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Safety Evaluation of Secretome Proteins from *Paenibacillus polymyxa* Kp10 and *Lactococcus lactis* Gh1 as the Potential Antimicrobial Therapeutic Agent

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Secretome proteins, *Paenibacillus polymyxa* Kp10, *Lactococcus lactis* Gh1, Safety evaluation and Antibiotic resistance

Abstract

The secretome proteins of *Paenibacillus polymyxa* Kp10 and *Lactococcus lactis* Gh1 were previously found to have the potential for controlling the antibiotic-resistant pathogen. Therefore, their safety evaluation to ensure consumer health is deemed important. This study was to evaluate the toxicity effect of both secretome proteins in human cells against Medical Research Council cell strain 5 (MRC5). Then, their antibacterial activities were tested in human serum to assess the stability of both secretome proteins. The results showed no cytotoxic effects of either secretome protein when MRC5 cells were treated up to the determined concentrations. Therefore, no IC50 was determined. In addition, human serum did not affect the antibacterial activity of both secretome proteins against Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE). In conclusion, both secretome proteins are potentially safe for future development as a potential therapeutic agent.

1. Introduction

Today, the uncontrolled use of conventional antibiotics has caused various problems in the medical field, resulting in the widespread emergence of antibiotic resistance worldwide. Vancomycin-resistant *Enterococcus* (VRE) and Methicillin-resistant *Staphylococcus aureus* (MRSA) are prominent antibiotic-resistant bacteria. They have resulted in significant patient deaths and financial burdens on healthcare systems (Ventola, 2015; Dadgostar, 2019; Zainal Baharin *et al.*, 2021). In addition, antibiotic resistance poses a significant risk to current medical advances (Ventola, 2015; Zainal Baharin *et al.*, 2021; Golkar, Bagasra & Pace, 2014). The efficacy of conventional antibiotics has dramatically deteriorated over time, and more effective therapeutics against infections caused by antibiotic-resistant bacteria are urgently needed (Golkar, Bagasra & Pace, 2014; Sengupta, Chattopadhyay & Grossart, 2013; Wright, 2014). Several studies are searching for alternative compounds that could replace existing antibiotics.

Secretome protein is known as an antimicrobial substance that has the ability to inhibit the growth of bacteria (Damayanti, Rusdiana & Wathoni, 2021) by releasing antimicrobial peptides (AMPs) such as cathelicidin, RNase3, human β-defensins, and calprotectin (Kasiri *et al.*, 2016). Secretome proteins appear to have enormous potential to replace the role of antibiotics (Damayanti, Rusdiana & Wathoni, 2021). Generally, AMPs released by secretome proteins act as host defences, where many of them have been isolated from living entities, such as bacteria, animals, plants, and fungi (Zainal Baharin *et al.*, 2021; Kumar, Kizhakkedathu & Straus, 2018). In previous studies, secretome proteins from *Paenibacillus polymyxa* Kp10 (Kp10) and *Lactococcus lactis* Gh1 (Gh1) were shown to display antimicrobial activity and could potentially replace antibiotics (Zainal Baharin *et al.*, 2022; Mokhtar *et al.*, 2020; Jawan *et al.*, 2021). A recent study showed that Gh1 exhibited antimicrobial activity against pathogenic *Staphylococcus aureus, Listeria monocytogenes, Salmonella* and *Bacillus cereus species* (Jawan *et al.*, 2020), while Kp10 was shown to demonstrate antimicrobial activity against *Escherichia coli* (Mokhtar *et al.*, 2020).

Our recent study has shown that secretome proteins of Kp10 and Gh1 have antibacterial activity against MRSA and VRE (Zainal Baharin *et al.*, 2022). Given their potential as an alternative antibiotic, we further conducted a preliminary safety evaluation on both the secretome proteins by using agar well diffusion assay in the presence of serum for their stability test and cytotoxicity test in MRC5 cells. In common practice,

registered pharmaceutical products are tested for safety and quality by the Ministry of Health through the Pharmaceutical Services Division (Department of Standards Malaysia, 2012). This coincides with the concept of *Halalan Toyyiban*, according to which Allah has commanded us to eat food that is not only halal but also wholesome (Hamdan & Hashim, 2022). Based on the great potential found in both secretome proteins of Kp10 and Gh1, the proteins are expected to be stable in the presence of human serum, and there will be no cytotoxicity effect when tested on human cells. Therefore, the safety assessments conducted in this study would fulfil the halal aspect that the secretome proteins are safe for humans and can be further developed as the potential therapeutic agent.

2. Materials and methods

2.1 Bacterial culture, growth, and cell conditions

Kp10 and Gh1 isolates producing secretome proteins were obtained from Professor Dr. Arbakariya Ariff of the Bioprocessing and Biomanufacturing Research Centre, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. Kp10 was cultured in M17 broth at 37°C while Gh1 were cultured in de Man Rogosa and Sharpe (MRS) broth at 30°C (Mokhtar *et al.*, 2020; Kasimin *et al.*, 2020). For Medical Research Council cell strain 5 (MRC5), the cells were obtained from UPM-MAKNA Cancer Research Laboratory (CanRes) Institute of Bioscience, Universiti Putra Malaysia and were cultured in Dulbecco's Modified Eagle Medium (DMEM) broth at 37°C. Two antibiotic resistant bacteria used in this study, Methicillin resistant *S. aureus* (MRSA) ATCC 700699 and Vancomycin resistant *Enterococci* (VRE*)* ATCC 700221 were obtained from the American Type Culture Collection (ATCC). The inoculates of MRSA and VRE were prepared using the colony suspension method, in which colonies were picked from cultures previously grown on Mannitol Salt Agar (MSA) and sheep blood agar for 24 hrs at 37°C (Arjyal, Kc & Neupane, 2020; Özsoy & Arzu, 2017), and transferred to Brain Heart Infusion (BHI) broth before incubating for 24 hrs at 37°C. All media were purchased from Oxoid, UK, except for DMEM, purchased from Thermo Fisher Scientific, USA.

2.2 Preparation of secretome proteins from cell-free culture supernatants of Kp10 and Gh1

Secretome proteins of Kp10 and Gh1 were isolated and purified from the bacteria's cell-free culture supernatant using a modified version of the ammonia sulphate precipitation technique (Duong-Ly & Gabelli, 2014). First, Kp10 and Gh1 were cultivated for 24 hrs at 37°C in M17 and MRS broth, respectively. Then, 1000 mL of a suspension of cultivated bacteria was aliquoted into 50 mL falcon tubes and centrifuged at 10,000 x g for 15 mins at 4°C. With the use of a magnetic stirrer, all cell-free supernatants were mixed into a sterile bottle, and 567 g $(NH_4)_2SO_4$ was added until it was dissolved (Thermo Fisher Scientific, USA). After that, the solvents were aliquoted into 50 mL tubes and incubated at 4°C overnight. After incubation, the tubes underwent a 15-minute, 10,000 x g centrifugation at 4°C. Upon removing the supernatant, the pellet was dissolved with distilled water and kept overnight at 4°C. Finally, all the tube contents were combined into one sizable beaker to prepare the inoculum.

2.3 Agar well diffusion assay for antibacterial activity and serum stability test

Modified agarose diffusion assay detected the antibacterial activities and serum stability test of secretome proteins from Kp10 and Gh1. Briefly, a single colony of each MRSA and VRE was grown in trypticase soy broth (TSB, 30 g L^{-1}) overnight at 37°C under aerobic conditions. 2 x 10^8 CFU mL^{-1} of the bacterial culture of each strain was added to warm (50–55°C) sterile agarose [1% agarose (low EEO, Sigma, St. Louis, MO), 0.03% nutrient broth, and 10 mM Phosphate Buffer Saline (PBS), pH 7.4 (1:100 *v/v*). For antibacterial activity, 10 µL samples of secretome proteins from Kp10 and Gh1 were added to 3 mm wells punched by agar punch (BioRad Laboratories, Hercules, Canada). Then, 0.2 mol L^{-1} sodium acetate (solvent) and 20000 IU penicillin-streptomycin solution of the same volume were added as a negative and positive control, respectively. After overnight incubation at 37°C, the diameter of each clean zone of growth inhibition was measured, indicating the antibacterial activity of Kp10 and Gh1 against MRSA and VRE strains. A stock of human serum was obtained from the Haematology Laboratory, Faculty of Medicine and Health Science UPM for the stability test. Then, the secretome proteins were diluted in PBS or human serum for sample preparation at a different minimum inhibitory concentration (MIC). Then, 10 µL of each sample was again added to 3 mm wells punched by agar punch (BioRad Laboratories, Hercules, Canada). Human serum without the addition of secretome proteins served as a negative control. The inhibition zone was measured after 24 hrs incubation at 37°C.

2.4 Determination of Minimum Inhibitory Concentration (MIC)

The Resazurin-based 96-well plate microdilution method was used to determine the MIC of each secretome protein (Elshikh *et al.*, 2016). Resazurin was prepared in distilled water at 0.02% (wt/vol), sterilised by filtration, and stored at 4°C for up to two weeks after preparation. The direct colony suspension method was used to prepare the organism's saline suspension at the McFarland 0.5 turbidity standard density, which corresponded to 1-2 x 10^8 CFU/ml. Plates were prepared aseptically, and a sterile 96 well plate was properly labelled. A volume of 100 µl of the test material was pipetted into the first row of the plate (Well 1). In the case of the other wells, 50 µl of Muller Hinton broth (MHB) was added (Wells 2-12). Serial dilution was performed using a multichannel pipette, starting from Well 1 and finishing at Well 10. Well 11 and 12 were used as negative control. The tested concentrations of the different samples were achieved through doubling serial dilution. Finally, 1µl of tested bacteria was added to each well. Following overnight incubation at 37°C, 30 µl of resazurin (0.02%) was added to all wells. This was followed by further incubation for 2-4 hrs to observe any colour change. The MIC is defined as the lowest concentration of the test material to inhibit growth. Columns with no colour change (the blue resazurin colour remained unchanged in no growth condition) were scored as MIC value after incubation. The presence of pink and purple colours indicated growth. The test was run in triplicate.

2.5 Cytotoxicity assays

MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was used to determine the potential cytotoxicity of the secretome proteins. Cell culture with 2 x 10^3 cells/ml concentration was prepared and plated (100µl/well) onto 96-well plates. The diluted ranges of sample extracts (100, 50, 25, 12.5, 5, 1, 0.5 (µg/ml) were added to each

well and further incubated for 72 hrs. MTT solution was added to the cell suspension by the end of incubation and continued for another 3-hrs incubation. After the solubilisation of the purple formazan crystals using Dimethyl sulfoxide (DMSO) was completed, the Optical Density (OD) of the plant extract was measured using an ELISA reader at a wavelength of 570 nm. The cytotoxicity was recorded as the drug concentration causing 50% growth inhibition of the cells (IC50 value) using the formula (Zahra *et al.*, 2020) given below. After determining the percentage of cell viability, graphs were plotted with the percentage of cell viability against their respective concentrations. The test was run in triplicate.

3. Results and discussion

3.1 Antibacterial activity and the stability test of Kp10 and Gh1 secretome proteins against antibiotic resistant pathogens

MRSA and VRE were observed to have developed resistance to many antibacterial agents, most notably vancomycin, which was considered one of the last treatment options for MRSA and VRE infections, complicating treatment (Liu *et al.*, 2011). This issue has sparked worldwide concern because resistance to many available conventional antimicrobial agents makes treating bacterial infections difficult. Many deaths have been associated with VRE, which was first identified in the mid-1980s, and MRSA in 1961s; both have since then spread rapidly to become a major health threat all over the world (Zainal Baharin *et al.*, 2021; Cetinkaya, Falk & Mayhall, 2000). The antibacterial activity of secretome proteins derived from Kp10 and Gh1 against *MRSA* and *VRE,* as demonstrated in our previous study (Zainal Baharin *et al.*, 2022), may highlight a new potential in overcoming the problem of antibiotic resistance.

Their antimicrobial susceptibility was tested using agar well diffusion method and MIC to demonstrate the potential use of the Kp10 and Gh1 secretome proteins. Both secretome proteins were active against both MRSA and VRE strains as shown in Figure 1 and 2. The secretome protein of Kp10 recorded the highest inhibition zone of 21.3 mm against MRSA, and 17.0 mm was recorded against VRE. Meanwhile, the secretome protein of Gh1 also recorded the highest inhibition zone of 18.0 mm against MRSA and the least inhibition zone of 6.0 mm against VRE. Secretome protein of Kp10 gave MIC of 0.1563 ± 0 µg/ml for MRSA and MIC of 0.3648 ± 0 µg/ml for VRE, whereas the secretome protein of Gh1 gave MIC of 2.95325 ± 0 µg/ml for MRSA and MIC of 1.47662 ± 0 µg/ml for VRE. These results show the antibacterial potentials of the secretome proteins as the new agents to combat antimicrobial resistance.

