The PCR amplicons of 215-230 bp were amplified (Figure 1). For 16S-KH, agarose gel electrophoresis showed that all species (cattle, chicken, fish, and pig) could generate PCR amplicons. The PCR amplicons of 112 -130 bp were amplified (Figure 2). For 16S-Ki, agarose gel electrophoresis showed that two species (cattle and pig) could generate PCR amplicons except other two species (chicken and fish). The PCR amplicons of 243-253 bp were amplified (Figure 3). Unfortunately, the results showed that 12S-Ki and 16S-Ki universal primer could not be used to amplify in fish and chicken species. This occurs may due to the several mismatches between primer sequences and the annealed target regions of these species.

Table 2. Detection of meat species from 4 animals' DNA by conventional PCR.

Universal Primer	Cattle			Chicken			Fish			Pig		
	1	2	3	1	2	3	1	2	3	1	2	3
12S-Ki	+	+	+	+	+	+	-	-	-	+	+	+
16S-KH	+	+	+	+	+	+	+	+	+	+	+	+
16S-Ki	+	+	+	-	-	-	-	-	-	+	+	+

(+) amplification, (-) no amplification

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Simultaneous identification of four meat species (cattle, chicken, fish, and pig) using next generation sequencing (NGS)

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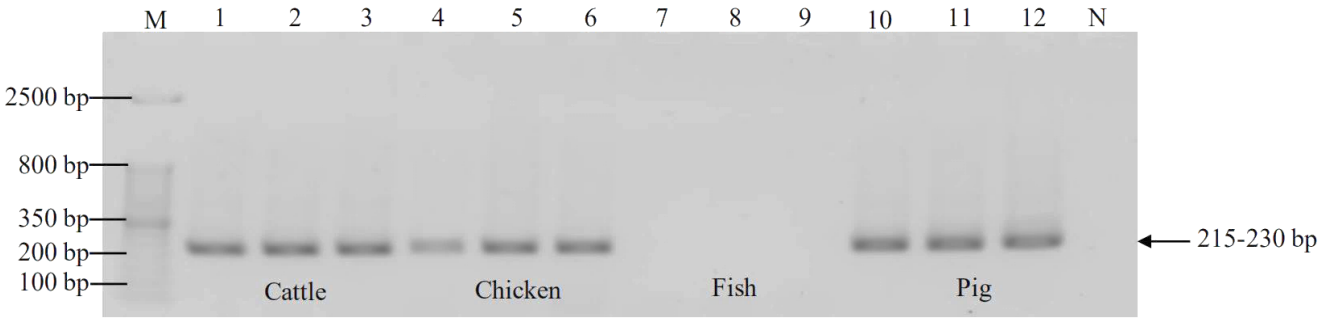

Figure 1. The image of PCR products using 12S-Ki universal primer from agarose gel electrophoresis. Lane M, 50bp DNA marker; Lane 1-3, cattle; Lane 4-6, chicken; Lane 7-9, Fish; Lane 10-12, pig; and Lane N, negative control.

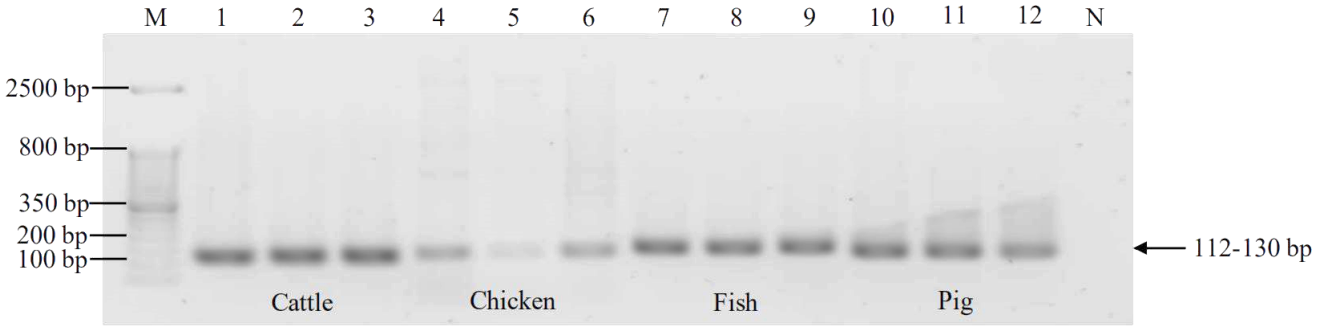

Figure 2. The image of PCR products using 16S-KH universal primer from agarose gel electrophoresis. Lane M, 50bp DNA marker; Lane 1-3, cattle; Lane 4-6, chicken; Lane 7-9, Fish; Lane 10-12, pig; and Lane N, negative control.

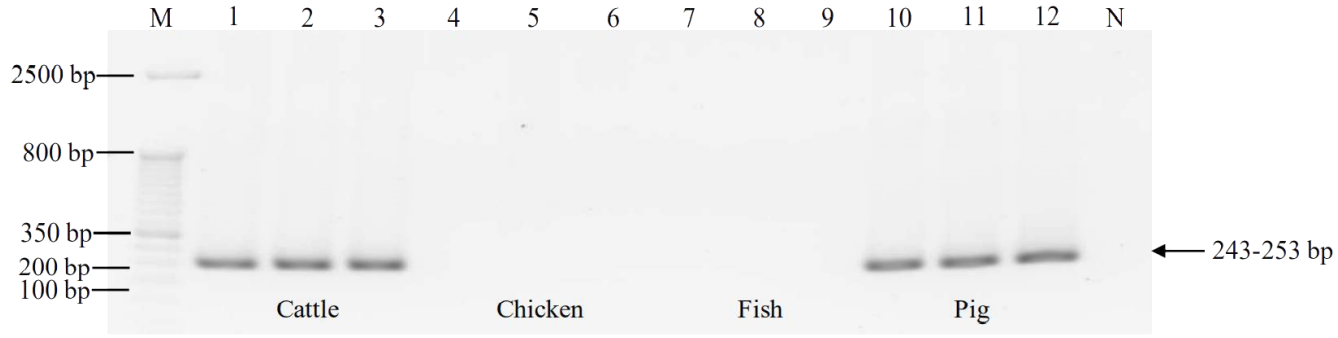

Figure 3. The image of PCR products using 16S-Ki universal primer from agarose gel electrophoresis. Lane M, 50bp DNA marker; Lane 1-3, cattle; Lane 4-6, chicken; Lane 7-9, Fish; Lane 10-12, pig; and Lane N, negative control.

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To verify the usable of NGS, the libraries of the amplified PCR amplicons were constructed and sequenced. The amplicons obtained from the conventional PCR step were prepared in library 1 to 12 and sequenced with the Ion Torrent PGM. The number of mapped reads to the corresponding reference mtDNA regions (12S-Ki, 16S-KH and 16S-Ki), without (Total reads) and with preliminary filtering steps (Valid reads) is shown in Table 3. For 12S-Ki, the amplicons with library 1 to 4 were sequenced. The results of BLAST showed 2,514 reads (37.91%) in cattle, 1,739 reads (26.23%) in chicken, 0 reads (0.00%) in fish, and 2,378 reads (35.86%) in pig. For 16S-KH, the amplicons with library 5 to 8 were sequenced. The results of BLAST showed 6,285 reads (22.56%) in cattle, 3,959 reads (14.21%) in chicken, 9,207 reads (33.05%) in fish, and 8,409 reads (30.18%) in pig. For 16S-Ki, the amplicons with library 9 to 12 were sequenced. The results of BLAST showed 10,848 reads (76.75%) in cattle, 0 reads (0.00%) in chicken, 0 reads (0.00%) in fish, and 3,286 reads (23.25%) in pig (Table 3 and Figure 4). These results were similar to the previous studies of Liu et al. (2021) and Bertolini et al. (2015). The NGS coupled with rRNA mitochondrial DNA gene had good detection effect on mammalian species, but it had poor detection effect on poulty species. Interestingly, amplicons amplified by 12S-Ki (fish) and 16SKi (chicken and fish) could not be mapped to the mtDNA database. This demonstrated that NGS coupled with rRNA mitochondrial DNA gene had a high potential to identify meat species since the amplicons could not be amplified by conventional PCR. In this study, 16S-KH showed better detection effect in all species comparing with others. This demonstrated that 16S-KH was reliable and usable for meat species identification. Therefore, it was chosen to further use in precision (reproducibility) of meat species identification using NGS.

Table 3. NGS sequencing results by 12S-Ki, 16S-KH, and 16S-Ki primer.

Primer name	Species	Base	Q20	Total Read	Valid read	mapped read
12S-Ki	Cattle	1,370,239	1,224,901	7,671	6,636	2,514
	Chicken	943,856	851,071	5,235	4,450	1,739
	Fish	2,435,841	2,188,886	13,540	11,714	0
	Pig	1,519,488	1,327,858	8,260	7,480	2,378
16S-KH	Cattle	1,089,953	1,004,640	10,961	9,267	6,285
	Chicken	840,607	766,884	7,548	6,991	3,959
	Fish	1,863,969	1,708,795	15,725	14,281	9,207
	Pig	1,614,247	1,442,022	16,106	13387	8,409
16S-Ki	Cattle	5,294,863	4,888,586	24,623	22,539	10,848
	Chicken	2,460,738	2,262,428	11,605	10,373	0
	Fish	2,202,543	2,035,535	10,305	9,280	0
	Pig	1,895,529	1,752,051	8,956	8,042	3,286

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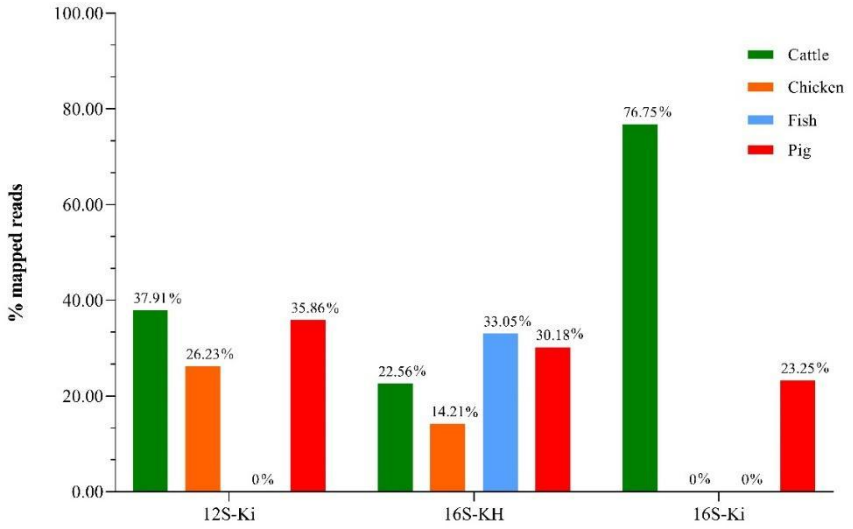

Figure 4. NGS sequencing results of 4 meat species (cattle, chicken, fish, and pig) amplified by 3 pairs of adopted primer.

For precision evaluation, the 16S-KH primer was further used to amplify the targeted PCR amplicons from the four meat species. The amplicons obtained from the conventional PCR step were prepared in library 1 to 4 and then sequenced with the Ion Torrent PGM. The experimental precision was directly investigated comparing the results obtained from library 1 to 4 that derives from DNA of four meat species which separately amplified for 3 different runs (Table 4). The number of mapped reads to the corresponding reference mtDNA regions (16S-KH), without (Total reads) and with preliminary filtering steps (Valid reads) is shown in Table 4. For 1st run, the amplicons with library 1 to 4 were sequenced. The results of BLAST showed 7,853 reads (24.93%) in cattle, 4,425 reads (14.04%) in chicken, 9,779 reads (31.04%) in fish, and 9,447 reads (29.00%) in pig. For 2nd run, the amplicons with library 1 to 4 were sequenced. The results of BLAST showed 7,573 reads (25.70%) in cattle, 4,461 reads (15.14%) in chicken, 9,422 reads (31.98%) in fish, and 8,007 reads (27.18%) in pig. For 3rd run, the amplicons with library 1 to 4 were sequenced. The results of BLAST showed 6,285 reads (22.56%) in cattle, 3,959 reads (14.21%) in chicken, 9,207 reads (33.05%) in fish, and 8,409 reads (30.18%) in pig. The percentage of mapped reads ranged from 14.04% to 31.04%, 15.14% to 31.98%, and 14.21% to 33.05% (1st, 2nd, and 3rd run, respectively) (Figure 5). As expected, the number and proportion of mapped reads of each species per run were similar. Besides, the number and proportion of mapped reads between three different runs were also concordant (Figure 5). This demonstrated that NGS coupled with rRNA mtDNA gene could be reliably implemented as a routine testing of meat inspection and authentication.

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Table 4. NGS sequencing results by 16S-KH primer.

Run no.	Species	Base	Q20	Total Read	Valid reads	Mapped reads
1st	Cattle	1,035,782	1,026,913	22,980	21,991	7,853
	Chicken	981,549	887,534	10,844	9,973	4,425
	Fish	1,588,948	1,500,638	20,541	18,368	9,779
	Pig	1,456,547	1,382,846	19,335	18,471	9,447
2nd	Cattle	1,163,981	1,017,913	20,367	19,992	7,573
	Chicken	1,230,290	1,148,544	11,744	10,584	4,461
	Fish	1,617,893	1,438,847	24,951	24,011	9,422
	Pig	1,241,337	1,036,884	28,561	27,877	8,007
3rd	Cattle	1,089,953	1,004,640	10,961	9,267	6,285
	Chicken	840,607	766,884	7,548	6,991	3,959
	Fish	1,863,969	1,708,795	15,725	14,281	9,207
	Pig	1,614,247	1,442,022	16,106	13,387	8,409

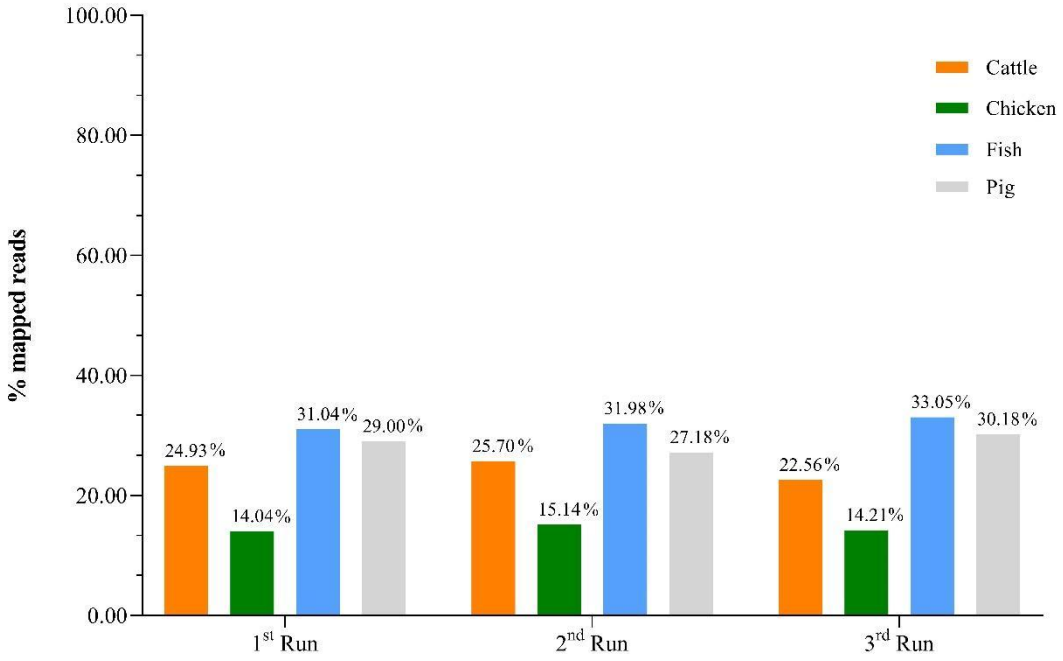

Figure 5. NGS sequencing results of 4 meat species (cattle, chicken, fish, and pig) amplified by 16S-KH primer.

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CONCLUSION AND FURTHER RESEARCH

In this study, we tested the possibility to use the NGS technology coupled with ribosomal RNA mitochondrial gene to identify meat species including cattle, chicken, fish, and pig. The results showed that NGS coupled with 16S-KH primer had a positive detection effect on all meat species. However, the values of mapped reads were still low. Hence, PCR quantity and quality protocol should be further optimized. In addition, the good laboratory practices should be implemented. DNA extraction, PCR, and NGS sequencing should be performed in different rooms to avoid accidental contamination between samples. Moreover, other halal and haram species should be further examined for comprehensive test of meat inspection and authentication. This innovative technique could be applied to employ as a monitoring tool for identifying food fraud and undeclared meat species. It may become a routine method for species identification in meat products and will build confidence in the safety of halal food products for consumers.

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REFERENCES

Akbar, A., Shakeel, M., Al-Amad, S., Akbar, A., Ali, A. K., Rahmeh, R., ... & Musharraf, S. G. (2021). A simple and sensitive NGS-based method for pork detection in complex food samples. *Arabian Journal of Chemistry,* 14(5), 103124.

Allard, M. W., Bell, R., Ferreira, C. M., Gonzalez-Escalona, N., Hoffmann, M., Muruvanda, T., ... & Brown, E. W. (2018). Genomics of foodborne pathogens for microbial food safety. *Current opinion in biotechnology*, 49, 224-229.

Amaral, J. S. (2021). Target and Non-Target Approaches for Food Authenticity and Traceability. *Foods*, 10, 172.

Bertolini, F., Ghionda, M. C., D'alessandro, E., Geraci, C., Chiofalo, V., & Fontanesi, L. (2015). A next generation semiconductor based sequencing approach for the identification of meat species in DNA mixtures. *PloS one*, 10(4), e0121701.

Denyingyhot, A., Phraephaisarn, C., Vesaratchavest, M., Dahlan, W., & Keeratipibul, S. (2021). A new tool for quality control to monitor contamination of six non-halal meats in food industry by multiplex high-resolution melting analysis (HRMA). *NFS Journal*, 25, 31-40.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Denyingyhot, A., Srinulgray, T., Mahamad, P., Ruangprach, A., Sa, S., Saerae, T., ... & Keeratipibul, S. (2022). Modern on-site tool for monitoring contamination of halal meat with products from five non-halal animals using multiplex polymerase chain reaction coupled with DNA strip. *Food Control*, 132, 108540.

Dobrovolny, S., Blaschitz, M., Weinmaier, T., Pechatschek, J., Cichna-Markl, M., Indra, A., ... & Hochegger, R. (2019). Development of a DNA metabarcoding method for the identification of fifteen mammalian and six poultry species in food. *Food chemistry*, 272, 354-361.

Fang, X., & Zhang, C. (2016). Detection of adulterated murine components in meat products by TaqMan© real-time PCR. *Food chemistry*, 192, 485-490.

Giusti, A., Tinacci, L., Sotelo, C. G., Marchetti, M., Guidi, A., Zheng, W., & Armani, A. (2017). Seafood identification in multispecies products: assessment of 16SrRNA, cytb, and COI Universal Primers' efficiency as a preliminary analytical step for setting up metabarcoding next-generation sequencing techniques. *Journal of agricultural and food chemistry*, 65(13), 2902-2912.

Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17(6), 333-351. Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., ... & Grant, K.

(2019). The use of next generation sequencing for improving food safety: translation into practice. *Food microbiology,* 79, 96-115.

Kappel, K., Haase, I., Käppel, C., Sotelo, C. G., & Schröder, U. (2017). Species identification in mixed tuna samples with next-generation sequencing targeting two short cytochrome b gene fragments. *Food chemistry*, 234, 212-219.

Karlsson, A. O. & Holmlund, G. (2007). Identification of mammal species using species-specific DNA pyrosequencing. *Forensic Sci Int*, 173, 16-20.

Kitano, T., Umetsu, K., Tian, W., & Osawa, M. (2007). Two universal primer sets for species identification among vertebrates. *International journal of legal medicine,* 121(5), 423-427. Liu, X., Liu, Z., Cheng, Y., Wu, H., Shen, W., Liu, Y., ... & Yang, J. (2021). Application of Next-Generation Sequencing Technology Based on Single Gene Locus in Species Identification of Mixed Meat Products. *Journal of Food Quality,* 2021.

Mahama, S., Waloh, N., Chayutsatid, C., Sirikwanpong, S., Ayukhen, A., Marnpae, M., ... & Dahlan, W. (2020). Postmarket Laboratory Surveillance for Forbidden Substances in Halal-Certified Foods in Thailand. *Journal of food protection*, 83(1), 147-154.

Pan, Y., Qiu, D., Chen, J., & Yue, Q. (2020). Combining a COI Mini-Barcode with Next-Generation Sequencing for Animal Origin Ingredients Identification in Processed Meat Product. *Journal of Food Quality*, 2020.

Piredda, R., Mottola, A., Cipriano, G., Carlucci, R., Ciccarese, G., & Di Pinto, A. (2022). Next Generation Sequencing (NGS) approach applied to species identification in mixed processed seafood products. *Food Control*, 133, 108590.

ISSN 2828-1500 (Online) | 2828-1985(Print)

Quainoo, S., Coolen, J. P., van Hijum, S. A., Huynen, M. A., Melchers, W. J., van Schaik, W., & Wertheim, H. F. (2017). Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. *Clinical microbiology reviews*, 30(4), 1015-1063.

Ribani, A., Schiavo, G., Utzeri, V. J., Bertolini, F., Geraci, C., Bovo, S., & Fontanesi, L. (2018). Application of next generation semiconductor based sequencing for species identification in dairy products. *Food chemistry,* 246, 90-98.