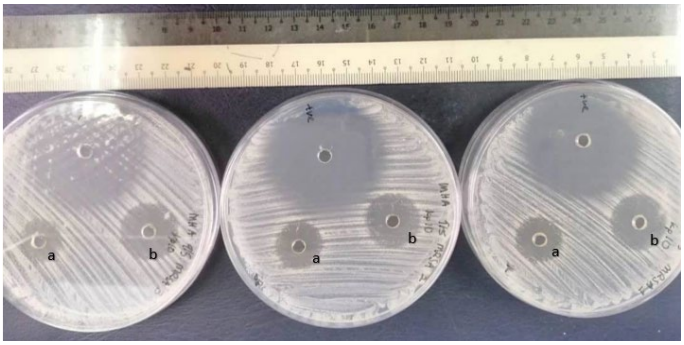

Figure 1: Agar well diffusion assay of secretome proteins extract of Gh1 (a) and Kp10 (b) against MRSA as indicator strain. Assay was conducted in triplicate.

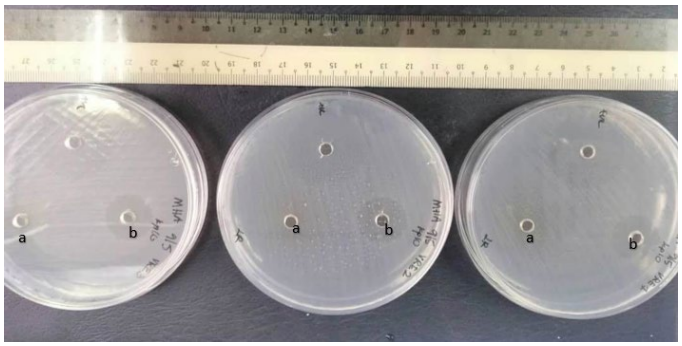

Figure 2: Agar well diffusion assay of secretome proteins extract of Gh1 (a) and Kp10 (b) against VRE as indicator strain. Assay was conducted in triplicate.

Meanwhile, in developing new drugs, the stability and safety of the proposed therapeutic agent must first be established (Kang & Lee, 2009). Concerns have been raised regarding their stability in human serum, although peptides released by secretome proteins are generally considered rather stable in many test assays (Jenssen & Aspmo, 2008). In this study, the preliminary test on the effect of human serum on the antibacterial activity of Kp10 and Gh1 secretome proteins was done using the agar well diffusion method at different concentrations of MIC values. The results are summarised in Table 1 and 2. There were no significant changes in the inhibition zone of secretome proteins in the presence and absence of the human serum within 24 hrs of incubation.

Therefore, we concluded that both secretome proteins were not interfered or affected by human serum, reflecting some safety features. This also gives an initial impression that the protein is safe to use in the human body as it does not interact with serum. However, more detailed studies, such as using HPLC and other related tests, should be carried out to validate the accuracy and efficacy of the treatment (Jenssen & Aspmo, 2008).

3.2 Cytotoxicity study of Kp10 and Gh1 secretome proteins on human cells, MRC5

Interest in the pharmacological effects of antimicrobial peptides on antibiotic resistant pathogens has increased dramatically over the past few years. Secretome proteins containing AMPs have been shown to possess numerous antibacterial activities through different cytotoxic effects against bacterial cells without exhibiting considerable damage to normal host cells (Lei *et al.*, 2019). Our observations on toxicity to MRC5 cells showed that the secretome proteins derived from Kp10 and Gh1 showed no toxicity to *MRC5*. This highlights the notion that the secretome proteins of Kp10 and Gh1 are not harmful to humans and can serve as potential therapeutic agents.

Nevertheless, it has been reported elsewhere that high concentrations of AMPs may cause severe toxicity to normal tissues, thus causing a significant side effect (Ventola, 2015; Mohammad, Thangamani & Seleem, 2015). More studies are therefore required to find the optimum range for human use. This study studied the inhibitory effect of Kp10 and Gh1 secretome proteins on MRC5 cells at different concentrations for 72 hrs. Results, as shown in Figure 3, suggested that no cytotoxic effect of secretome protein derived from Kp10 and Gh1 was observed when treated on MRC5 cells up to the identified concentrations. Hence, the IC50 was not determined

Table 1: Serum stability test of Kp10 and Gh1 against MRSA
(Data presented as mean ± standard deviation values of triplicate measurements)

Type of AMPs	Zone of inhibition on negative control (Human serum) (mm)	Zone of inhibition on 0.5 X MIC against MRSA (mm)	Zone of inhibition on 0.5 X MIC against MRSA with Human Serum (mm)	Zone of inhibition on 1 X MIC against MRSA (mm)	Zone of inhibition on 1 X MIC against MRSA with Human Serum (mm)	Zone of inhibition on 2 X MIC against MRSA (mm)	Zone of inhibition on 2 X MIC against MRSA with Human Serum (mm)
Kp10	-	12	11	21	22	40	42
Standard deviation	-	1	1	0.5	0.5	1	1
Gh1	-	9	9	17	18	30	29
Standard deviation	-	0.5	0.5	0	1	1	1

Table 2: Serum stability test of Kp10 and Gh1 secretome proteins against VRE
(Data presented as mean ± standard deviation values of triplicate measurements)

Type of AMPs	Zone of inhibition on negative control (Human serum) (mm)	Zone of inhibition on 0.5 X MIC against VRE (mm)	Zone of inhibition on 0.5 X MIC against VRE with Human Serum (mm)	Zone of inhibition on 1 X MIC against VRE (mm)	Zone of inhibition on 1 X MIC against VRE with Human Serum (mm)	Zone of inhibition on 2 X MIC against VRE (mm)	Zone of inhibition on 2 X MIC against VRE with Human Serum (mm)
Kp10	-	14	14	16	18	20	20
Standard deviation	-	1	0.5	0	1	1	1
Gh1	-	-	-	2	2	4	3
Standard deviation	-	-	-	1	0	1	0.5

in the tested secretome proteins. Thus, Kp10 and Gh1 secretome proteins that can induce apoptosis in MRSA and VRE (Zainal Baharin *et al.*, 2022) could be a potential therapeutic agent with a safety feature. Further studies *in vivo* environment and clinical trials need to be conducted to establish Kp10 and Gh1 secretome protein as safe agents for antibiotic resistance treatment.

4. Conclusion

All in all, data from this study is of interest, as it suggests that both secretome proteins of Kp10 and Gh1 are nontoxic and have safety characteristics on human cells, which fulfil the *Halalan Toyyiban* principle to be applied as therapeutic agents for antibiotic resistant pathogens. As Muslims, we comply that curing diseases is in the power of *Allah*. However, we must put

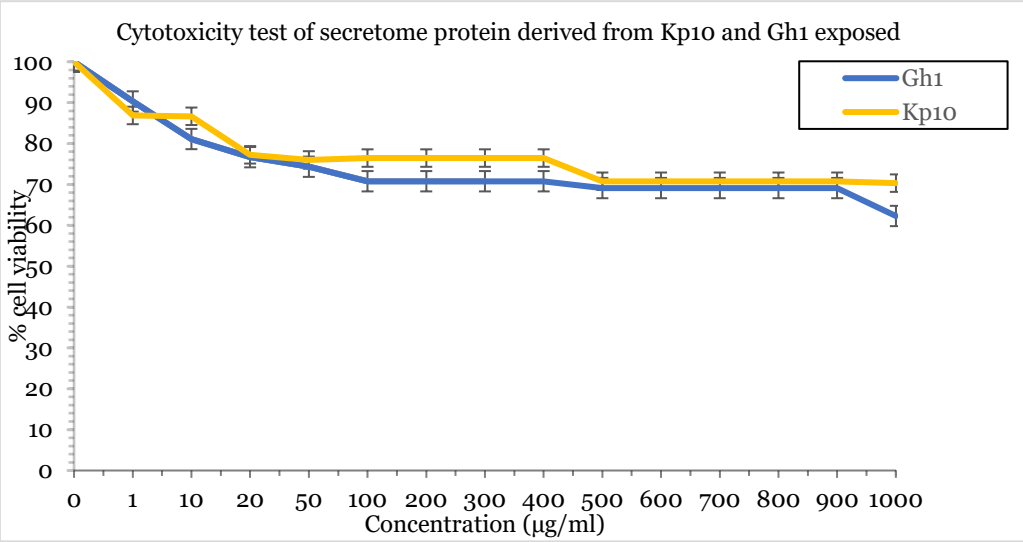

Figure 3: Cytotoxicity test to analyse the effect of secretome proteins of Kp10 and Gh1 on MRC5 cells after 72 hrs exposure. (Data presented as mean ± standard deviation values of triplicate measurements. There is no IC50 determined in tested secretome proteins).

an effort to find a cure that does not contradict Islamic principles. The materials used as medicine must also be in accordance with Islamic philosophy, that is, clean and harmless.

To align with the halal concept, apart from being absence of haram elements, the safety assessment of the selected agent is also an important criterion before being used for human consumption. In this study, the safety status of the secretome proteins was furnished by the cytotoxicity and serum stability tests. We did not observe any effect of cytotoxicity of the secretome proteins on human cells. In addition, we also found that the serum did not affect the antibacterial activity of both secretome proteins. Further improvements are warranted to use the proteins, including the halal requirement. For the later, avoiding materials of non-animal origin in the process of the product may encourage the production of halal pharmaceutical commodities for medicinal use not only dedicated for use among Muslims who are concerned about the use of haram animal derivatives but this innovation can also benefit the public globally regardless of religion.

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References

Arjyal, C., Kc, J., Neupane, S. (2020) Prevalence of methicillin-resistant *Staphylococcus aureus* in shrines. International Journal of Microbiology, 7981648.

Cetinkaya, Y., Falk, P., Mayhall, CG. (2000). Vancomycin-resistant *Enterococci*. Journal of Clinical Microbiology, *Rev,* 13, 686–707.

Dadgostar, P. (2019) Antimicrobial Resistance: Implications and Costs. Infection and Drug Resistance, 12:3903-3910.

Damayanti, RH., Rusdiana, T., Wathoni, N. (2021) Mesenchymal Stem Cell Secretome for Dermatology Application: A Review. Clinical, cosmetic and investigational dermatology, (14):1401–1412.

Department of Standards Malaysia. (2012) MS2424:2012 Halal Pharmaceuticals – General Guidelines.www.halal.gov.my.

Duong-Ly, KC., Gabelli, SB. (2014) Salting out of proteins using ammonium sulfate precipitation. Methods in Enzymology, 541: 85-94.

Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., *et al.* (2016) Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters, 38(6): 1015-1019.

Golkar, Z., Bagasra, O., & Pace, D.G. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. The Journal of Infection in Developing Countries, 13, 8(2), 129-36.

Hamdan, NF., Hashim, N. (2022) The Practices of Halalan Thayyiban Aspect in Japanese Restaurants in Brunei Darussalam, Journal of Halal Science and Technology, 1(1), Page 67–73.

Jawan, R., Abbasiliasi, S., Tan, JS., Kapri, MR., Mustafa, S., Halim, M., Ariff, AB. (2021) Evaluation of the Estimation Capability of Response Surface Methodology and Artificial Neural Network for the Optimization of Bacteriocin-Like Inhibitory Substances Production by *Lactococcus lactis* Gh1. Microorganisms, 9(3):579.

Jawan, R., Abbasiliasi, S., Tan, JS., Mustafa, S., Halim, M., Ariff, AB. (2020) Influence of Culture Conditions and Medium Compositions on the Production of Bacteriocin-Like Inhibitory Substances by *Lactococcus lactis* Gh1. Microorganisms, 8(10):1454.

Jenssen, H., Aspmo, S.I (2008) Serum Stability of Peptides. In: Otvos, L. (eds) Peptide.

Kang, JS., Lee, MH. (2009) Overview of therapeutic drug monitoring. The Korean Journal of Internal Medicine, 24(1):1-10.

Kasimin, ME., Mohd Faik. AA., Jani, J., Abbasiliasi, S., Ariff, A., Jawan, R. (2020) Probiotic properties of antimicrobial-producing lactic acid bacteria isolated from dairy products and raw milk of Sabah (Northern Borneo), Malaysia. Malaysian Applied Biology, 49(3): 95-106.

Kasiri, MM., Beer, L., Nemec, L., *et al.* (2016) Dying blood mononuclear cell secretome exerts antimicrobial activity. *European Journal of Clinical Investigation,* 46(10):853–863.

Kumar, P., Kizhakkedathu, JN., Straus, SK. (2018) Antimicrobial peptides: Diversity, mechanism of action and strategies to improve the activity and biocompatibility *in vivo*. Biomolecules, 8(1): 4.

Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D.H., & He, Q.
(2019). The antimicrobial peptides and their potential clinical applications. American Journal Translational Research, 11, 3919–3931.

Liu, C., Bayer, A., Cosgrove, S.E., Daum, R.S., Fridkin, S.K., Gorwitz, R.J., Kaplan, S.L., Karchmer, A.W., Levine, D.P., Murray, B.E.J, Rybak, M., Talan, D.A., & Chambers, H.F. (2011). Infectious Diseases Society of America. Clinical practice guidelines by the infectious diseases' Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clinical Infectious Diseases, 52:e18–55.

Mohammad, H., Thangamani, S., Seleem, MN. (2015). Antimicrobial peptides and peptidomimetics-potent therapeutic allies for staphylococcal infections. Current Pharmaceutical Design, 21(16):2073-88.

Mokhtar, NFK *et al.* (2020) The Discovery of New Antilisterial Proteins From *Paenibacillus polymyxa* Kp10 via Genome Mining and Mass Spectrometry. Frontiers in Microbiology, 1–11.

Özsoy, S., Arzu, İ. (2017) Detection of Vancomycin-Resistant Enterococci (VRE) in Stool Specimens Submitted for *Clostridium difficile* Toxin Testing. Brazilian Journal of Microbiology, 48(3): 489–92.