Suryawan, G. Y., Suardana, I. W., & Wandia, I. N. (2020). Sensitivity of polymerase chain reaction in the detection of rat meat adulteration of beef meatballs in Indonesia. *Veterinary World,* 13(5), 905.

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M. X., Huang, T., & Li, R. (2014). Species identification through mitochondrial rRNA genetic analysis. *Scientific reports,* 4(1), 1-11.

Yin, R., Sun, Y., Wang, K., Feng, N., Zhang, H., & Xiao, M. (2020). Development of a PCR-based lateral flow strip assay for the simple, rapid, and accurate detection of pork in meat and meat products. *Food chemistry,* 318, 126541.

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Physicochemical Properties of Cellulose extracted From Hom Thong Banana Peels

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Abstract

Bananas are one of the most popular fruits in the world, yet only around 12% of them are consumed, posing an environmental problem. The goal of this research is to extract Hom Thong banana cellulose, which is the major component of banana peels. Fat analysis was used to extract and bleach Hom Thong banana cellulose, followed by soaking in 15% hydrogen peroxide for 3 h. The Hom Thong banana peel cellulose was washed and dried at 60 °C for 10 h. The obtained Hom Thong banana cellulose was characterized in terms of fatty acid profile, intermolecular interactions, and thermal analysis by using gas chromatography, FT-IR, and DSC techniques, respectively. The results showed that the content of palmitic acid (C16:0) in the post-evaporated ethanolic extract is larger than in pre-evaporated ethanolic extract, with a ratio of 44.91% and 38.62%, respectively. At a ratio of 26.19% and 31.56%, the post-evaporation of the ethanolic extract contained less linoleic acid (18:2cis) than the pre-evaporation of ethanolic extract. Intra-molecular interactions between OH groups of cellulose were shown by FT-IR spectra. DSC thermograms revealed that the extracted cellulose had good thermal characteristics and was appropriate for the food and cosmetic industries.

Keywords: *Hom Thong banana peel, Cellulose, Fatty acid profile, Bleaching*

INTRODUCTION

Banana is one of the world's most favorite fruits, accounting for 40% of all fruit commerce. Due to Thailand's geographical advantage and favorable environment, banana is one of the most significant fruits exported. Specifically, the Hom Thong Banana (*Musa acuminata*, AAA group) is a Thailand geographical indicator or GI (Geographical Indication) (Amnuaysin et al., 2020). There is a lot of cultivation in many provinces such as Chanthaburi, Kanchanaburi, Suphan Buri, Nakhon Sawan, Kamphaeng Phet, Bueng Kan, Phatthalung and Chumphon. Groups of countries that import bananas from Thailand are China, the United Kingdom, Japan, and the United States, and ASEAN. In addition, bananas are also processed into various products to add value, such as banana chips, salted bananas, butter bananas, and unsweetened bananas, allowing the market has expand rapidly both in Thailand and abroad. As a result, farmers are more interested in cultivating bananas. However, the edible section of the banana only accounts for 12% (w/w) of the plant,

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posing an environmental issue. One approach is to convert banana peels into cellulose, which is a more valuable product that might be used more broadly in the food industry. The goal of this study was to extract cellulose from Ham Thong banana peels and test its functional properties in food and cosmetic applications.

LITERATURE REVIEW

Forest wastes, agricultural residues, algal waste, and industrial byproducts can all be used to make cellulose (Yu et al., 2021). Banana peel is one of the abundant cellulose sources from fruit waste. From the processing of banana products, the banana peels (35-40% of all bananas) will be leftover, which is an agricultural waste. Many studies have shown that banana peels contain many chemical constituents, including cellulose (75%), carbohydrates (53%), fiber (34%), proteins, fats, and also antimicrobial agents, antioxidants, etc. (Tibolla et al., 2018). Therefore, processing waste banana peels into value-added materials from local sources should be cheaper than importing materials from abroad.

Banana peels are known as a source of lignocellulose, with varying main amounts of cellulose, hemicellulose, and lignin, depending on the plant species. Cellulose, the major component of plant fiber cell walls, is a glucose polymer with β-1, 4 glycosidic bonds, resulting in great structural complexity owing to intermolecular hydrogen bonding interaction (Veeramachineni et al., 2016, Tibolla et al., 2014). Furthermore, hemicellulose, an insoluble heteropolysaccharide, is made up of monosaccharides such as xylose, mannose, fructose, galactose, glucuronic acid, and arabinose that bind to glycosidic links in the polysaccharide backbone directly or by branching. When cellulose and hemicellulose interact with lignin, more complexation or crystalline form is produced (Palacios et al., 2017). Cellulose has been useful for many applications with natural, renewable, and biodegradable properties. It has a high degree of crystallinity, as well as good mechanical strength and stiffness.

RESEARCH METHODOLOGY

Materials

Hom Thong bananas (Musa acuminata) were obtained from the market in Pathum Thani, Thailand. Hydroxyl ethylcellulose (HEC), methanol, n-hexane, dichloromethane, and potassium chloride were obtained from Merck (Darmstadt, Germany). Boron trifluoride-methanol purchased from Sigma-Aldrich (Switzerland). Methyl-2-hydroxyethyl cellulose (MHC), hydroxypropyl methylcellulose (HMC), sodium chloride, and the supelco 37 Component FAME Mix purchased from Sigma-Aldrich (St. Louis, MO, USA).

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Hom Thong banana cellulose preparation and fat extraction

Fresh Hom Thong banana peels with a maturity rating of 7 (a yellow peel with a few brown spots) were cut into 0.3 x 2.5 cm pieces and then dried in a hot air oven at 55 °C for 10 h (Singanusong et al., 2014). Banana peel samples (3 g) were placed in a screw cap tube, and then 10 ml of methanol/dichloromethane (1:2 v/v) was added before the sample tubes were left for 1 h. The samples were filtered using Whatman filter paper no. 1, and then 0.1 M potassium chloride (approximately 20% of total volume) was added to the samples, which were then combined and centrifuged at 2,000 rpm for 10 min at 25 °C. For the methylation reaction, the lower phase of the organic solvent was collected.

a) Methylation

200 µL of the lipid samples in the screw cap tube were added 1 ml of 0.5 M NaOH-methanol, then mixed and Boiled at 100 °C, for 15 min, followed by adding 2 ml of 14% BF_3/methanol. The samples were then heated at 100 °C for 1 min before being cooled to room temperature. In addition, 500 µl of hexane and 5 ml of saturated NaCl solution were added to the samples and then centrifuged at 1,000 rpm for 5 min. Finally, the upper phase was pipette into a vial for gas chromatography analysis. (Lepage and Roy, 1984, Lepage and Roy, 1986).

b) General experimental condition

Gas chromatograph-mass spectrometer (GC-MS) (Thermo scientific®, Model Trace 1310, ISQ 7000, Sci Spec company limited). The capillary column used was TR-FAME; length: 30 m, i.d.: 0.25 mm, and film: 0.25 µm. The analysis conditions for GC-MS were set as follows: carrier gas: helium (1 ml/min), flow rate: 1.0 ml/min, detector temperature: 240 °C, column temperature: 240 °C, injector temperature: 240 °C, split ratio: 15, split flow: 18 ml/min, running time: 16 min, starting temperature was 30 °C, then increased to 240 °C with a final hold at 240 °C for 3 min. The analysis parameters of the mass spectrometry detector were: ion source temperature: 200 °C, mass spectrometry transfer line: 220 °C (Mordi et al., 2016).

c) Bleaching of the Cellulose

The defatted and protein-free banana peel powder was soaked in 15% of hydrogen peroxide solutions for 3 h. The bleached samples were washed in triplicate with distilled water, separated using Whatman paper No. 4, and finally dried for 10 h in a hot air oven at 60 °C (Singanusong et al., 2014).

Properties of Hom Thong Banana Cellulose

a) Fourier-transform infrared spectroscopy (FT-IR) Analysis

FT-IR analyses were recorded on a FT-IR spectrophotometer (HTS-XT, Sensor II, Bruker, Karlsruhe,

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Germany) equipped with a universal attenuated total reflectance device. FT-IR analysis was performed in the infrared region, with 16 scans in wavenumbers ranging from 4,000 to 400 cm^{-1} and spectral resolution of 4 cm^{-1}.

b) Differential scanning calorimetry (DSC) Analysis

A differential scanning calorimeter (Thermo plus Evo2, Rigaku, Japan) was performed to determine the thermal characteristics of the materials. Each sample (4.0-5.0 mg) was accurately weighed into aluminum pans, and an empty pan was utilized as a reference. The scan speed was set at 10 °C/min from room temperature to 450 °C. The DSC analyses were carried out three times.

FINDINGS AND DISCUSSION

Hom Thong banana peel has brownish yellow color after fat extraction and bleaching process. Fatty acid profile of Hom Thong banana peel in ethanolic extract of both pre-and post-evaporation was analysed by using a gas chromatograph-mass spectrometer. The result in table 1 showed that post-evaporation of the ethanolic extract contained a higher amount of palmitic acid (C16:0) than Pre-evaporation of ethanolic extract at the ratio of 44.91% and 38.62%, respectively. Post-evaporation of ethanolic extract had a lower amount of linoleic acid (18:2cis) than pre-evaporation of ethanolic extract at the ratio of 26.19% and 31.56 %, respectively. Besides, α-linolenic acid (C18:3n3) was also found at the ratio of 19.94% and 8.12%, respectively (Mordi et al., 2016, Erdogan Orhan et al., 2008). Evaporation of a minor fatty acid might result in a difference in the fatty acid content of ethanolic extracts. Furthermore, the number of fatty acids in the Hom Thong banana peel changed with age. Palmitic acid content increased as the fruit matured, whereas linoleic and linolenic acids were greatest in stage 2 of fruit development. They, however, gradually decreased as the fruit matured (Khawas et al., 2016).