Sengupta, S., Chattopadhyay, M.K., & Grossart, H.P. (2013). The multifaceted roles of antibiotics and antibiotic resistance in nature. Frontiers in Microbiology, 12; 4:47.

Ventola, CL. (2015) The Antibiotic Resistance Crisis Part 1: Causes and Threats. 40(4): 277–83.

Wright, GD. (2014). Something old, something new: revisiting natural products in antibiotic drug discovery. Canadian Journal of Microbiology, 60(3), 147-154.

Zahra, I., Kamyar, S., Mikio, M., Hirofumi, H., Shaza, EM., Katayoon, K., Siti, HMT., Elisa, R. (2020). Cytotoxicity assay of plant-mediated synthesized iron oxide nanoparticles using Juglans regia green husk extract. Arabian Journal of Chemistry, Volume 13, Issue 1.

Zainal Baharin, NH., Khairil Mokhtar, NF., Mohd Desa, MN., Gopalsamy, B., Mohd Zaki, NN., Yuswan, MH., *et al.* (2021) The characteristics and roles of antimicrobial peptides as a potential treatment for antibiotic-resistant pathogens: A review. PeerJ, 9: e12193.

Zainal Baharin, NH., Khairil Mokhtar, NF., Mohd Desa, MN., Dzaraly, ND., Muthanna, AR., Al-Obaidi. MMJ *et al.* (2022) Inhibition mechanism of secretome proteins from Kp10 and Gh1 against MRSA and VRE. Asian Pacific Journal of Tropical Biomedicine, 12(11): 483-494.

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Ultrasonic-assisted Extraction Technique of Fixed Oils from Sudanese Seeds

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Abstract

The extraction of oils from Sudanese seeds is of great economic and traditional significance. This study investigated the use of ultrasonic-assisted extraction to extract oils from five different Sudanese seeds: desert date (*Balanites aegyptiaca* L), baobab (*Adansonia digitata*), peanut (*Arachis hypogaea* L.), watermelon (*Citrullus lanatus*), and roselle (*Hibiscus sabdariffa*). The extraction process used two organic solvents, *n*-hexane and *n*-heptane. The results showed that *n*-heptane was a more effective solvent for extracting oils from Sudanese seeds than *n*-hexane. The highest oil yield was obtained from *B. aegyptiaca* seeds, with a yield of 29%. The optimal seed-to-solvent mass ratio for *B. aegyptiaca* was 1:6, and the optimal extraction time was 1 hour (60 mins). A thermodynamic study was conducted to validate the experimental results. The larger area in the nonpolar region for n-heptane suggests a higher extraction capacity for the seed oils. These results are consistent with the experimental findings of this study. In conclusion, ultrasonic-assisted extraction is a promising method for extracting oils from Sudanese seeds. *N*-heptane is a more effective solvent than *n*-hexane for this purpose, and the optimal seed-to-solvent mass ratio and extraction time are 1:6 and 1 hour (60 mins), respectively. The results of this study could be used to develop new commercial products based on Sudanese seed oils. However, further research is needed to investigate the effects of other factors, such as temperature and pressure, on the extraction of oils from Sudanese seeds.

1. Introduction

The extraction of active compounds is a pivotal initial step in preparing traditional herbal remedies. Historically, the extraction of seed oils has involved roasting seed kernels using a mortar and pestle or between two stones and mixing the crushed mass with water (Olaniyan & Yusuf, 2012). While effective, these traditional methods are often time-consuming and require large quantities of solvents.

In recent years, modern sample-preparation techniques have revolutionised the extraction process. These techniques offer significant advantages over conventional methods, including shorter extraction times, higher yields, optimised solvent volumes, and improved quality of extracts (Gupta *et al.*, 2012). Such advancements are crucial in ensuring the availability of high-quality herbal products to consumers worldwide.

Conventional oil extraction methods, such as solvent extraction and mechanical processing, are commonly used to derive seed oils, leaving minimal residual oil in the cake or meal (Dutta *et al.*, 2015). However, modern techniques like microwave-assisted extraction, ultrasound-assisted extraction, pressurised liquid extraction, and supercritical fluid extraction have been developed to address the major shortcomings of traditional methods like Soxhlet extraction (Kemper, 2005; Pavlić *et al.*, 2018).

Among these modern techniques, ultrasonic-assisted extraction (U.A.E.) has emerged as a green extraction method that reduces energy consumption and extraction time while increasing processing throughput (Kalhor & Ghandi, 2019; Žlabur *et al.*, 2016). The U.A.E. method operates on the principle of processing samples under specific frequencies and amplitudes. The sound waves produced generate vacuum bubbles in the liquid, which collapse upon reaching a critical size, releasing a high amount of energy in the liquid medium.

This energy can be harnessed for mixing, extracting, and grinding (Prabuthas *et al.*, 2011).

The Conductor-like Screening Model for Real Solvents (COSMO-RS) has been utilised in various studies to extract oil from plant seeds. For instance, a study by Hizaddin *et al.* (2022) explored the interaction between Deep Eutectic Solvents (D.E.S.s), phenol, *n*-heptane, and toluene using COSMO-RS. The study suggested that D.E.S.s, considered green solvents, could serve as alternatives to conventional organic solvents and ionic liquids to extract phenolic compounds from pyrolysis oil.

This study aims to determine the yield of oil extracted using U.A.E. from different plant seeds, such as *Hibiscus sabdariffa, Citrullus lanatu, Arachis hypogae, Adansonia digitata,* and *Balanites aegyptiaca*, using different solvents. After identifying the highest extraction yield and the most effective solvent, the factors affecting the extraction, including mass ratio and extraction time, will be investigated. The study will also conduct a thermodynamic analysis to support the selection of solvents. The findings from this study will contribute to the body of knowledge on optimising oil extraction from plant seeds using modern extraction techniques. U.A.E. demonstrated higher yield, less time, lower temperature and less solvent consumption; it was selected to optimise the oil extraction yield from several Sudan seeds. This study aims to determine the yield of oil extracted using U.A.E. from different plant seeds, such as (*Hibiscus sabdariffa, Citrullus lanatu, Arachis hypogae, Adansonia digitata and Balanites aegyptiaca,* using different solvents. After screening for the highest extraction yield and best solvent, factors affecting the extraction, including mass ratio and extraction time, will be investigated. The optimisation, evaluation of the optimum mass ratio and extraction time are also conducted in this study. After that, a thermodynamic analysis was conducted to support the selection of solvents.

2. Methodology

2.1 Preparation of seed

Five different types of dried seeds, Desert date, Peanuts, Baobab, Watermelon, and Roselle, were prepared for extraction. The dried seeds were ground using a mortar and pestle to crush the seeds into granules. This process was undertaken to increase the surface area available for oil extraction. The granularity of the crushed seeds was carefully controlled to ensure a consistent size across all samples. The resulting seed powder was of a fine consistency, optimal for the ultrasonic-assisted extraction process.

2.2 Screening of solvents and seeds

Ten (10) g of seed powder were weighed and placed individually into conical flasks. Following that, 60 g of *n*-heptane was added into each of the flasks to make a sample with a seed-to-solvent mass ratio of 1:6. Each of the conical flasks was then covered with aluminium foil to avoid the evaporation of the solvent. Next, the conical flasks containing the sample solution were placed in the water bath of the sono-reactor for 60 mins at 35°C with 70 rpm agitation speed. After 1 hour, each sample was transferred from conical flasks into centrifuged tubes and centrifuged for 10 mins. At the same time, the weight of the empty beakers (W_1) was measured and labelled respectively. After centrifugation, each sample solution was filtered into the labelled empty beakers to obtain only solvent with extracted oil. Subsequently, the beakers were placed in the fume hood for evaporation of *n*-heptane. The beakers with the extracted oil were weighed and recorded as (W_2). The steps were repeated by using *n*-hexane as the extracting solvent. Lastly, the oil yield (Y) was calculated based on the equation below. (See Figures 1A to 6A in the supplementary file).

$$\% \text{ yield of oil (Y)} = \frac{W_2 - W_1}{W} \times 100 \qquad \text{Eq (1)}$$

Where:

W_2 = weight of beaker with extracted oil after evaporation (g)
W_1 = weight of empty beaker before evaporation (g)
W = initial weight of seeds (g)

Figure 1: Plant seeds powders after grinding.

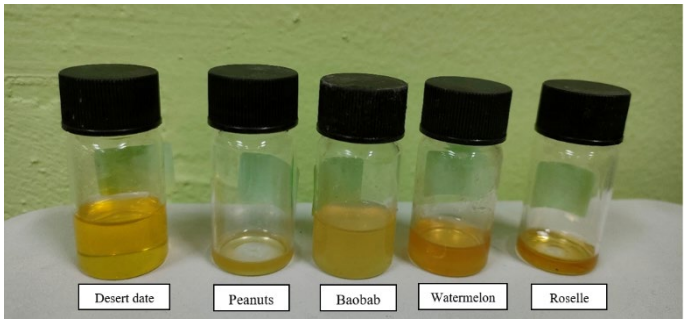


Figure 2: Oil extracted from Desert date, Peanuts, Baobab, Watermelon and Roselle seeds (from left to right) after evaporation.

2.3 Study of factors affecting the extraction

2.3.1 Mass ratio

Firstly, the selected seed powder was added with the solvent into a conical flask with a mass ratio 1:2, where 10 g of seed powder and 20 g of solvent were weighed. The steps were repeated using different mass ratios where the weight for seed powder was kept constant at 10 g, and the weight of the solvent varied from 40 g, 60 g, and 80 g. The steps demonstrated in Section 2.2 were followed to carry out the extraction.

2.3.2 Extraction time

The selected seed powder with the highest yield was added with the solvent into 4 different conical flasks according to a mass ratio of 1:2, where 10 g of seed powder and 20 g of solvent were weighed. The samples were placed into the sono-reactor for various extraction times of 20 mins, 40 mins, 60 mins and 80

mins. Samples were processed using the same steps as demonstrated in Section 2.2

2.4 Thermodynamics of mixing and solubility

This work used COSMO-RS to screen the relative solubility of the fatty acids in hexane and heptane. The geometric optimisation and solvent representation in COSMOtherm were performed according to the methods described in previous work (Hayyan *et al.*, 2023; Salleh *et al.*, 2017).

3. Result and discussion

3.1 Screening of plant seeds and solvents

A preliminary screening of plant seeds and solvents was conducted to select the plant seed and solvent which gives the highest oil yield using an ultrasound-assisted oil extraction method. The result of the preliminary screening is presented in Figure 3.

A graph of oil yield against the type of seeds using *n*-heptane and *n*-hexane as the extracting solvent was plotted to determine the plant seed and solvent producing the highest oil yield. From that, the plant seeds of desert date (*Balanites aegyptiaca*) and solvent (*n*-heptane) were selected for optimisation.

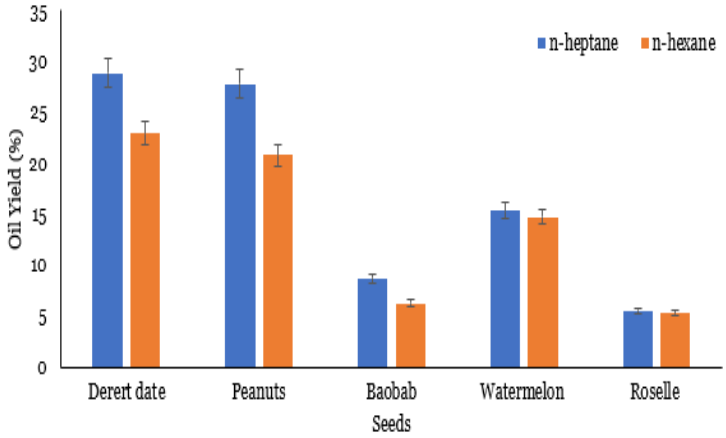


Figure 3: Oil yields from different plant seeds in different solvents (*n*-hexane and *n*-heptane). The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

This study selected edible seeds such as Desert date, Peanut, Watermelon, Baobab and Roselle seeds to evaluate the oil yield. Figure 3 shows that oil desert date seed recorded the highest oil yield, followed by peanut, watermelon, baobab and roselle seeds when using *n*-heptane and *n*-hexane. It also demonstrated that when *n*-heptane is used as the extracting solvent, a higher amount of oil is extracted from the same plant seed than when using *n*-hexane as the extracting solvent. The highest oil yield (29%) was obtained by extracting the oil from *Balanites aegyptiaca* seed using *n*-heptane as the oil-extracting solvent. Therefore, *Balanites aegyptiaca* seed and *n*-heptane were selected for further optimisation.

It was reported by (Alostad *et al.*, 2022) that *n*-heptane could extract a wider mass range of compounds. Heptane was also used to extract oil and bioactive compounds from *Cucumis melo* L. seeds (Mallek-Ayadi *et al.*, 2018). Furthermore, *n*-heptane is less polar and has a higher boiling point than *n*-hexane, making it a better choice for extracting oils that are more soluble in nonpolar solvents. N-heptane is also less volatile and flammable than *n*-hexane, making it a safer choice for some applications. This fact can be significant from an economic perspective in the process sector since it allows for the more effective extraction of fixed oils from plum seeds using nonpolar solvents (*n*-hexane and *n*-heptane) (Savic *et al.*, 2020).