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Table 1. Fatty acid profile from pre-and post-evaporation of ethanolic extracts of Hom Thong banana peel

Pre-evaporation						Post-evaporation					
	Compound	**1**	**2**	**3**	**mean**		**Compound**	**1**	**2**	**3**	**mean**
1	C12:0	-	3.51	8.28	3.93	1	C12:0	1.07	0.76	0.31	0.71
2	C14:0	0.07	2.03	3.89	2.00	2	C14:0	1.23	1.08	0.81	1.04
3	C16:0	13.03	54.99	47.85	38.62	3	C16:0	49.83	47.59	37.31	44.91
4	C16:1	0.07	-	-	0.07	4	C16:1	-	-	0.27	0.09
5	C17:0	0.07	-	-	0.07	5	C17:0	-	-	0.38	0.13
6	C18:0	3.06	3.16	4.18	3.47	6	C18:0	2.78	2.85	2.78	2.80
7	C18:1 cis	24.65	5.29	6.38		7	C18:1 cis	3.06	3.08	4.16	3.43
8	C18:2 cis	55.17	20.67	18.84	31.56	8	C18:2 cis	25.05	24.95	28.58	26.19
9	C18:3 n6	0.17	-	-	0.06	9	C18:3 n6	-	-	0.15	0.05
10	C18:3 n3	3.41	10.35	10.59	8.12	10	C18:3 n3	16.98	19.68	23.17	19.94
11	C20:1	0.2	-	-	0.07	11	C20:0	-	-	0.92	0.31
12	C20:2	0.09	-	-	0.03	12	C22:0	-	-	0.34	0.11
13	C24:0	-	-	-	-	13	C24:0	-	-	0.82	0.27
	Total	99.99	100	100.01	100.00		**Total**	100	99.99	100	100.00

Physicochemical Properties of Hom Thong Banana Cellulose

a) Fourier-transform infrared spectroscopy (FT-IR)

The intermolecular chemistry of banana peel cellulose was characterized by using the FT-IR technique (Fig. 1 (A)). Because of the -OH groups in the hydrophilic components, the FT-IR spectra showed a large absorption peak ranging of 3,500-3,000 cm^{-1}, which was assigned to intramolecular hydrogen bonding of hydroxyl groups in cellulose. The absorption peak at 2,884.11 cm^{-1} was caused by the aliphatic -CH stretching vibration in cellulose and also hemicellulose. The C=O bonds of aldehydes or carboxylic stretching of hemicelluloses were suggested by a band in the spectra of banana peel cellulose at 1,647.70 cm^{-1}. Due to the elimination of hemicellulose by chemical treatment, the absorption peak of the ester group

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around 1730 cm-1 was not detected in commercial celluloses (HEC, HMC, and MHC). The C-O-C cellulose bond was seen in the band at 1,020.06 cm-1, indicating a significant cellulose concentration (Pelissari et al., 2017). As shown in Fig. 1 (B), the hydroxyl modification of commercial celluloses resulted in increasing the absorption intensity of the methylene group ataround 2874-2891 cm^{-1}, leading to the decreasing intensity of their H-bonding interaction at 3,500-3,000 cm^{-1}.

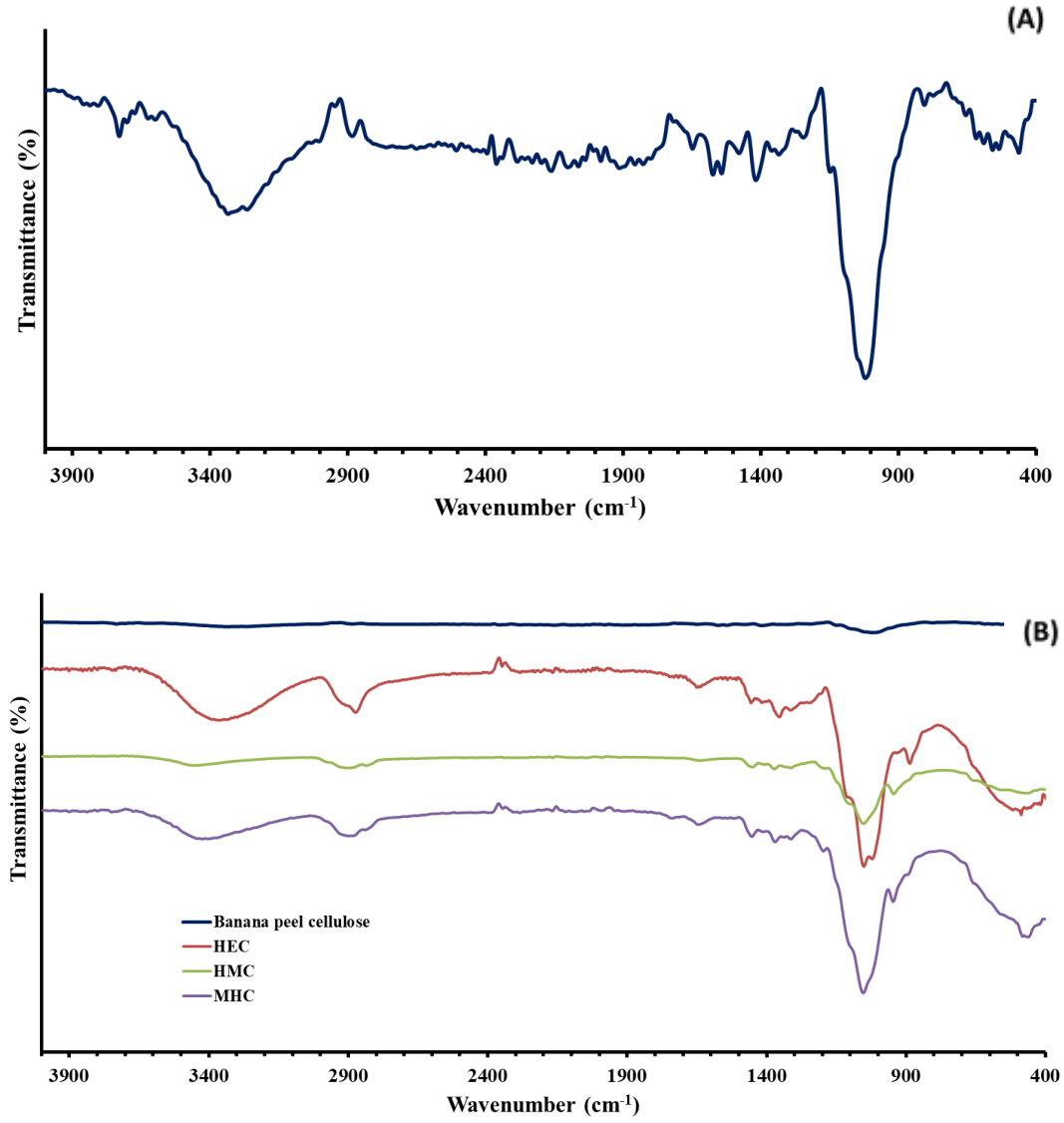

Figure 1. FTIR spectroscopy analyses of Hom Thong Banana Cellulose (A) and HEC, HMC, MHC standards (B)

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b) Differential scanning calorimetry (DSC)

Hom Thong banana cellulose was thermally analyzed by the DSC technique. As shown in Fig. 2, the result suggested that the water was evaporated around 90-130 °C with an endothermic reaction (19.838 J/g). Hemicellulose possibly degraded in the range of 170-190 °C (125.835 J/g), due to having an amorphous structure from the random structural arrangement of various polysaccharides (galactose, glucose, mannose) than those commercial celluloses (HEC, MHC, and HMC) that were degraded in range of 202-210 °C. Furthermore, cellulose would be depolymerized and then destroyed by exothermic reaction at a temperature ranging of 320-350 °C (31.157 J/g) because it might be interacted with lignin, resulting in a highly organized structure (Pelissari et al., 2014, Pelissari et al., 2017). Moreover, degradation of lignin with amorphous cross-linked structure would have occurred above 350 °C.

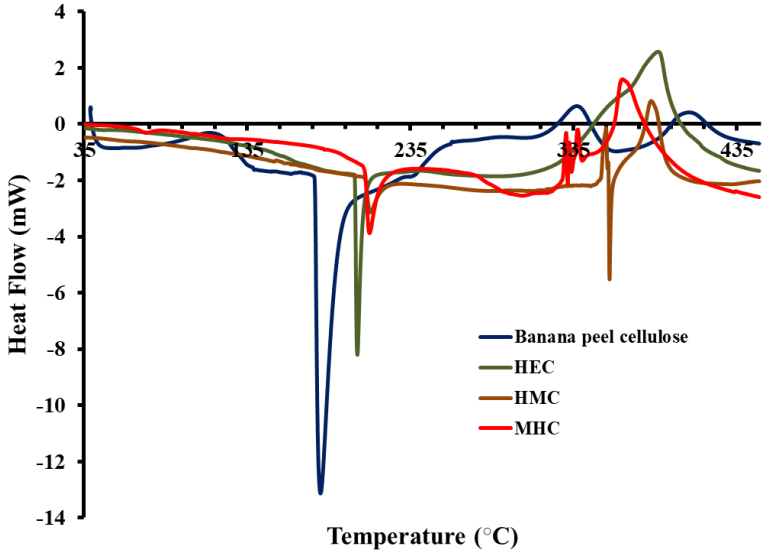

Figure 2. DSC thermograms of the Hom Thong Banana Cellulose and HEC, HMC, MHC standards

CONCLUSION

This preliminary study investigated the possibility of Hom Thong banana peel waste as an alternative cellulose source for food and cosmetic applications due to its low cost and availability. The chemical composition of fatty acid can be measured by GC-MS depending on the maturity and species of banana peel source. When cellulose extracted from Hom Thong banana peels was compared to cellulose standards using FT-IR and DSC techniques, the results exhibited varied functional groups as well as different physicochemical microstructures. Screening characterization of the obtained cellulose indicates the natural complex structure of cellulose that has the potential properties for food and cosmetic applications. However, the optimized condition of cellulose extraction, bleaching process, purification, and also mechanical properties would be further studied.

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REFERENCES

Amnuaysin, N., Seraypheap, K. & Kidyoo, M. 2020. Delayed Softening Of 'Hom Thong'banana Fruits During Postharvest Storage Following Hot Water Treatment.

Erdogan Orhan, I., Küsmenoğlu, Ş. & Sener, B. 2008. Fatty Acid Profile Of Fresh And Dried Banana (Musa Sapientum L. Var. Cavendishii Lamb.) Peel Oils : Taze Ve Kurutulmuş Muz (Musa Sapientum L. Var. Cavendishii Lamb.) Kabuk Yağlarinin Yağ Asiti Profi. *Ankara Universitesi Eczacilik Fakultesi Dergisi*, 0103-019.

Khawas, P. & Deka, S. C. 2016. Comparative Nutritional, Functional, Morphological, And Diffractogram Study On Culinary Banana (Musa Abb) Peel At Various Stages Of Development. *International Journal Of Food Properties,* 19, 2832-2853.

Lepage, G. & Roy, C. C. 1984. Improved Recovery Of Fatty Acid Through Direct Transesterification Without Prior Extraction Or Purification. *J Lipid Res,* 25, 1391-6.

Lepage, G. & Roy, C. C. 1986. Direct Transesterification Of All Classes Of Lipids In A One-Step Reaction. *J Lipid Res,* 27, 114-20.

Mordi, R. C., Fadiaro, A. E., Owoeye, T. F., Olanrewaju, I. O., Uzoamaka, G. C. & Olorunshola, S. J. 2016. Identification By Gc-Ms Of The Components Of Oils Of Banana Peels Extract, Phytochemical And Antimicrobial Analyses. *Research Journal Of Phytochemistry,* 10, 39-44.

Palacios, S., Ruiz, H. A., Ramos-Gonzalez, R., Martínez, J., Segura, E., Aguilar, M., Aguilera, A., Michelena, G., Aguilar, C. & Ilyina, A. 2017. Comparison Of Physicochemical Pretreatments Of Banana Peels For Bioethanol Production. *Food Science And Biotechnology,* 26, 993-1001.

Pelissari, F., Sobral, P. & Menegalli, F. C. 2014. Isolation And Characterization Of Cellulose Nanofibers From Banana Peels. *Cellulose,* 21.

Pelissari, F. M., Andrade-Mahecha, M. M., Sobral, P. & Menegalli, F. C. 2017. Nanocomposites Based On Banana Starch Reinforced With Cellulose Nanofibers Isolated From Banana Peels. *J Colloid Interface Sci,* 505, 154-167.

Singanusong, R., Tochampa, W., Kongbangkerd, T. & Sodchit, C. 2014. Extraction And Properties Of Cellulose From Banana Peels. 21, 201-213.

Tibolla, H., Pelissari, F. & Menegalli, F. C. 2014. Cellulose Nanofibers Produced From Banana Peel By Chemical And Enzymatic Treatment. *Lwt - Food Science And Technology,* 59, 1311–1318.

Tibolla, H., Pelissari, F. M., Martins, J. T., Vicente, A. A. & Menegalli, F. C. 2018. Cellulose Nanofibers Produced From Banana Peel By Chemical And Mechanical Treatments: Characterization And Cytotoxicity Assessment. *Food Hydrocolloids,* 75, 192-201.

Veeramachineni, A. K., Sathasivam, T., Muniyandy, S., Janarthanan, P., Langford, S. J. & Yan, L. Y. 2016. Optimizing Extraction Of Cellulose And Synthesizing Pharmaceutical Grade Carboxymethyl Sago Cellulose From Malaysian Sago Pulp. *Applied Sciences,* 6, 170.

Yu, S., Sun, J., Shi, Y., Wang, Q., Wu, J. & Liu, J. 2021. Nanocellulose From Various Biomass Wastes: Its Preparation And Potential Usages Towards The High Value-Added Products. *Environmental Science And Ecotechnology,* 5, 100077.

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The development of a multi-dimensional reporting system for monitoring operations and the decision of the administrators. study case of Halal Science Center Chulalongkorn University, Pattani Office

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Abstract

The Halal Science Center Chulalongkorn University at Pattani Office was established in 2009 with the primary mission of developing areas according to the Indonesia-MalaysiaThailand Growth Triangle (IMT-GT) through the project to increase personnel potential from the border provinces in the southern region until now. In addition, must report the performance of the various activities Performance in finance, supplies, content, people, public relations, Etc. Through weekly reports. The Executive Committee of The Halal Science Center Chulalongkorn University's monthly report and the report found that have the problem of the past operations cannot see the overall picture of the whole operation. Therefore, this research was conducted to develop a system for monitoring and reporting the performance in a multi-dimensional format and testing the users' satisfaction. So, results of the study showed that the multi-dimensional performance tracking and reporting system had been developed with Microsoft Excel that can reduce the operating time reduce about 70.00%, while Users of the monitoring and reporting system have an outstanding level of satisfaction from 15 total users.

Keywords: *Dashboard; Monitor, Office dashboard, Excel dashboard*

INTRODUCTION

Nowadays, the economy is motivated by digital information becoming the biggest asset of an organization. Business operations and work processes are relied on information to create added value. Then for absolute competitive advantage, companies in all industries develop strategies for information management. Knowledge management was used to different priorities and harvest and leverage information to make business decisions [3]. Information and knowledge This is therefore extremely important for management's decision-making. Suppose accessed information Manage and select information efficiently. Therefore, the system for supporting information management to be accurate and complete will support the management and decision-making with the most correct and effective.

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The Halal Science Center Chulalongkorn University (HSC) has expanded the responsibility in the southern border part of Thailand since 2009 by establishing a Pattani office that works on "Project to increase personnel from in southern border Thailand. The projects that have been commissioned from the center must report the performance of various activities, financial performance, supplies, content, people, and public relations, Etc., through weekly reports. Monthly report and report of the Executive Committee of the Halal Science Center Chulalongkorn University Period to acknowledge the problems and needs of the management of the Halal Science Center.

Reporting problems

a) There is much information from different parties to be collected and scattered among different parties. It cannot be used
b) There is no linkage of information from different parties. That is a puzzle in different parts, which are not projected overall picture of the operation.
c) Unable to keep track of the operations of various departments, the current operational status, must wait for reporting to be informed of the current state of operations.
d) Lack of multi-dimensional reporting and inability to analyze data for organizational decision-making.
e) There was a delay in reporting, doing the job out of date, incapability to analyze data, and forecast operations trends.

Executive needs

a) Overview of the current state of the organization.
b) Past operating trends and future decisions of the activities.
c) Visualize the performance in each section. Linked to a big picture to follow up on problems and resolve them promptly.

Objective

a) To develop the performance monitoring and reporting system in a multi-dimensional format to monitor operations and support management decisions.
b) To test the satisfaction of users of the system to track and report the performance in a multi-dimensional format

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RESEARCH METHODOLOGY

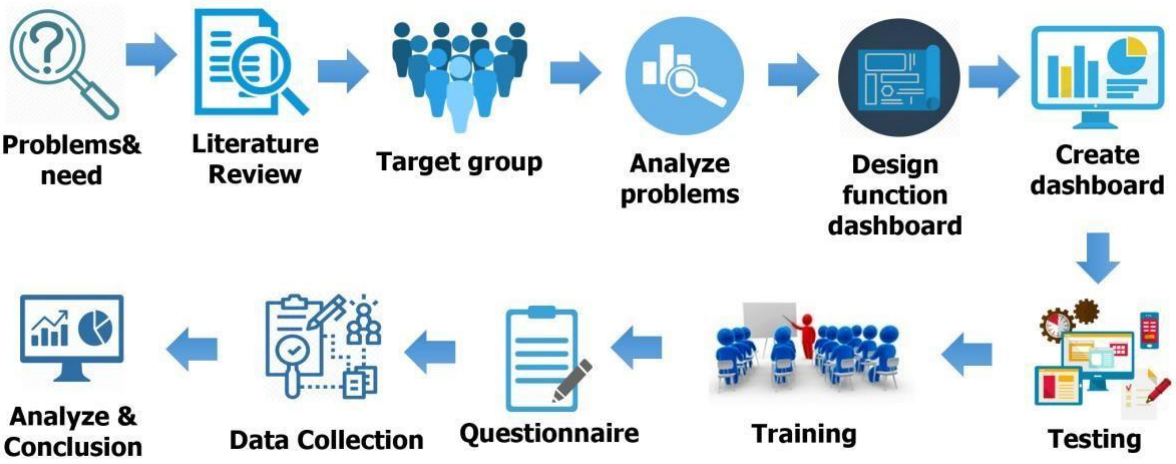

Figure 1 The Methodology Framework of The Study

FINDINGS AND DISCUSSION

This research can be summarized as follows

Development of monitoring and reporting system on the performance in a multidimensional format.

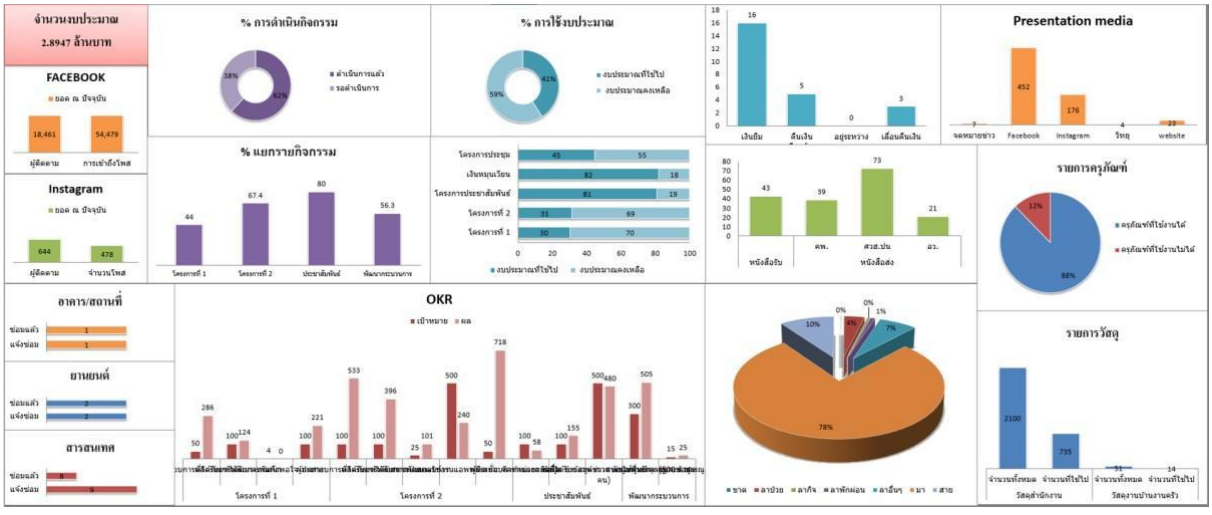

Figure 2 Monitoring and reporting dashboard presented in multidimensional format.

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Reducing time to follow up and report on the performance of The Halal Science Center Chulalongkorn University (Pattani office).

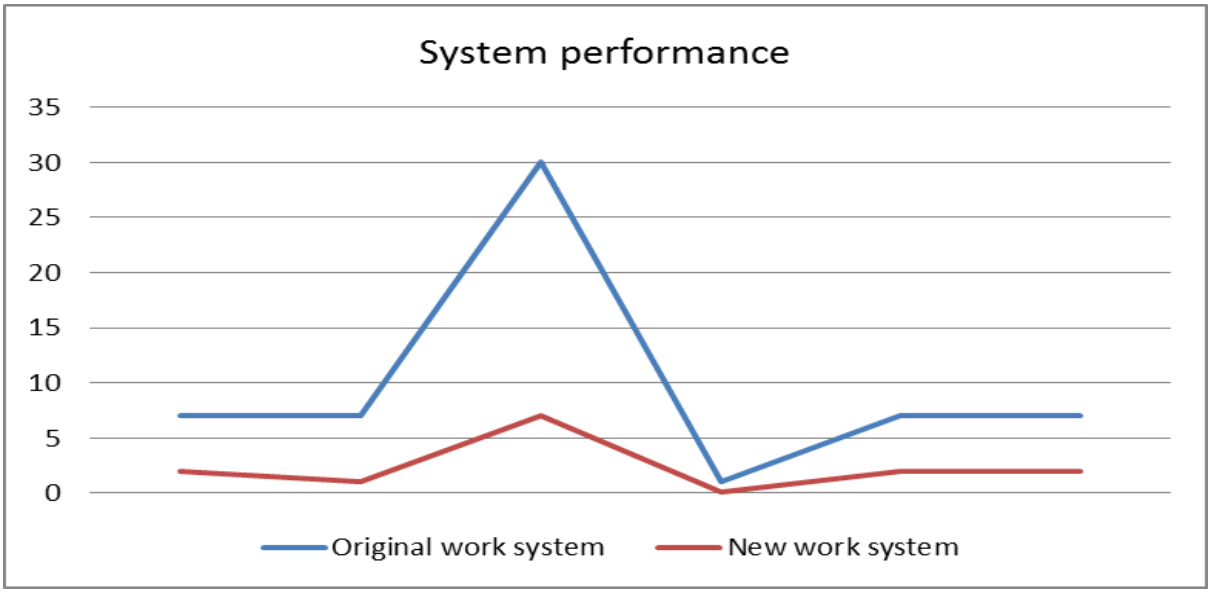

Figure 3 Line graph showed system performance

Reduction of time monitoring and reporting of performance comparing pre-adjustment and post-adjustment operations the follow-up and reporting of results were improved by ten days after improvement to 3 days, which reduced the tracking and reporting time by 70.00%.