3.2 Effect of mass ratio

The influence of seed-to-solvent mass ratio was investigated by extracting oil from *Balanites aegyptiaca* seed using 20 g, 40 g, 60 g and 80 g of solvent with a constant seed weight of 10 g to obtain seed to solvent mass ratio of 1:2, 1:4, 1:6, and 1:8. Meanwhile, other conditions were maintained constant. The results for the optimisation of seed to solvent mass ratio are shown in Figure 4.

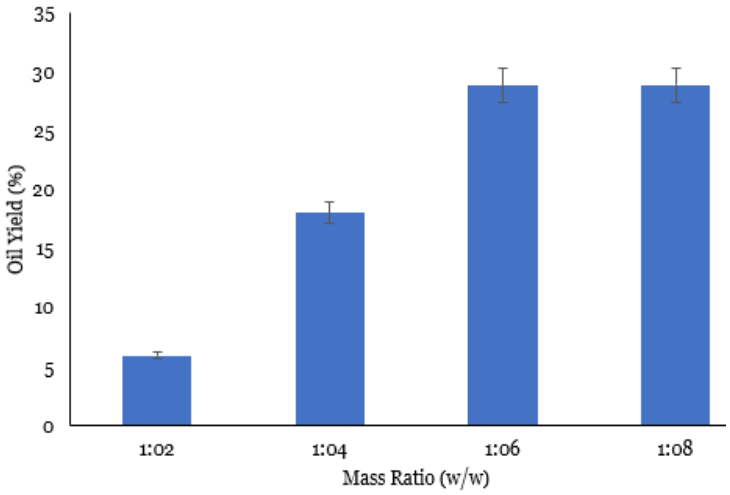


Figure 4: Effect of seed to solvent mass ratio on oil yield at 60 mins extraction time, 35°C extraction temperature, and 75 rpm agitation speed. The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

Figure 4 depicts that oil yield increases with the seed-to-solvent mass ratio until the 1:6 mass ratio. A higher seed-to-solvent mass ratio led to a larger concentration gradient between the solid phase (seed) and liquid phase (solvent), where mass transfer of seed oil from powder to the solvent is favourable (Goula, 2013). Next, by observing Figure 4, beyond the 1:6 mass ratio, no significant improvement in oil yields was observed. This is because the extracting solution, the seed and solvent mixture, has achieved the saturation concentration (Hayyan *et al.*, 2022).

According to the results, a seed-to-solvent mass ratio of 1:6 is sufficient for extracting the maximum oil yield. Since raising the ratio to 1:8 would only result in a minor increase in oil yield, thus, seed to solvent mass ratio of 1:8 is not considered as it would not be economical to use a higher amount of *n*-Heptane for obtaining an insignificant increase in oil yield. Hence, 1:6 (10 g of plant seed to 60 g of solvent) was selected as the optimum seed-to-solvent mass ratio.

3.3 Effect of extraction time

The relationship of extraction time on oil yield was examined by extracting oil from Balanites seed at different extraction times of 20 mins, 40 mins, 60 mins and 80 mins in a sono-reactor with a constant seed-to-solvent mass ratio of 1:6 and extraction temperature of 35°C. With that, the result for optimisation of extraction time is displayed in Figure 5.

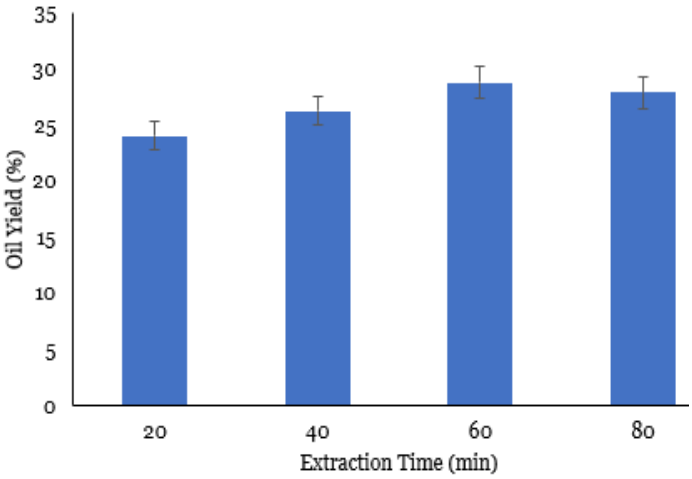


Figure 5: Effect of extraction time on oil yield at 1:6 seed to solvent mass ratio, 35°C extraction temperature and 75 rpm agitation speed. The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

According to Figure 5, it is observed that the yield of oil increases with the extraction time until 60 mins. Increasing the extraction time gives adequate time for the seeds to be exposed to ultrasonic waves. At the same time, the solvent has more time to disrupt and break the cell wall of the seed, allowing a longer penetration time of solvent into the seed to extract the oil content from the seed (Hayyan *et al.*, 2022).

Results show that the maximum extracted oil yield (29%) was achieved at 60 mins. A slight decrease in oil yield after 60 mins was observed. This is likely because a longer extraction time causes oil oxidation and degradation (Torii *et al.*, 2019). In addition, the oil that is dissolved in the solvent tends to reabsorb the seed's granules that have a relatively large surface due to a longer extraction time. Therefore, 60 mins (1 hour) is selected as the optimum time for oil extraction using a sono-reactor as a long time did not result in a significant yield. More prolonged exposure to air can cause the oil to oxidise, leading to the formation of unwanted byproducts and a decrease in oil quality.

Moreover, over-extraction may occur if the extraction process continues too long, removing more than just the desired oil and reducing overall yield (Cai *et al.*, 2021; Lam *et al.*, 2019). Furthermore, some oil components may break down over time, reducing the amount of oil obtained. In addition, longer extraction times may also increase temperature, which can cause thermal degradation of the oil, leading to a reduction in yield (Chemat *et al.*, 2019; Kusuma *et al.*, 2018).

3.4 Thermodynamics of mixing and solubility

Understanding the thermodynamics of mixing and solubility in oil extraction from *Balanites aegyptiaca* seeds is crucial. Solubility, as defined within the framework of Gibb's free energy of mixing, is the ability of solute molecules (in this case, the oils within the seeds) to dissolve and form a homogeneous solution with a solvent, such as *n*-heptane. This solubility is a key determinant of the efficiency of the extraction process.

The composition of *Balanites aegyptiaca* seed oil predominantly includes palmitic acid (16.68%), oleic acid (22.85%), linoleic acid (47.84%), and stearic acid (11.67%) (Murthy *et al.*, 2020). When these oils act as solutes in *n*-heptane, their solubility is governed by the Gibbs free energy of mixing between the solute and solvent. A negative mixing energy signifies that the solute will dissolve and form a homogeneous solution, while a positive mixing energy indicates that the solute will not dissolve in the solvent. As shown in Figure 6, all oil compounds are soluble in *n*-heptane, validating the effectiveness of *n*-heptane in extracting these oils from *Balanites aegyptiaca* seeds.

To further elucidate the interaction between the solute and solvent, we analysed the sigma (σ) profile of each component involved in the mixing process. In COSMO-RS, the σ-profile describes electron density distribution around a solute molecule in a solvent environment, providing insights into the solvation structure and the interaction between the solute and solvent. This information is instrumental in predicting a solution's thermodynamic and transport properties.

The σ-profile is divided into three regions: the polar hydrogen bond donor region when $\sigma < -0.0084$ eA−2, the nonpolar region when σ is between −0.0084 and 0.0084 eA−2, and the polar hydrogen bond acceptor (H.B.A.) region when $\sigma > 0.0084$ eA−2. The presence of peaks in all three regions, as shown in Figure 7(a), suggests that both polar and nonpolar solvents have the potential for extraction. However, nonpolar solvents like *n*-heptane are preferred due to the dominance of peaks in the nonpolar region, indicating higher solubility for these oils.

Figure 7(b) further compares the σ-profiles when the dominant oils in Balanites are combined with the solvents *n*-heptane and *n*-hexane. The larger area under the peak in the nonpolar region for *n*-heptane suggests a higher extraction capacity for the seed oils, aligning with the experimental findings of this study.

The thermodynamics of mixing and solubility play a crucial role in oil extraction from seeds. The solubility of the oil in the solvent determines the efficiency of the extraction process. A study by Mauliza *et al.* (2021) investigated the effect of extraction time on the yield of oil from Amla seeds using hexane as a solvent. They found that the extraction time significantly impacted the oil yield, highlighting the importance of understanding the thermodynamics of the extraction process to the yield.

Similarly, Evangelista *et al.* (2022) evaluated the oil extraction from (*Euphorbia lagascae* Spreng.) by pre-pressing followed by solvent extraction. They assessed the effect of starting seed moisture content and heating temperature on oil extractability and quality. Their findings underscored the importance of understanding the thermodynamics of mixing and solubility in achieving efficient oil extraction and maintaining the quality of the extracted oil. Fetzer *et al.* (2021) reported on extracting oil conditions for maximum yield. A study on the fatty acid profile and anti-Alzheimer's disease activity reported by Wei *et al.* (2022) highlighted the importance of selecting the appropriate extraction method to preserve the beneficial properties of the extracted oil.

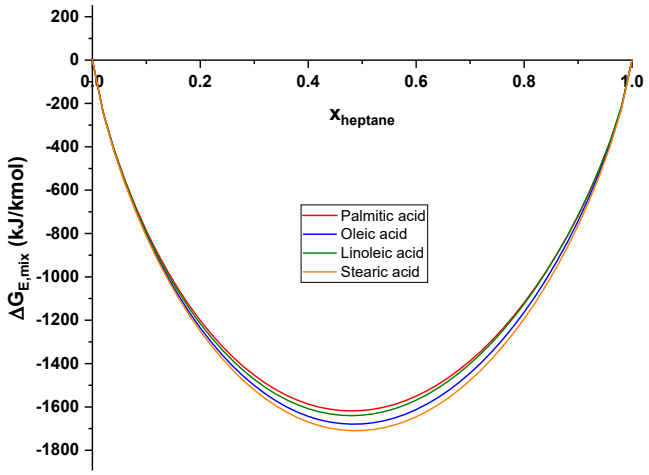
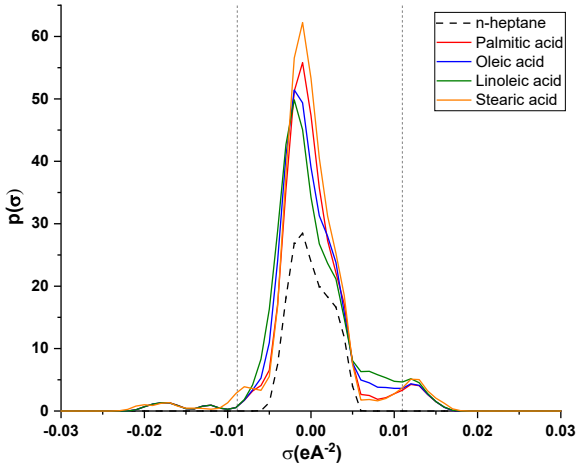
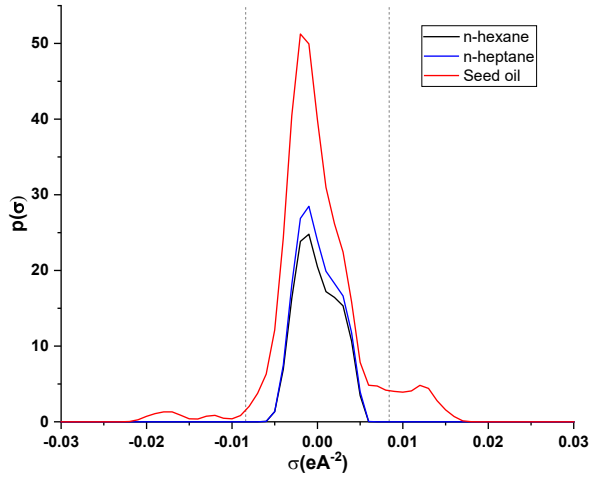


Figure 6: Gibbs free energy of mixing between oil species in *Balanites aegyptiaca* and *n*-heptane.



(a)



(b)

Figure 7: σ-profiles of *n*-hexane and/or *n*-heptane in comparison with the (a) individual oil species, and (b) combined oil species.

In conclusion, the thermodynamics of mixing and solubility play a significant role in the extraction process. The choice of solvent, its interaction with the solute, and the solubility of the oils in the solvent all contribute to the efficiency of oil extraction. This study's findings underscore the importance of these factors in optimising the extraction process for *Balanites aegyptiaca* seeds.

4. Conclusion

The study established that *n*-heptane is a more effective solvent for oil extraction from seeds, particularly from desert date (*Balanites aegyptiaca* L.) seeds, yielding up to 29% oil. The optimal extraction conditions were identified as a seed-to-solvent mass ratio of 1:6 and an extraction time of 1 hour. The superior performance of *n*-heptane is attributed to its higher nonpolarity, enhancing its oil solubility. Further research could explore using other nonpolar solvents to verify if they can yield even better results than *n*-heptane. Studies could also be conducted on different types of seeds to broaden the understanding of oil extraction processes. Investigating the environmental impact and economic viability of using *n*-heptane as a solvent on a large scale would be beneficial. Future research could also focus on refining the extraction process to further increase the yield, such as by optimising temperature conditions or exploring enzyme-assisted extraction methods.

References

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. Current Research in Food Science, 4, 200–214.

Alostad, L. K., Palacio Lozano, D. C., Gannon, B., Downham, R. P., Jones, H. E., & Barrow, M. P. (2022). Investigating the influence of *n*-Heptane versus *n*-Nonane upon the Extraction of Asphaltenes. Energy & Fuels, 36(16), 8663–8673. https://doi.org/10.1021/acs.energyfuels.2c01168

Ameer, K., Shahbaz, H. M., & Kwon, J. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. Comprehensive Reviews in Food Science and Food Safety, 16(2), 295–315.

Cai, Z., Li, K., Lee, W. J., Reaney, M. T. J., Zhang, N., & Wang, Y. (2021). Recent progress in the thermal treatment of oilseeds and oil oxidative stability: A review. Fundamental Research, 1(6), 767–784.

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V., & Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges. TrAC Trends in Analytical Chemistry, 118, 248–263.

Dutta, R., Sarkar, U., & Mukherjee, A. (2015). Soxhlet extraction of Crotalaria juncea oil using cylindrical and annular packed beds. International Journal of Chemical Engineering and Applications, 6(2), 130.

Evangelista, R. L., Isbell, T. A., Todd, J., & Cermak, S. C. (2022). Euphorbia lagascae seed oil obtained by pre-pressing and solvent extraction. Industrial Crops and Products, 180, 114799. https://doi.org/10.1016/j.indcrop.2022.114799

Fetzer, D. L., Hamerski, F., Errico, M., & Corazza, M. L. (2021). Extraction of cumaru seed oil using compressed propane as

solvent. The Journal of Supercritical Fluids, 169, 105123. https://doi.org/10.1016/j.supflu.2020.105123

Goula, A. M. (2013). Ultrasound-assisted extraction of pomegranate seed oil–Kinetic modeling. Journal of Food Engineering, 117(4), 492–498.

Gupta, A., Naraniwal, M., & Kothari, V. (2012). Modern extraction methods for preparation of bioactive plant extracts. International Journal of Applied and Natural Sciences, 1(1), 8–26.

Hayyan, A., Loo, F., Qing, W., Zulhaziman, M., Salleh, M., Basirun, W. J., Hamid, D., Saleh, J., Aljohani, A. S. M., Alhumaydhi, F. A., Zulkifli, M. Y., al Abdulmonem, W., Yeow, A. T. H., Roslan, M., Nor, M., Hashim, A., & Al-Sabahi, J. N. (2023). Encapsulated paracetamol-based eutectic solvents for the treatment of low-grade palm oil mixed with microalgae oil. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2023.116322

Hayyan, A., Samyudia, A. v, Hashim, M. A., Hizaddin, H. F., Ali, E., Hadj-Kali, M. K., Aldeehani, A. K., Alkandari, K. H., Etigany, H. T., & Alajmi, F. D. (2022). Application of deep eutectic solvent as novel co-solvent for oil extraction from flaxseed using sono-energy. Industrial Crops and Products, 176, 114242.

Hizaddin, H. F., Wazeer, I., Huzaimi, N. A. M., El Blidi, L., Hashim, M. A., Lévêque, J.-M., & Hadj-Kali, M. K. (2022). Extraction of phenolic compound from model pyrolysis oil using deep eutectic solvents: computational screening and experimental validation. Separations, 9(11), 336. MDPI AG. Retrieved from http://dx.doi.org/10.3390/separations9110336

Kalhor, P., & Ghandi, K. (2019). Deep eutectic solvents for pretreatment, extraction, and catalysis of biomass and food waste. Molecules, 24(22), 4012.

Kemper, T. G. (2005). Oil extraction. Bailey's Industrial Oil and Fat Products, 1–38.

Kusuma, H. S., Altway, A., & Mahfud, M. (2018). Solvent-free microwave extraction of essential oil from dried patchouli (Pogostemon cablin Benth) leaves. Journal of Industrial and Engineering Chemistry, 58, 343–348.

Lam, S. S., Mahari, W. A. W., Ok, Y. S., Peng, W., Chong, C. T., Ma, N. L., Chase, H. A., Liew, Z., Yusup, S., & Kwon, E. E. (2019). Microwave vacuum pyrolysis of waste plastic and used cooking oil for simultaneous waste reduction and sustainable energy conversion: Recovery of cleaner liquid fuel and techno-economic analysis. Renewable and Sustainable Energy Reviews, 115, 109359.

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. Process Safety and Environmental Protection, 113, 68–77. https://doi.org/https://doi.org/10.1016/j.psep.2017.09.016

Mauliza, N. P., Rosnelly, C. M., & Supardan, M. D. (2022, December). Solvent extraction of Amla (Phyllanthus emblica) seed oil using hexane. In I.O.P. Conference Series: Earth and Environmental Science (Vol. 1116, No. 1, p. 012037). I.O.P. Publishing.

Murthy, H. N., Yadav, G. G., Dewir, Y. H., & Ibrahim, A. (2020). Phytochemicals and biological activity of desert date (Balanites aegyptiaca (L.) Delile). Plants, 10(1), 32.

Olaniyan, A. M., & Yusuf, K. A. (2012). Mechanical Oil Expression from Groundnut (Arachis hypogaea L) Kernels using a Spring-Controlled Hydraulic Press. Journal of Agricultural Research and Development, 11(2), 235–248.

Pavlić, B., Savić, D., Vladić, J., Vidović, S., & Zeković, Z. (2018). Comparative analysis of the essential oils of three Lamiaceae species obtained by conventional and microwave-assisted hydrodistillation. Journal on Processing and Energy in Agriculture, 22(4), 174–179.

Petigny, L., Périno-Issartier, S., Wajsman, J., & Chemat, F. (2013). Batch and continuous ultrasound assisted extraction of boldo leaves (Peumus boldus Mol.). International Journal of Molecular Sciences, 14(3), 5750–5764.

Prabuthas, P., Majumdar, S., Srivastav, P. P., & Mishra, H. N. (2011). Standardisation of rapid and economical method for neutraceuticals extraction from algae. J Stored Prod Postharvest Res, 2(5), 93–96.

Salleh, Z., Wazeer, I., Mulyono, S., El-blidi, L., Hashim, M. A., & Hadj-Kali, M. K. (2017). Efficient removal of benzene from cyclohexane-benzene mixtures using deep eutectic solvents – COSMO-RS screening and experimental validation. Journal of Chemical Thermodynamics, 104, 33–44. https://doi.org/10.1016/j.jct.2016.09.002

Savic, I., Savic Gajic, I., & Gajic, D. (2020). Physico-chemical properties and oxidative stability of fixed oil from plum seeds (Prunus domestica Linn.). Biomolecules, 10(2), 294.

Torii, S., Hashimoto, T., Do, A. T., Furumai, H., & Katayama, H. (2019). Repeated pressurisation as a potential cause of deterioration in virus removal by aged reverse osmosis membrane used in households. Science of The Total Environment, 695, 133814.

Wei, G., Zhang, Z., Fu, D., Zhang, Y., Zhang, W., Zu, Y., Zhang, L., & Zhang, Z. (2021). Enzyme-assisted Solvent Extraction of High-yield Paeonia suffruticosa Andr. Seed Oil and Fatty Acid Composition and Anti-Alzheimer's Disease Activity. Journal of oleo science, 70(8), 1133–1146. https://doi.org/10.5650/jos.ess21040

Vermaak, I., Kamatou, G. P. P., Komane-Mofokeng, B., Viljoen, A. M., & Beckett, K. (2011). African seed oils of commercial importance—Cosmetic applications. South African Journal of Botany, 77(4), 920–933.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry, 8(3), 303–313.

Žlabur, J. Š., Voća, S., Dobričević, N., Pliestić, S., Galić, A., Boričević, A., & Borić, N. (2016). Ultrasound-assisted extraction of bioactive compounds from lemon balm and peppermint leaves. International Agrophysics, 30(1), 95–104.

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Mangosteen (*Garcinia mangostana*): Extraction, Purification, Bioactivities and Toxicities

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Abstract

Mangosteen (*Garcinia mangostana*) is a popular tropical fruit known as 'the queen of fruits' due to its delicious flavour. It comprises phytochemicals, flavonoids, and polyphenols such as xanthones, anthocyanins, and phenolic acids. The *G. mangostana* pericarp, seed, and flesh bioactive compounds have been shown to have anticancer, antitumor, wound healing, anti-inflammatory, antigenotoxic, antioxidative, antimicrobial, and antiviral properties. This review focuses on the bioactivity of *G. mangostana*, with the most common bioactive compound discovered being xanthone, which is frequently used in cosmetics, antimicrobials, antioxidants, and pharmaceutical research. Various extraction methods, both conventional and non-conventional, have been used in studies, depending on the goal of the research. This review concludes by using a computerised database search technique to discuss the extraction methods available and the properties of the bioactive compounds found in various parts of *G. mangostana*, including the pericarp, seed, and flesh.

1. Introduction

G. mangostana is one of the most popular tropical fruits, known as the 'queen of the fruits' due to its juicy white flesh that has a slightly acidic and sweet taste consumed by many people (Yang *et al.*, 2017). This *G. mangostana* belongs to the Family Clusiaceae and genus Garcinia. Garcinia is a genus of plants native to Asia and Africa, with over 300 species from which bioactive chemicals such as xanthones, flavonoids, triterpenoids, and benzophenones have been isolated and described. Although all fruits from Garcinia species are edible, *G. mangostana* has gotten the most recognition in the real economy. *G. mangostana* fruit is round, dark purple or reddish, and the trees mature at 6 to 25 meters. Fruit production usually takes ten years or more, with a yield of roughly 400 fruits per tree that increases with age (Gutierrez-Orozco & Failla, 2013). Since antiquity, *G. mangostana* has been grown in Java, Sumatra, Indochina, and the southern Philippines. In Indonesia, it is a common dooryard tree. In 1855, the *G. mangostana* fruit in English greenhouses and its culture were later brought to the Western Hemisphere, where it became established in various West Indian islands, most notably Jamaica. It was later created in Guatemala, Honduras, Panama, and Ecuador on the mainland. It is also possible to cultivate it in southern Florida. *G. mangostana* does not grow well outside the tropics and is only fresh in local markets. The fruit was banned from importation until 2007 in the United States due to fears of introducing the Asian fruit fly; imported *G. mangostana* must be irradiated first to remove the pest (Article, 2021). *G. mangostana* is a valuable plant since its parts can be used in medicine and food. Parts of the fruit, pericarps, and stems of *G. mangostana* plants have been frequently utilised, whereas *G. mangostana* leaves have not been commonly employed as ingredients in traditional medicine. However, *G. mangostana* leaves contain antibacterial chemicals. Flavonoids, tannins, alkaloids, and saponins are the four active components in *G. mangostana* leaves (Suhartati *et al.*, 2019a). Figure 1 summarises all the topics discussed in this review paper.

Every method that has been used has its limitations and weaknesses. In liquid chromatography, using organic solvents and other additives, which are rarely compatible with bio or biochemical tests, is one of its limitations. Gradient-based separations, in which the solvent composition changes dramatically, are particularly problematic. In this instance, evaporation is frequently the most effective technique to remove interferences. Volatile chemicals, on the other hand, may be lost. Another issue is quick separations, which often do not correspond to the period required for the bioassay. When combined with mass spectrometry, gas chromatography separation for volatile chemicals is unparalleled due to its efficiency and excellent detection sensitivity. Only a few biochemical systems can be employed (Weller, 2012).