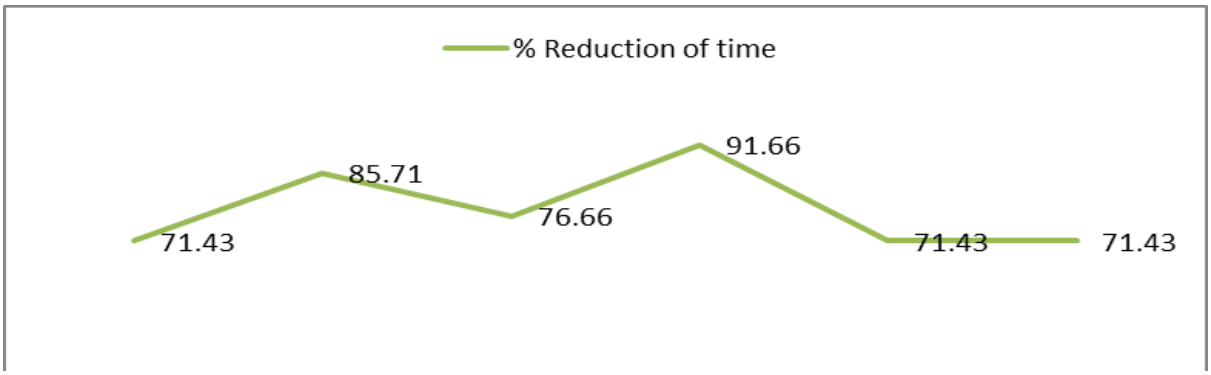

Figure 4 Line graph showed reduction of time (percent)

Satisfaction with using multi-dimensional tracking and reporting system.

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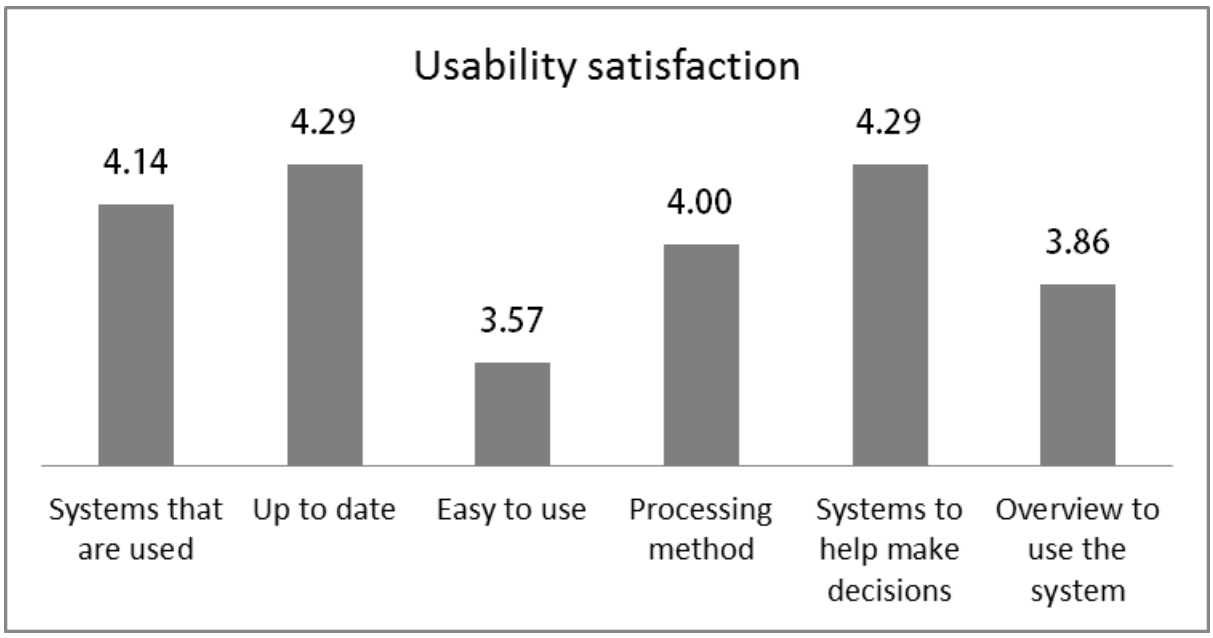

Figure 5 Bar chart showed usability satisfaction

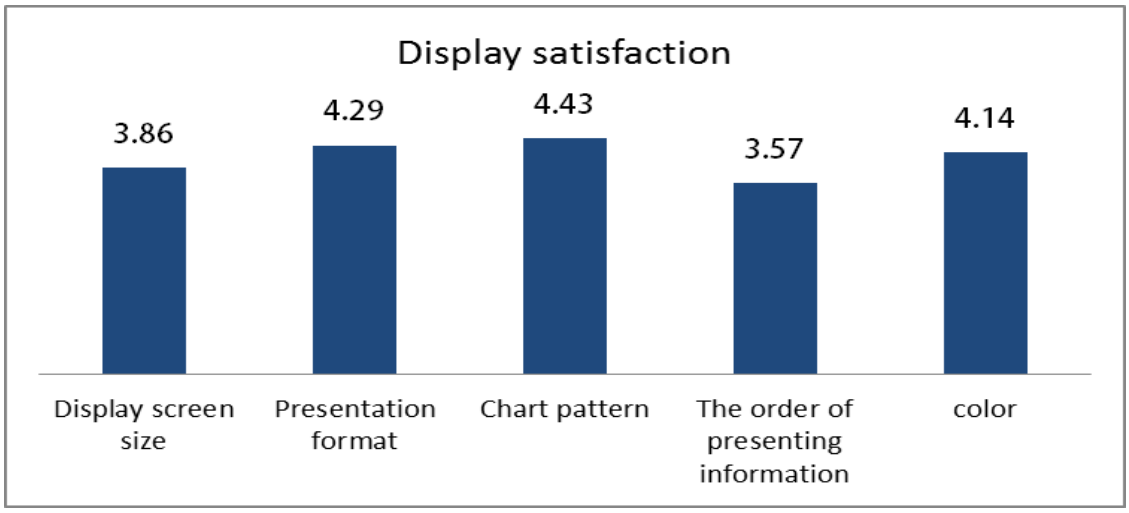

Figure 6 Bar chart display 5 category of satisfaction

The satisfaction level of the use of multi-dimensional monitoring and reporting systems to monitor management operations and decisions is shown in the figure. From the population of 15

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people of the Halal Science Center Chulalongkorn University Pattani Office, it was found that the satisfaction level of the system users was outstanding. It accounted for 80.80%.

DISCUSSION

The development of a monitoring and reporting system is necessary for improving the performance of the Halal Science Center Chulalongkorn University Pattani Office to manage the project. A multi-dimensional reporting system provided the dashboard to see the overall picture of the operation and follow up to solve the operational problems on time. Figure 2 shows the reduction of the operating time to 7 days, with 70.00 percent of the monitoring and reporting time prior to system development. However, the satisfaction level of the monitoring and reporting system was outstandingly presented in 80.80 percent of the total population of 15 people by this research reducing the operating time. In addition, it provided an overview of current project execution and agency status and helped management decision-making drive further work.

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REFERENCES

Chalump Supawadee(2019). Leader, “6 Steps Required in Data Management in Organizations”., Retrieved 2 November 2021. From. https://www.theeleader.com/digitaltransformation/6-steps-needed-to-manage-data-in-the-organization/

Devender Maheshwari & Marijn Janssen (2014): ACM digital library, “Dashboards for Supporting Organizational Development: Principles for the Design and Development of Public Sector Performance Dashboards”.

Rattana Suwanwichanee and Pralee Maneerat, “The development of a multi-dimension report system to support policy decisions of the National Research Council of Thailand,” Sripatum review of science and technology Edition, Vol. 9 (Jan.-Dec.), pp. 66 – 75, 2017.

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The Model Development for Early Lung Cancer Analysis by Using Image Processing and Neural Network

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Abstract

Lung cancer is the leading mortality disease for men and women compared to other organ cancers. The leading cause is smoking until the patient begins to show symptoms. Therefore come to see a doctor determine that the disease has spread in the last stage, causing cumbersome and complex treatment. Therefore, early screening of patients is very important to allow patients to enter receive treatment in a timely manner and have a chance to recover from the disease. This research has developed a model for early lung cancer analysis by using a CXR image that can screen a large number of patients when they are asymptomatic. Let's improve the image enhancement to reduce the noise with median filter and then go into image processing by image segmentation with Active contour algorithm, image edge detection with Laplacian of Gaussian (LoG) algorithm, and image extraction with Shape and GLCM in combine with data classification with a neural network using MLP compared against SVM classifiers. Training and testing the performance of the model by the result of MLP provides a better time and up to 99% accuracy.

Keywords: *Image Processing, Lung Cancer, Neural Network, Support Vector Machine*

INTRODUCTION

Lung cancer has the highest mortality rate in both men and women compared to other organ cancers. The main cause is smoking, together with other environmental factors, including the inevitable increase in air pollution. Although evolution continues to aid the diagnosis of early and metastatic lung cancer, the mortality rate did not decrease because patients' access to primary lung cancer screening is limited, and the symptoms of early-stage lung cancer are unclear. Patients visit their doctor when they have symptoms. The disease has spread so much that it is difficult to treat. There are cases where doctors accidentally detect nodules in the lungs when the patient goes for a check-up. Therefore, proactive screening is important to allow asymptomatic patients to be screened early if abnormalities are found in the early stages. Doctors will be able to cure the disease in time to prevent spread to other organs, and the patient has a chance to recover.

From studying and exploring the problems that arise, Innovative information technology has been used to develop a model for the analysis of lung X-ray images of lung cancer patients and lung cancer patients with image processing and neural networks. Let's start by using CXR images, which are the

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primary source of important information. Many diseases can be screened to improve image quality, known as image preprocessing to reduce noise. The resulting image is processed using image segmentation using Active Contour algorithm, image edge detection using Laplacian of Gaussian (LoG) algorithm, and image feature extraction using Shape and GLCM combined with neural network classification using MLP classifier and the most popular is Support Vector Machine (SVM) classifier to compare performance evaluation results. The objective is to develop a chest X-ray image analysis model using image processing techniques and neural networks for the early screening of lung cancer abnormalities. It will be an important guideline for physicians to plan treatment and follow-up in the next steps.