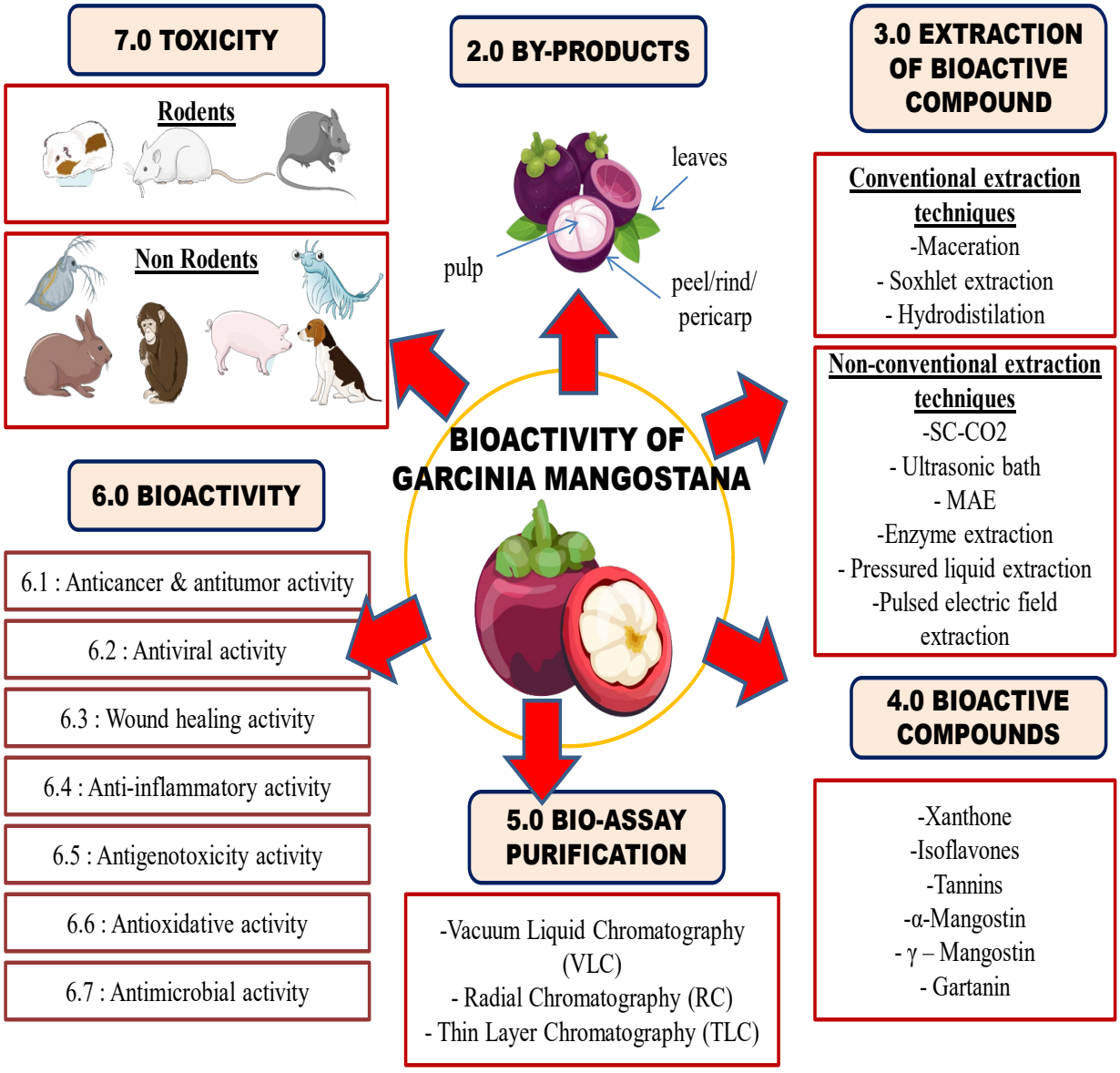


Figure 1: Bioactivity of *Garcinia mangostana*.

Parts of *G. mangostana* fruit, such as pericarps, pulps, stems, and leaves, are essential sources of bioactive components with various properties that will provide many benefits. Early descriptions of infusions and decoctions of its pericarps and seeds showed they were used to treat gastrointestinal and urinary tract infections. They were also used as anti-scorbutic, laxative, and anti-fever agents dating back nearly 200 years. In line with the rise of the times, *G. mangostana* is used modernly to treat infection-related symptoms like diarrhoea, stomach pain, and fever and complaints associated with inflammatory and immunological illnesses such as acne, food allergies and arthritis (Ovalle-Magallanes *et al.*, 2017). In Southeast Asia, the pericarp of the *G. mangostana* fruit has been used as a traditional medicine to treat infection, wounds and diarrhoea for ages (Gutierrez-Orozco & Failla, 2013). According to numerous in vitro and in vivo investigations, *G. mangostana* pericarp has a wide range of pharmacological actions, including antioxidant, anti-inflammatory, antibacterial, and anthelmintic due to its bioactive compound, xanthones (Markowicz *et al.*, 2019). Bioactive components in *G. mangostana* also have been found to have antiproliferative, pro-apoptotic, antiobesity, anti-carcinogenic and antimicrobial activities (Gondokesumo *et al.*, 2019). The pericarp of *G. mangostana* is thought to contain medicinal characteristics, making it a possible natural therapeutic agent. It also can be used in various ways and be included in the diet to help fight infections (Datta *et al.*, 2014). Although there are many studies on the bioactivities of *Garcinia mangostana*, most of these studies have focused on common bioactivities such as antioxidant, antimicrobial, anti-inflammatory and wound healing. There is still a lack of up-to-date information on toxicity studies, antiallergy activity, and the anti-mutagenic activity of *G. mangostana*.

G. mangostana extract has been widely used since ancient times due to its several properties, which many researchers have proved in vitro and in vivo. On the other hand, the possible toxicity of extracts and formulations, including *G. mangostana* pericarps, pulps, and leaves, is still a minor worry as relevant data on toxicity are still lacking. Therefore, microbiological and toxicity tests must first determine their potential toxic effects. The goal of an acute toxicity test on *G. mangostana* pericarp

extract is to find out how dangerous it is and what the lethal dosages are (LD50). Dose levels are determined by preliminary information on symptoms, likely effects on target organs, and species sensitivity. The dangers of its usage or exposure to humans and a reference for designing additional safety and toxicity testing are calculated based on this outcome (Sunarjo, 2017). In addition, halal-related issues are also a significant concern when involving consumers among Muslims. Generally, Muslims are permitted to eat everything except those forbidden in the *Qur'an* or the *Hadith*. These *Shari'ah* (Islamic law) regulations provide people with the freedom to eat and drink whatever they like, as well as it is not haram (prohibited) (Khattak *et al.*, 2011).

This review paper evaluated the properties of the bioactive compounds found in various parts of *G. mangostana*, including the pericarp, seed, and flesh, and the available extraction methods used by a computerised database search technique.

2. *Garcinia mangostana* by-products

The *G. mangostana* tree grows to six to twenty-five meters, with lushes of thick leathery leaves covering the tree. Meanwhile, the fruit is round with thick skin, also known as pericarp and ripens seasonally, changing colours from green to yellow to pink spotted and full purple. The pericarp contains the edible part of the fruit, mainly composed of three to eight septa (also known as aril), which is white and has a sweet-sour taste. Its seeds are also found in one or two septa per fruit and are considered abrasive, cold-sensitive, and drying-resistant. This fruit's seeds often grow apomictically, without relying on sexual reproduction, and require a long planting period before bearing (usually 7 to 9 years), limiting the agronomic improvement and cross-breeding opportunities (Aizat *et al.*, 2019). Figure 2 shows the pericarps, pulps and leaves of the *G. mangostana*.

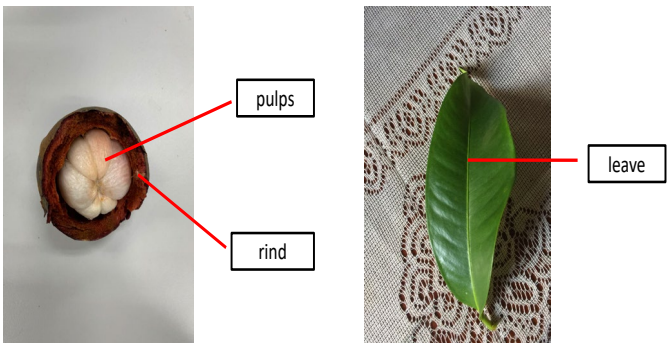


Figure 2: *Garcinia mangostana* pericarps, pulps, and leaves.

From 19 million tonnes of *G. mangostana* production in 2019 (FAOSTAT, 2019), 65% of the output, equalising 12 million tonnes, are the outer and inner pericarps (Chaovanalikit *et al.*, 2012). These underexplored by-products have gone to disposal, although they possess various bioactivities. According to Moopayak (2020), *G. mangostana* pericarp functions as an antioxidant, antitumoral, anticancer, antifungal, antibacterial, antiviral, antiallergy and anti-inflammatory agent. The seeds have antioxidant, antibiotic, and anticancer activities beneficial in pharmaceutical and cosmetic products. Both *G. mangostana* pericarp and seed have been used as bio-filters to produce natural rubber latex; for instance, medical gloves, rubber toys, rubber health care products and rubber transdermal patches. The antimicrobial properties of *G. mangostana* pericarp protected the medical glove against undesired water and liquid molecule absorption and were effective against a virus (Moopayak, 2020).

In other studies, *G. mangostana* pericarp has been extracted into a gel and used for a patient with chronic periodontitis as additional therapy, which has improved clinical epithelium attachment in chronic periodontitis and reduced pocket depth and gingival inflammation after mechanical treatment such as scaling and root planing (Hendiani *et al.*, 2017). In food applications, the *G. mangostana* pericarp extract has also been used as anti-lipid oxidation in yoghurt drinks to hinder rancidity (Wibawanti *et al.*, 2019). All these bioactivity compounds act accordingly due to specific bioactive compounds in the *G. mangostana* by-products. Parts of the fruit, pericarps, and stems of *G. mangostana* plants have been commonly used, although *G. mangostana* leaves have not been widely used as ingredients in traditional medicine. However, *G. mangostana* leaves contain antibacterial compounds. *G. mangostana* leaf extract inhibits *E. coli* bacteria development, and the optimum concentration of *G. mangostana* leaf extract for inhibiting *E. coli* bacteria growth is 80% concentration with an average diameter of 32.75 mm (Suhartati *et al.*, 2019). *G. mangostana* seed contains several bioactive compounds, including oil, lipid, carbohydrate, moreollin and gambolic acid. *G. mangostana* pericarp contains xanthones which act as antibacterial and anti-inflammatory. The presence of xanthones in *G. mangostana* pericarp showed a higher radical scavenging activity. Flavonoids, tannins, alkaloids, and saponins are the four active compounds in *G. mangostana* leaves that render antibacterial potency.

3. Extraction of bioactive compounds of *Garcinia mangostana*

The extraction method is a process to separate the essential components from plant parts, such as antibacterial and antioxidant compounds. The characteristics of the compound to be extracted must be considered when choosing an extraction process. In a nutshell, extraction is one of the critical steps in creating medically beneficial extracts with the highest concentration of active constituents. The first prerequisite for producing an acceptable yield of active components in extracts is an appropriate extraction method and a suitable solvent. The attributes of the compound to be extracted must be considered when choosing an extraction process. The solvent and polarity index types, extraction times, solvent concentration and ratio, temperature, and particle size influence the extraction process's effectiveness and performance. The solvent and extraction time will have the most significant impact on the amount of extractable bioactive. When choosing a solvent for extraction, various factors must be considered, including availability, low cost, physical and chemical stability, neutral reaction, and potency to bioactive compounds (Kusmayadi *et al.*, 2018). Industrial legislation also mandates a reduction in petrochemical solvents and volatile organic compounds.

Furthermore, the lack of risk during extraction and the products' quality are significant concerns, prompting calls for greener solvents such as water and ethanol (Bundeesomchok *et al.*, 2016). Green extraction is a design of extraction procedures that can minimise energy consumption, allow for the use of alternative safe solvents and sustainable natural resources, and ensure a safe and high-quality extract. The polarity and concentration of the extraction solvents were essential factors in the extraction of α-mangostin from the *G. mangostana* pericarp. Various solvents, such as methanol, ethanol, and acetone, are widely used to remove secondary metabolites from

plant sources. The plant variety determines the type of organic solvent to use in the extraction process of the compounds to be extracted (Ghasemzadeh *et al.*, 2018).

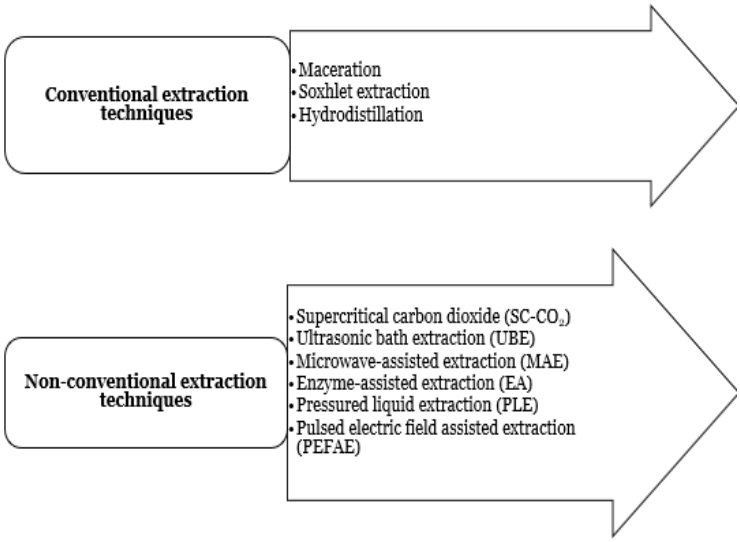
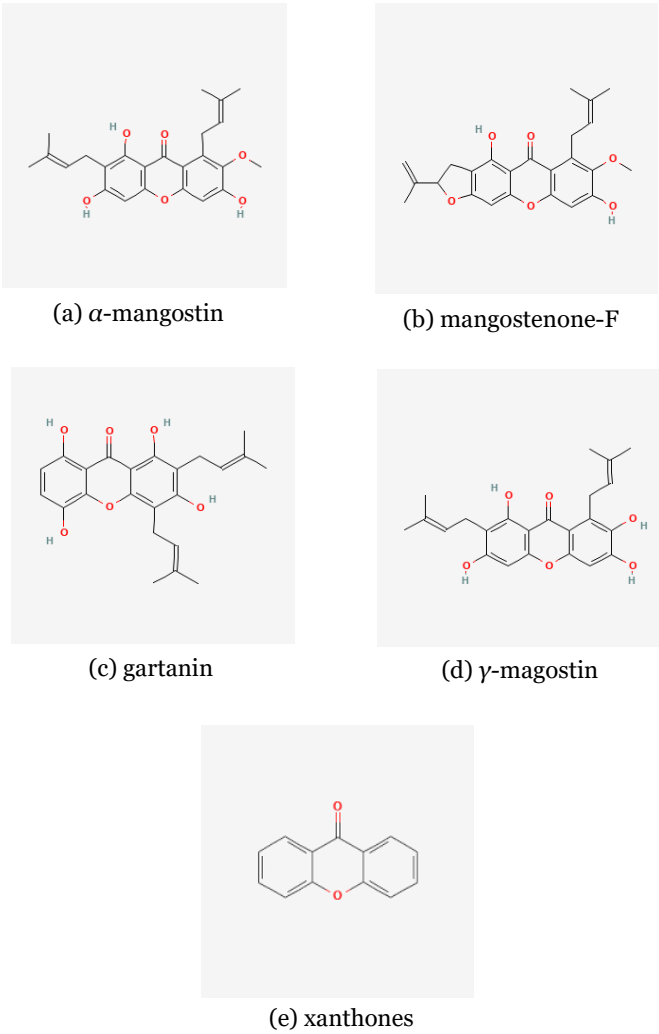


Figure 3: Type of extraction techniques.

Extraction techniques can be divided into two categories: conventional and non-conventional extraction techniques, as stated in Figure 3. Conventional extraction techniques include maceration in a water bath, soxhlet extraction (Mohammad *et al.*, 2019), and hydrodistillation (Azmir *et al.*, 2013). According to Ramesh *et al.* (2017), soxhlet extraction has been used to isolate Garcinone E from *G. mangostana* pericarp because it has been recognised to have a cytotoxic effect on the Sp2/0 cell lines. Sp2/0 cells are mouse-myeloma cell lines from B lymphocytes. They can be used as fusion partners for B cells to form hybridomas because they can develop indefinitely. On the other hand, conventional extraction techniques also have a few downsides, especially the maceration technique, which requires a high quantity of solvent and prolonged extraction time, especially during maceration in a water bath. Therefore, there has a variety of non-conventional techniques have been explored and can be used in the extraction of bioactive and phenolic compounds, such as ultrasonic-bath extraction (UBE) (Ahmad *et al.*, 2019), pressured-liquid extraction (PLE), microwave-assisted extraction (MAE) (Mohammad *et al.*, 2019), pulsed- electric-field-assisted extraction (PEFAE), enzymatic extraction (EA) and supercritical-carbon-dioxide extraction (SC-CO2) (Chhouk *et al.*, 2016).

Regarding Mohammad *et al.* (2019), the microwave-assisted extraction (MAE) method has been used to extract bioactive compounds, especially xanthones, from *G. mangostana* pericarp and analysed their total phenolic content and antioxidant activity. This method has been used for its superiority, such as less time consumption, little solvent consumption, and fast energy transfer, especially for preparing antioxidant-rich plant extract. The solvent is well diffused within the extraction medium during irradiation. However, potential influences on the extraction method include solvent types, volume, power, temperature, irradiation time, and raw material size. These variables impact the extracted yield and total phenolic content (TPC). The ultrasound-assisted extraction method was also used to compare its effectiveness with microwave-assisted extraction (MAE) in extracting bioactive compounds. These comparisons have been made because both techniques were used in previous studies (M'hiri *et al.*, 2015) to compare the different effects of different extraction conditions. Both methods can extract the highest amount of total phenolic content (TPC), making them the most common extraction methods over others, such as traditional solvent extraction (CSE), SC-CO2, and high-pressure extraction (HPE).

Other methods can also be used to extract bioactive compounds from *G. mangostana* pericarp, as Chhouk *et al.* (2016) used the supercritical carbon dioxide method in their study. On the other hand, supercritical carbon dioxide (SC-CO2) has been used in conjunction with subcritical water to overcome the disadvantages of the traditional process. The formation of carbonic acid lowers the pH of water when it is mixed with SC-CO2, particularly at high pressure. This makes the environment more conducive to extracting natural materials, usually attracted to acidic solvents. Using hydrothermal processes, SC-CO2 hydrolyse natural products like corn stover and hesperidin.



(a) *α*-mangostin (b) mangostenone-F

(c) gartanin (d) *γ*-magostin

(e) xanthones

Figure 4: Chemical structure of active compounds in *Garcinia mangostana*.

4. Bioactivities and active compounds of *Garcinia mangostana*

Bioactive substances with medicinal qualities, including anti-inflammatory, antibacterial, anti-inflammatory, antitumoral,

antioxidant, HIV inhibitory, antilipidemic, wound healing and analgesic properties, are abundant and beneficial in the Garcinia species. These bioactivities are present due to the reported existence of polyisoprenylated benzophenones, flavonoids, and xanthones. Xanthones are chemical compounds found in *G. mangostana* pericarps that have antibacterial properties and can potentially halt cell multiplication in bacteria. *G. mangostana*'s major component, *α*-mangostin, has shown significant pharmacological effects, including antioxidant action in treating age-related macular degeneration and retinal protection against light damage (Espirito Santo *et al.*, 2020). Besides these active compounds, other compounds such as mangostenone-F, gartanin, *γ*-magostin, etc., in Figure 4 render specific bioactivities (Espirito Santo *et al.*, 2020), which will be discussed further in the following subtopics.

4.1 Anticancer and antitumor activities of *Garcinia mangostana*

Apart from antioxidant, anti-inflammatory, and antimicrobial activities, *α*-mangostin and xanthone of *G. mangostana* exhibit antitumor activity in vitro and in vivo studies. This is evident since it showed that it could inhibit cancer cell proliferation and metastasis and significantly reduce tumour growth through the induction of apoptosis and cell cycle arrest (Markowicz *et al.*, 2019). Metastasis occurs at first when the tumour cells separate from their original tissue. This calls for modifying both the cancer cell and the tissue of origin. Cancer cells in circulation must develop survival mechanisms after detaching (Patel *et al.*, 2011). The process of metastasis can occur when cancer can spread to other organs by invading any body area. Cancer is a condition brought on by a loss of control over cell growth brought on by cells that proliferate abnormally, spread, and grow beyond their normal boundaries (Guerrero-Pepinosa *et al.*, 2021).

According to Kurniawan *et al.* (2021), the type, number, and position of the connected functional groups in the xanthone framework determine the anticancer activity of xanthone derivatives. Multiple protein receptors, including cyclooxygenase, protein kinase, and topoisomerase, have been shown to bind with xanthone derivatives, indicating their anticancer action. Numerous xanthone derivatives for anticancer activities have been developed and tested. Anticancer agents based on simple oxygenated xanthones are being studied extensively. The anticancer effect of the 1, 3-dihydroxyxanthone against cancer cell lines is well recognised. Simple oxygenated xanthone showed potential anticancer action against the KB and MCF-7 cell lines via an antiproliferative mechanism among the produced xanthones. The anticancer activity of these xanthone derivatives was shown to be enhanced by nitrogen atoms in the heterocyclic rings. Since the intracellular enzyme may hydrolyse back to dihydroxyxanthone, the acetylated group increased anticancer action. Simple oxygenated xanthone showed substantial inhibitory activity against KB cells, with IC_{50} values of 2.40, 0.90, and 1.05 μM, respectively, while these compounds had IC_{50} values of 1.3, 0.8, and 0.9 μM against the MCF-7 cancer cell line. These were significantly lower than doxorubicin, with IC_{50} values of 25.0 and 25.7 μM for KB and MCF-7, respectively.

On the other side, panaxanthone (a combination of α-mangostin and γ-mangostin) was found to inhibit DNA replication and cause cancer cells to enter the G1 phase of the cell cycle. Subcutaneous injections of panaxanthone dramatically suppressed the metastatic growth of BJMC3879 cancer cells in mice. The inhibitory function was linked to the activation of caspase-3 and caspase-9 proteins, with the loss of cytochrome c from the mitochondria resulting in the collapse of cancer cells' mitochondria membrane potential. Subcutaneous injection of xanthone extract from *G. mangostana* fruit rinds containing 81% α-mangostin and 16% *γ*-mangostin dramatically reduced HCT-116 tumour volume in mice. The inhibitory action was linked to apoptosis induction, cancer cell motility, invasion, and clonogenicity suppression.

4.2 Wound healing activity of *Garcinia mangostana*

G. mangostana pericarp is traditionally used to treat sicknesses like wounds because it consists of many bioactive compounds such as xanthones, terpenes, anthocyanins, tannins, and phenols (Ovalle-Magallanes *et al.*, 2017; Shan *et al.*, 2011). Interference of cellular and anatomic continuity of tissue with or without microbial infection is the best term to define the wound meaning. Physical, chemical, thermal, immunological, and microbial exploitation causes the occurrence, which disrupts the epithelial tissue of the skin with the disturbance of functional continuity of living tissue in the wound (Shafy *et al.*, 2019). Cutaneous wound healing is a complicated and essential physiological process that relies on the interaction of cytokines, growth factors, chemokines, and chemical mediators from cells to carry out regulatory functions. Tissue regeneration and repair begin when an acute injury occurs, and any stimulation disrupts functional tissues' physical continuity. There have four stages in the wound healing process for acute wounds, including hemostasis, inflammation, proliferation and migration of the cells and tissue remodelling or resolution. Sometimes the coagulation and inflammatory processes are lumped together. The function and histological properties of these phases are unique. There is a logical path from injury to coagulation, inflammation, cell migration, and tissue remodelling in an acute wound (Freiesleben *et al.*, 2017; Sombolayuk *et al.*, 2019).

G. mangostana pericarp extract was reported to accelerate the wound healing process as its bioactive compound, such as xanthone. The most abundant xanthone presented in *G. mangostana* pericarp was known as *α*-mangostin, which was mainly mediated to give the wound healing effect. Coagulation, platelet aggregation, and fibrin clot formation are the first steps in inflammation. These mechanisms protect cellular materials from being lost, work as a physical barrier to prevent microbe access, and act as a provisional matrix, cytokine deposit, and growth factors necessary for the future healing phases to continue. Neutrophils are recruited within hours of injury and mediate tissue damage by releasing proteases, cytokines, and other substances housed in cytoplasmic granules. These cells produce reactive oxygen species (ROS) and antimicrobial proteases (cathepsins, defensins, lactoferrin, and lysozyme), which kill potentially harmful microbes. Neutrophils also have a variety of matrix metalloproteinases (MMPs) involved in the breakdown of the extracellular matrix. Uncontrolled neutrophil migration initiates a cycle of recruitment activation of these cells. This result produces excessive production of reactive oxygen species (ROS) and proteases, causing extracellular matrix (ECM) degradation and additional tissue damage, which may progress to chronic inflammation. This occurrence results in defective collagen deposition, reduced tissue resistance, late reepithelialisation and limited wound healing (Sombolayuk *et al.*, 2019).

Table 1: Wound healing graded score (Sombolayuk *et al.*, 2019)

Wound Healing Assessed	Graded Score (1-3)		
	1	2	3
Granulation tissue formation (GTF)	0-25% GTF with inflammatory cell domination	50-70% GTF with wide neovascularisation and less fibroblast	>75% GTF with domination of collagen and fibroblast
Reepithelialisation (RE)	No RE	RE<50%	RE>50%
Inflammatory cells count (ICC)	Mild macrophage and neutrophil	Moderate macrophage and neutrophil	Abundant macrophages and neutrophils

4.3 Anti-inflammatory activity of *Garcinia mangostana*

Inflammation is a vital part of the host's defensive system, including various activities in response to external stimuli such as pathogen infection, bacterial endotoxin exposure, and chemical exposure. Changes in blood flow, increased vascular permeability, tissue damage via activation and migration of leukocytes, and the creation of reactive oxygen derivatives (oxidative burst) and local inflammatory mediators, are all part of the inflammation process. Inflammatory mediators such as interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-, and Nitric Oxide (NO), as well as anti-inflammatory mediators such as IL-10, are secreted as a primary reaction to inflammation, in addition to leukocyte recruitment. Inflammation is linked to several disorders, including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, Alzheimer's, and cancer. Anti-inflammatory is necessary to combat the risk of chronic inflammation that comes with chronic disease (Widowati *et al.*, 2016).

According to Tatiya-aphiradee *et al.* (2019), *α*-angostatin is the most prevalent xanthone in the *G. mangostana* pericarp. It has been shown to have antimicrobial and anti-inflammatory properties against various bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes*. Using a tape stripping model, the anti-inflammatory activity of *G. mangostana* pericarp extract against methicillin-resistant *Staphylococcus aureus* (MRSA)-induced superficial skin infection in mice was examined. Topically administered *G. mangostana* pericarp ethanolic extract (GME) and its component, *α*-mangostin, to mice with MRSA-induced superficial skin infection. *Staphylococcus aureus* secretes the extracellular adherence protein in cutaneous infections, an anti-inflammatory agent to prevent leukocyte recruitment. Extracellular vesicles produced by *Staphylococcus aureus* trigger cytokine production and accelerated the inflammatory process, resulting in the formation of an abscess.

Several investigations have shown that *G. mangostana* and α-mangostin have been shown to have anti-inflammatory properties in cell cultures. *G. mangostana* extract (GME) and α-mangostin decreased the release of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) in RAW264.7 macrophage-like cells, while *α*-mangostin inhibited TNF- and IL-4. Furthermore, α-mangostin suppressed TNF-, IFN-, IL-6, IL-8, and IL-1 expression in human U397 macrophage-like cells and human primary adipocytes, as well as decreased levels of inflammatory cytokines such as TNF-, IL-4, and IL-8 in human enterocyte-like Caco-2, human colorectal adenocarcinoma (HT-29), macrophage-like THP. Interestingly, the study shows that *G. mangostana* extract (GME) and α-mangostin have anti-inflammatory effects in MRSA-induced superficial skin infection in mice by reducing the expression of pro-inflammatory cytokines such as TNF-, IL-6, and IL-1, as well as TLR-2.