LITERATURE REVIEW

Nadkarni et al. (2019) detect lung cancer by image feature extraction of shape only and classification with SVM. No other classification data is compared. Jena et al. (2019) detect lung cancer by image extraction of shapes and textures combined with an SVM classifier. The acquisition of primary image processing data by image segmentation does not exist, and no other classification data is compared. M. Tech et al. (2018) detect lung nodules by image feature extracting with GLCM for texture only classified by MLP vs. KNN. Chellan et al. (2018) Detect lung cancer with Region Base Active Contour image segmentation, GLCM feature extraction for texture data only, no comparison of other classification data. Kasinnathan et al. (2017) Detect lung tumor with watershed segmentation and GLCM feature extraction for texture only combined with classification SVM, and no other classification data is compared. All of the above research uses data from CT scans that have limited access to patient screening.

In this study, an easily accessible lung X-ray image source was used for the initial screening not only to monitor treatment but also to cover the entire lung segmentation process as well as the extraction of image characteristics with both spatial and surface characteristics and distinguishing them with popular classifiers, both MLP and SVM classifiers, in order to obtain the optimal classifier with the best accuracy and time.

RESEARCH METHODOLOGY

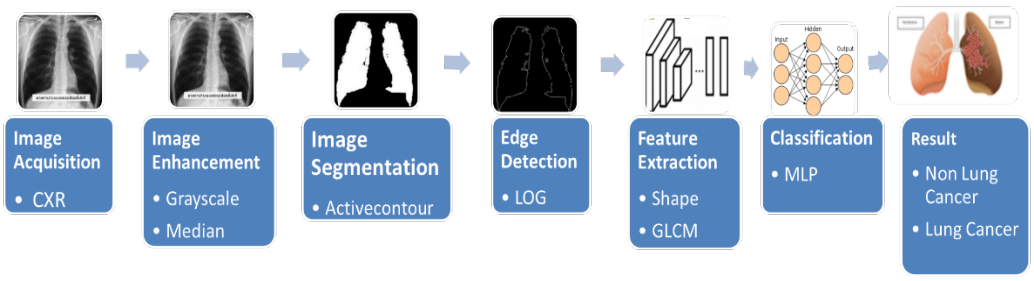

Figure 1. Early Lung Cancer Analysis Model

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This research is a model designed to develop the analysis of Chest x-ray images. To be screened for early lung cancer using image processing and neural network as shown in Figure 1. Each step is detailed as follows.

A. Preparation Data

1*) Data Collection* start collecting CXR images from JSRT Digital Image Database by downloading Nodule 154 images file and NonNodule 93 images file. The original image file is in the format of.IMG with image size 2084*2084 pixels, save all data to a computer for further review.

B. Image Pre-Processing

1) Image Acquisition is the process of importing data both nodule and nonnodule CXR images, image size 2048*2048, grayscale, selecting 100 samples into the computer database as JSRT Dataset file name, enter to MATLAB R2021a program when import data convert the original.IMG file to .jpg file for use in the program's processing with the open file, read the file, write file, close file, and then the image is saved in the workspace for the next process.

2) Image Enhancement after going through image acquisition and then image enhancement with Median Filter when A is an input image, grayscale also filter the image with Median Filter to reduce noise that has the appearance of Salt and pepper well with the command B = medfilt2(A) where A is a 2D variable represents a pixel grayscale image 2048*2048*uint8 8-bit integer format. Since the received image is large, it is scaled down to speed up processing with the command B = imresize(A, scale) when the scale is the scaling ratio is 0.25. After resizing, the output image will be a pixel grayscale image 512*512*uint8 format, 8-bit integer, the resulting image after filtering will be more smooth and preserve the details without loss of data.

C. Image Processing

1) Image Segmentation with Active Contour. Once the image has been enhanced, it goes into image segmentation with Active Contour algorithm with function of bw = activecontour(A,mask,n) segments the 2-D grayscale image A into foreground is lung and background regions is boundary of non-pulmonary using active contour segmentation when the mask is a binary image same size as A image 512*512*double that specifies the initial state of the active contour represent the boundaries of lung regions (white) with function of mask = zeros(size(A)) replace value with 0 is background (black) in mask define the initial contour position used for contour evolution to segment the image as the object of interest (lung) with function m(140:155, 140:155)=1;m(375:390, 140:155)=1; replace value with 1 is foreground (white) both left and right of lung, n is maximum number of iterations = 800 and the output image bw is a binary image where the foreground(lung) is white and the background is black obtain faster and more accurate segmentation results, specify an initial contour position that is close to the desired object boundaries.

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2) Image Edge Detection with Laplacian of Gaussian (LoG) is a necessary process to find the edge image using the Laplacian of Gaussian algorithm of the image interested (lung). The image edge detection represents an area of the edge of an image using function bw = edge(I, method, threshold) when I am an input variable. This research is the output image from the Active Contour algorithm. The technique used is Laplacian of Gaussian ('log'), and the threshold value used is 0.001. The output image (bw) is 512*512*logical, black and white logical type shown in Figure 2.

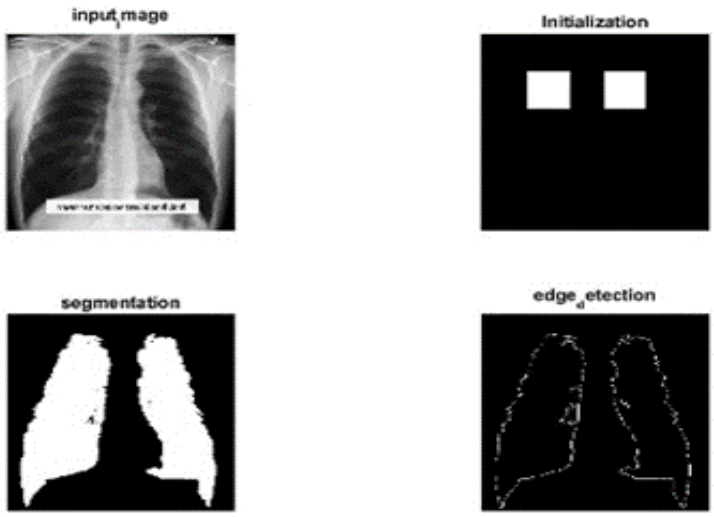

Figure 2. (Input image), mask (initialization), image segmentation, image edge detection

3) Image Feature Extraction with Shape and GLCM. After edge detection, it goes into image feature extraction for extracts important information of image in the Shape section by using with function stats = regionprops (BW,properties) when BW represents a binary input image variable and properties represents a group of connected pixels with similar is selected 4 attributes: Area, Perimeter, Eccentricity, Solidity and then texture analysis using the GLCM (gray level co-occurrence matrix) by begin generating a GLCM of the same size as the input image using the function glcm = graycomatrix(I) when I represents the input binary image, graycomatrix generates value GLCM by calculating the frequency of pixels with grayscale values (Gray level intensity) After that, use function stats = graycoprops(glcm, properties) to calculate the statistics specified in the properties from glcm to analyze the texture these statistics provide information about the texture of an image feature the selected values have 4 attributes: Contrast, Correlation, Energy and Homogeneity. When in collected total 8 attribute values in 1 image were obtained saved to workspace for data classification in next process

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D. Classification

After going through the process of image feature extraction then create A input variables were collected from image feature extraction is numerical type included all 8 attributes consist of Area, Perimeter, Eccentricity, Solidity, Contrast, Correlation, Energy, Homogeneity and target variables (predictor) represents of the lung cancer is 1 and non-lung cancer is 0 include total 100 samples in the file name DATASETA. The variable names are column aligned, and the observation part is in the row. When entering neural network processing, specify the name of the input variable (ip01) by converting dataset array with input variable display in a row aligned, and observation values display in a column aligned and specify the name of the target variable (tg01) by converting dataset array with target variable display in a row aligned and observation values display in a column aligned after that the input (ip01) and target (tg02) variables are stored in the workspace enter to the Apps. Neural Net Pattern Recognition for MLP classifier and classification learner for SVM classifier for next process.

1) Neural Network with the MLP (multilayer perceptron) classifier, the dataset of lung cancer and non-lung cancer patients is imported all 100 samples obtained from the image feature extraction of all 8 attributes, and the target result is lung cancer and non-lung cancer. Then the network begins to train data to create a network model to practice learning the network throughout validation of the data to find the best model until an acceptable error value is obtained; therefore, stop learning and use that best model to test the network by importing the same dataset as training after that to train data until is obtained appropriate value and can evaluate network the accuracy performance with a confusion matrix.

a) Select Data to define the input data and target data values of the neural network saved in the workspace, replace the input data with ip01 and the target data with tg01 of the network and select the dataset as matrix columns.

b) Validation and Test Data select a percentage at random validation 15%, testing 15% to be used to evaluate performance and 70% as training data, learners should have values covering the range of Validation and Testing for creating a system of model shown in Figure 3.

c) Network Architecture customize the hidden layer of the network where this panel provides the default number of hidden neurons is 10 after training the network if it still does not work well can become back to this panel to change the number of neurons until the result of neural network processing provides highest accuracy is obtained considering the acceptable error value after training data shown in Figure 4.

d) Train the Network after customizing the number of hidden neurons of the network will start to train for creating a network to practice learning the network throughout validation the data to find the best model until an acceptable error value is obtained therefore stop learning shown in Figure 5.- 6.

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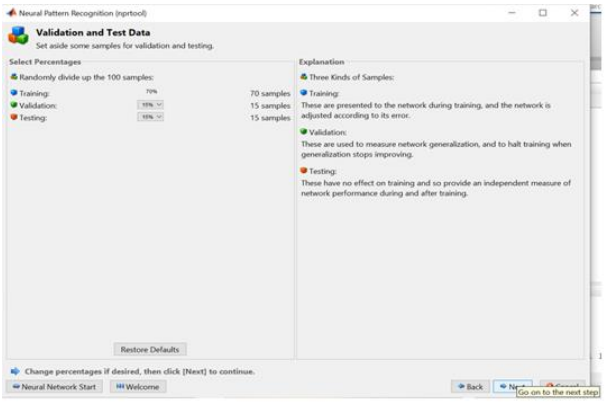

Figure 3. Validation and Test data

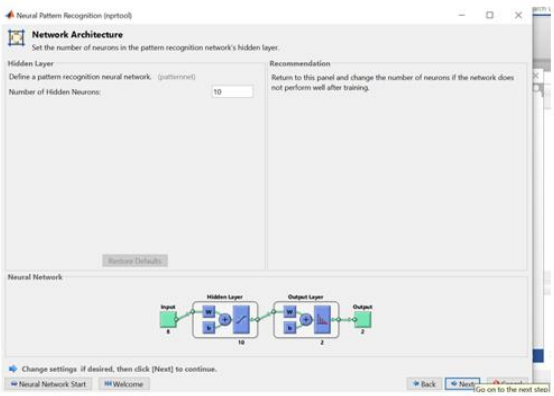

Figure 4. Network Architecture

Figure 5. Train Network

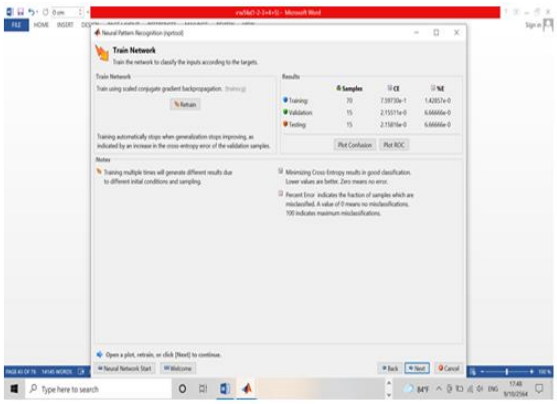

Figure 6. The error value is minimal (%E)

e) Performance Evaluate Network after training the network to the best model, the network performance is assessed for accuracy by testing the error-measurement network independent of all previously used segmented data before. When importing the same sample dataset as the training dataset, both the input data and the target sample data samples are in matrix column format for network testing. Error-values are calculated, and the test performance evaluation results are displayed with Confusion Matrix tables can be found as shown in Figure 7.

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Figure 7. Confusion Matrix Test Neural Network

2) Support Vector Machine to start imported datasets from the numeric matrix format of the DATASETA file. Select the cross-validation format. Then select a model type that contains all the SVM training data to find the best kernel as linear at the training accuracy values are shown in Figure 8. The model is then tested by importing the same dataset. To determine the accuracy obtained from the test, as shown in Figure 9.

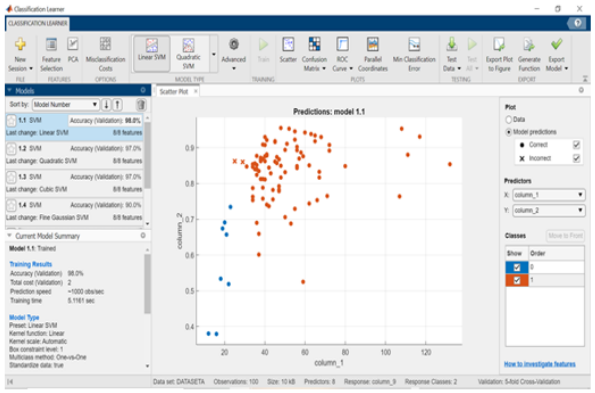

Figure 8. Training SVM

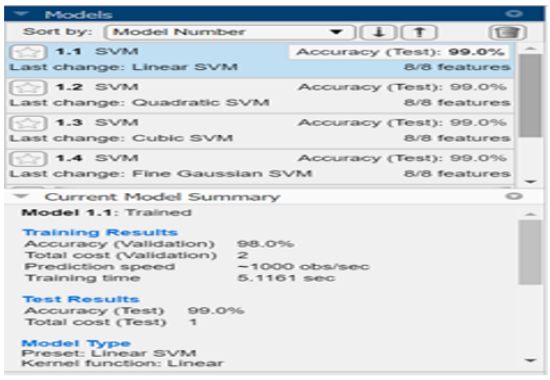

Figure 9. Testing SVM

3) Performance Evaluation CXR image classification of lung cancer and non-lung cancer patients with the best classifier of a neural network and SVM, the results of the correct probabilities are evaluated using the Confusion Matrix method to show the test validity values shown in Table 1.

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Table 1. Confusion Matrix

	Predict Positive	Predict Negative	
Actual	True Positive (TP)	False Negative (FN)	Positive
Actual	False Positive (FP)	True Negative (TN)	Negative

Accuracy is the amount of data that accurately predicts the data in all group classifications

$$= \frac{\text{true positive + true negatives}}{\text{true positive + false positive + false negative + true negative}} \quad (1)$$

where,
true positive (TP) is the result of the prediction that it is in persons with a disease
true negative (TN) is the result of the prediction that it is not in persons without a disease
false positive (FP) is the result of the prediction that it is in persons without a disease
false negative (FN) is the result of the prediction that it is not in persons with a disease

FINDINGS AND DISCUSSION

A study for CXR images of patients with lung cancer and non-lung cancer number of 100 samples using MATLAB R2021a program result of image processing shown in Table 2.

Table 2. Input Variable by Image Feature Extraction and Target Variable by Predictor

NO.	Area	Eccentricity	Solidity	Perimeter	Contrast	Correlation	Energy	Homogeneity	Predictor
1	39	0.812907	0.322314	46.198	0.013615	0.352011	0.96556	0.993192729	1
4	40	0.9073443	0.333333	47.422	0.01282	0.2973912	0.969099	0.993590234	1
5	40	0.9295226	0.344828	43.741	0.007	0.3687	0.9821	0.9965	1
6	36	0.8245825	0.36	40.764	0.008004	0.3879577	0.978984	0.995998196	1
7	56	0.8434234	0.264151	64.748	0.013683	0.330124	0.966077	0.993158329	1
8	62	0.7441517	0.295238	67.138	0.008478	0.3456748	0.978638	0.995761222	1
9	37	0.8348882	0.293651	41.194	0.010908	0.3449775	0.972557	0.994545774	1
10	44	0.7818214	0.321168	50.49	0.014073	0.3182831	0.965481	0.992963399	1
11	18	0.5332149	0.4	21.172	0.012567	0.316557	0.969202	0.993716365	0
12	25	0.8621657	0.431034	29.616	0.009609	0.401672	0.974424	0.995195542	0
14	20	0.6916028	0.4	22.95	0.014241	0.2647902	0.966591	0.992879311	0
15	16	0.3788861	0.421053	19.212	0.009158	0.3457139	0.976929	0.995421049	0

From Table 2. It shows the numerical statistics of the property selected from the image feature extraction of all 8 attributes is input variable consists of: area, perimeter, eccentricity, solidity, energy, contrast, correlation, homogeneity including target variable predictor value 1 represents lung cancer patients and 0 represents is non lung cancer patients total of 100 observation. All statistical data were collected in the DATASETA file name

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to enter the classification the neural network MLP and the SVM classifier to further assess the validity of the two classifiers, as shown in Table 3.

Table 3. The classification was tested with neural network MLP and classification learner SVM based on the accuracy method.

Classifier	MLP	SVM
True Positive	89	90
False Positive	0	1
False Negative	1	0
True Negative	10	9

From Table 3. Show that MLP neural network, together with the image feature extraction data, was able to classification CXR images correctly TP up to 89 images, misclassification FN 1 image from total 90 lung cancer images, and classifiers images correctly TN 10 images, misclassification FP 0 image from total 10 non-lung cancer images, while SVM in combine with image extraction data, was able to classification CXR images correctly TP up to 90 images, misclassification FN 0 image from total 90 lung cancer images, and classifiers images correctly TN 9 images, misclassification FP 1 image from total 10 non-lung cancer images. If considering the Confusion Matrix values in the classification of MLP and SVM neural networks, the test results are shown in Table 4.

Table 4. Confusion Matrix values from MLP and SVM classifiers.

Classifier	**Accuracy**	**Precision**	**TPR**	**TNR**	**FPR**	**FNR**	**Time/sec**
MLP	99%	100%	98.89%	100%	0%	0.01%	0
SVM	99%	98.9%	100%	90%	0.1%	0%	5.1161

Table 4. Show confusion matrix values of the neural network MLP classifier and SVM classifier, showing various efficacy values obtained from result predicting for lung cancer and non-lung cancer patients both the MLP classifier provides accuracy 99% , precision 100%, TPR recall or (sensitivity) 98.89%, TNR(specificity)100%, FPR 0%, FNR 0.01% and time 0 seconds, while the classifier SVM provides accuracy 99%, precision 98.9%, TPR 100%, TNR 90%, FPR 0.1%, FNR 0% and time 5.1161 seconds. When comparing the two classifiers for 100 observation, this study chose a classifier MPL is used in a model to optimize system operation which provides very high performance at the better time.

This research has been found that when image segmentation with Active contour, both lung positioning and appropriate mask size were used to accurately contour the lung area, and upon edge detection, this study found with (LoG), the edge locating efficiency is greatly improved in the detection of pulmonary nodules. In neural network classification of lung cancer and non-lung cancer patients, the MLP classifier compared to the SVM classifier gave similar values, where MLP had a better time.

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CONCLUSION

This research presents the development of a model for early lung cancer screening from CXR images by image processing and Neural Network. The input variables consisted of area perimeter eccentricity solidity energy correlation contrast homogeneity and target variables predictive lung cancer and non-lung cancer. Select an appropriate model, use Cross-Validation, start training, validation, and testing the model for evaluating efficiency from the Confusion Matrix. The results of Neural Network accuracy prediction depend on the selection of the variables in the study. Selection of good information to train the network and choosing a suitable model. Limitations of this research are the number of samples and setting the proper position of the mask for Active Contour segmentation when the data sources are different, and in the future, further disease stages can be analyzed.This model to analyze CXR images of patients with lung cancer and non-lung cancer by image processing and Neural Network MLP classifier with Confusion Matrix value TPR = 98.89% TNR = 100% FPR = 0% FNR = 0.01% Accuracy = 99%.

REFERENCES

Gonzalez, R. C. & Woods, R. E. (2002), *Digital Image Processing*, 2nd edn, Prentice Hall, New Jersey.

Gonzalez, R. C. & Woods, R. E. (2008), *Digital Image Processing*, 3rd edn, Prentice Hall, New Jersey.

Gonzalez, R. C. & Woods, R. E. (2018), *Digital Image Processing*, 4th edn, Pearson.

Kass, M., Witkin, A., & Terzopooulos, D. (1988), *Snake: Active contour models*, *International Journal of Computer Vision 1*, pp.321-331.

Nadkarni, N.S. & Borkar, S. (2019), "Detection of Lung Cancer in CT Images using Image Processing", *International Conference on Trends in Electronics and Informatics (ICOEI)*, pp.863-866, IEEE database, doi: 10.1109/ICOEI.2019.8862577

Jena, S.R., George, T., & Ponraj, N. (2019), "Texture Analysis Based Feature Extraction and Classification of Lung Cancer", *International Conference on Electrical, Computer and Communication Technologies (ICECCT),* IEEE database, doi: 10.1109/ICECCT.2019.8869369

Potghan, S., Rajamenakshi, R., & Bhise, A. (2018), "Multi-Layer Perceptron Based Lung Tumor Classification", *International Conference on Electronics, Communication and Aerospace Technology (ICECA)*, pp.499-502, IEEE database.

Chellan, T.D. & Chellappan, A. (2018), "Novel computer-aided diagnosis of lung cancer using bag of visual words to achieve high accuracy rates", eISSN 2051-3305, pp.1941-1946.

Kasinnathan, G. & Selvakumar, J. (2017), "Lung tumor area recognition and classification using EK-mean clustering and SVM", *International Conference on Nextgen Electronic Technologies: Silicon to Software (ICNETS2)*, IEEE database, doi: 10.1109/ICNETS2.2017.8067906

Avinash, S., Manjunath, K., & Kumar, S.S. (2017), "An improve image processing analysis for the detection of lung cancer using Gabor filters and watershed segmentation technique", *International Conference on Inventive Computation Technologies (ICICT)*, IEEE database, doi: 10.1109/INVENTIVE.2016.7830084

MathWorks, (2021), Available: **<https://www.mathworks.com>.** Accessed 15 December 2021.

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