4.4 Antigenotoxicity activity of *Garcinia mangostana*

Genotoxicity is known as the capacity of various chemicals to cause damage to genetic material. Moreover, the genetic material is damaged not only in terms of DNA but also in terms of cellular components that affect the operation and behaviour of chromosomes inside the cell. Proteins involved in the repair, condensation, and decondensation of DNA in chromosomes, or other structures responsible for chromosome distribution during cell division, such as the mitotic spindle, are examples. Genotoxic or genotoxins can cause genetic toxicity whereas antigenotoxicity is the inverse of this (López-Romero *et al.*, 2018).

According to Carvalho-Silva *et al.* (2016), the genotoxicity and antigenotoxicity of hydroethanolic *G. mangostana* extract (HEGM) were tested on *Saccharomyces* cerevisiae yeast. The antigenotoxicity activity of hydroethanolic *G. mangostana* extract (HEGM) was determined by comet assay using human peripheral blood cells (leukocytes). Pure *α*-mangostin extracted from the pericarp of *G. mangostana* was used to test for DNA antigenotoxicity. The finding was discovered that a concentration of 12.5 μM α-mangostin protected DNA from H2O2 damage in a dose-dependent manner. Cellular cytotoxicity was observed at higher dosages. As a result, the findings imply that some components in the pericarp of *G. mangostana* L., particularly α-mangostin, protect DNA against H2O2-induced damage. Cells were preloaded for 1 to 4 hours with HEGM (160, 320, 640 μg/mL), centrifuged, and washed twice with saline solution before being resuspended in RPMI 1640 medium and exposed for 5 minutes to H_2O_2 (1 mM) at 37°C. Alternately, cells were preloaded with *α*-mangostin 12.5 μg/mL for 1 to 4 hours, centrifuged, washed, and resuspended as before, but then exposed to H2O2 (1, 0.5, or 0.25 mM) at 37°C. The test revealed that it was effective against DNA damage caused by H_2O_2 in dosages up to 320 g/mL. (1, 2, and 4 hours exposure). When doubling the dose of HEGM from 160 to 320 μg/mL and comparing preloading up to 4 hours of exposure, it was discovered that improved DNA-induced damage protection was observed as a reduction in the quantity of identified DNA damage. Potential antioxidants in HEGM may protect cells by neutralising DNA damage-inducing H_2O_2-derived free radicals, preventing DNA-strand breaks identified by the Comet assay. Identifying compounds that can protect genetic material against genotoxic agents is critical because it indicates the ability to prevent diseases caused by genetic damage, such as cancer. Up to 640 μg/mL exposure concentration, the hydroethanolic extract of *G. mangostana* (HEGM) showed no genotoxicity/mutagenicity. Hence it was capable of preventing DNA against damage caused by free radicals produced by H_2O_2. Because of the lack of genotoxicity and the demonstrated antioxidant in vivo characteristics, extracts of *G. mangostana* are safe and even helpful to people since the incidence of oxidative stress-induced genetic damage may be reduced. *G. mangostana* extract, or isolated active components thereof, may offer promise for pharmaceutical or

nutraceutical applications by helping to prevent DNA damage-related disease by neutralising free radicals produced by cellular metabolism or external factors.

4.5 Antioxidative activity of *Garcinia mangostana*

An antioxidant compound in *G. mangostana* pericarp extract has been reported to be vital in promoting health benefits (Gondokesumo *et al.*, 2019). In the food industry, adding antioxidants is essential to inhibit the deterioration of food quality. Xanthones, prenylated and oxygenated xanthones, are the antioxidants in *G. mangostana* pericarp extract. Their various maturity levels of fruit skin affected the total bioactive compound content and antioxidant activities of Garcinia mangostana pericarps. The total flavonoid content has been found to be higher in the mature fruit pericarp (4.08g QE/100g) compared to the young fruit pericarp (2.91g QE/100 g) as it may result in higher antioxidant activity (Suttirak & Manurakchinakorn, 2014). Research also evaluated the antioxidant activity of *G. mangostana* pericarp can also be determined by their skin colour related to the concentration of phenolics and flavonoids (Lourith & Kanlayavattanakul, 2011). Compounds from the pericarp of *G. mangostana* were extracted using n-hexane, EtOH, and deionised water with different concentration ratios and periods of maceration. The most prolonged maceration period had the most *G. mangostana* colour, and the best solvent was water, followed by EtOH and n-hexane. Water and EtOH colour extracts were further examined for biological activity based on their colour preferences. The brilliant yellow EtOH extract had much higher antioxidant activity than the darker brown-yellow water-extracted colour. The proportion of phenolic and flavonoid components in the extracts, including xanthones, influences colour differences, although the EtOH colour extract's DPPH scavenging activity was 2-fold lower than the control. Furthermore, the EtOH extract had a higher total phenolic content but a lower flavonoid concentration than the other extracts. (Lourith & Kanlayavattanakul, 2011).

According to Palapol *et al.* (2009), the maturity levels of *G. mangostana* pericarp have been differentiated using Liquid chromatography-mass spectrometry (LC-MS/MS). The six different maturation levels of *G. mangostana* pericarp were classified based on their skin colour, as mentioned in Figure 5. However, the different maturity levels of *G. mangostana* fruit affected the bioactive compound content and antioxidant activity of the *Garcinia mangostana* pericarp extract due to the different xanthones concentrations (Pothitirat *et al.*, 2010; Suttirak & Manurakchinakorn, 2014). The xanthones concentration between each maturity level is different because of water loss of the fruit pericarp as their weight was reduced due to the ripeness process. The chlorophyll disappeared, and the carotenoid progressed, causing discolouration of the fruit pericarp from yellow, orange, red or purple during the maturation and ripening process (Gondokesumo *et al.*, 2019). Gartanin, γ-mangostin, smeathxanthone and garcinone-E are the standard compound present in all the maturity levels of *G. mangostana* pericarp extract, and the highest total of xanthones was reported in level 6 maturity of *G. mangostana* pericarp (Gondokesumo *et al.*, 2019). Antioxidant capacity was determined using electron spin resonance (ESR) analysis and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical standard. The EPR spin trapping approach includes trapping reactive short-lived free radicals in a diamagnetic EPR passive compound (spin trap) via a spin trap double bond, resulting in a more stable radical product (spin adduct). According to the analysis, different stages of *G. mangostana* pericarp maturity have varying antioxidant activity, as seen by decreased free radical intensity. Compared to the DPPH free radical, each maturity stage of *G. mangostana* pericarp extract has a different g value. Different g values showed different types of free radicals while decreasing g values suggested decreased free radical intensity. *G. mangostana* pericarp, with maturity levels 4, 5, and 6, had the highest decrease in g value (Gondokesumo *et al.*, 2019).

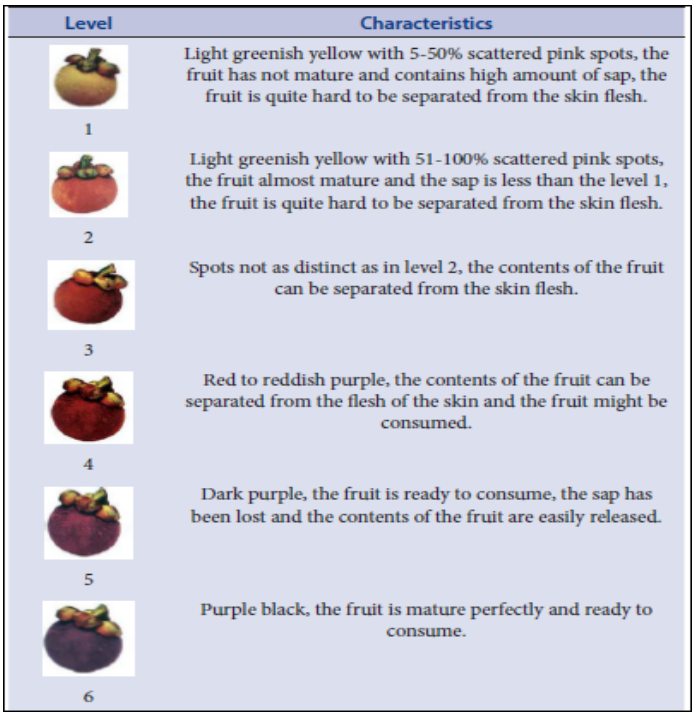


Figure 5: Level of *Garcinia mangostana* and its characteristic. Source: (Gondokesumo *et al.*, 2019)

The ability of electron spin resonance (ESR) spectroscopy to discover antioxidative chemicals is well established. ESR spectroscopy is a one-of-a-kind approach for detecting free radicals and other compounds that do not affect the registered signal, and it reflects the researched material's actual antioxidative characteristics. The absorption of electromagnetic radiation was measured with an ESR spectrometer to detect antioxidant activity. The standard or stable free radical was DPPH (2,2-diphenyl-1-picrylhydrazyl), compared to DPPH plus six different maturity levels of *G. mangostana* pericarp extract. Compared to DPPH, the standard free radical, each maturity level has a different g value. The g value difference suggests a different sort of free radical. The antioxidant's ability to donate hydrogen atoms to trap the DPPH radical is measured by DPPH radical scavenging activity (DPPH). The structure, position, and degree of hydroxylation on the ring structure and the electron and hydrogen donating activity of polyphenols contained in *G. mangostana* pericarp extract all influence DPPH scavenging capacity. Maturation level is likely to impact the differences in antioxidant activity among the *G. mangostana* fruit. The flavonoid content of the mature fruit pericarp was higher than that of the young fruit pericarp. In conclusion, the higher total flavonoid content of the ripe fruit pericarp may explain its higher antioxidant action (Gondokesumo *et al.*, 2019).

4.6 Antimicrobial activity of *Garcinia mangostana*

G. mangostana extract has been proven through several studies to have antimicrobial abilities that are very effective in inhibiting the couples of gram-positive and gram-negative bacteria (Boonmak *et al.*, 2018; Panawes *et al.*, 2017; Soetikno *et al.*, 2016). The part that is often used to study its microbial activity is the pericarp part because it is always considered a waste product. However, it has many bioactive components that give many effective antibacterial effects (Datta *et al.*, 2014).

Referring to Saepudin *et al.* (2019), *G. mangostana* pericarp extract has been determined to have antibacterial activity against bacteria of the *Xanthomonas oryzae p.v. oryzae* (Xoo) that causes HDB disease (bacterial leaf blight) in rice and antibacterial components from the active extract has been identified. Bacterial leaf blight (HDB) is one of the most common rice diseases, affecting rice ecosystems worldwide. Pathogen Xoo infects rice plants during all stages of their development, from the nursery through the harvest. Xoo infects rice plants and degrades leaf chlorophyll by infecting portions of the leaves through leaf wounds or natural openings in the form of stomata. This infection reduces the ability of plants to carry out photosynthesis, resulting in death in immature plants and a less complete grain filling in generative phase plants.

Temperature and fruit storage duration substantially impact the antibacterial activity of *G. mangostana* pericarp extract. With an inhibition zone diameter of 24.46 mm and a minimum inhibitory concentration (MIC) value of 25%, *G. mangostana* fruit refrigerated for seven days at 13.5°C demonstrated the best antibacterial activity. Each treatment combination's inhibition zone of *G. mangostana* pericarp extract was higher than the chloramphenicol control inhibition zone. This result clearly shows that *G. mangostana* pericarp extract can prevent the growth of Gram-negative bacteria *Xanthomonas oryzae p.v. oryzae*. *G. mangostana* pericarp extract has a more substantial inhibitory effect than chloramphenicol antibiotics. Several active substances in the *G. mangostana* pericarp extracted with 95% ethanol, including xanthone, flavonoid, tannin, terpenoids, and saponins, determine this inhibitory effect. Xanthones are chemical compounds found in *G. mangostana* pericarp that have antibacterial properties and can potentially halt cell multiplication in bacteria. They are also high in antioxidants. Saponins work as antibacterials by interfering with the stability of bacteria's cell membranes, causing them to dissolve. The cell wall will be stretched severely, disrupting cell membranes, and releasing numerous essential components (proteins, nucleic acids, and nucleotides) required for bacterial life. Terpenoid is a lipophilic phenolic molecule. Terpenoids cause cell membrane damage as a mode of action. Tannin can stop protein transport enzymes from moving across cell membranes. Flavonoids are the most common type of phenol chemical with potent antiviral, antibacterial, and antifungal activities. Flavonoids tend to attach to proteins, causing metabolic disruption.

Regarding Li *et al.* (2020), natural compounds from *G. mangostana* pericarp have been identified to act as antibacterial action against *Ralstonia solanacearum* and thus find a way to employ this waste stream as a biological control for bacterial wilt. *Ralstonia solanacearum* causes bacterial wilt, one of agriculture's most severe bacterial diseases. Although chemical pesticides are used to prevent this illness, they may cause significant environmental pollution problems. Natural plant products can be a rich and environmentally compatible source of bacteria biological control across a broad spectrum. The pericarp of the *G. mangostana* was investigated utilising bioactivity-guided fraction analysis and liquid chromatography-mass spectrometry combined with multivariate analysis to identify markers of active fractions.

The antibacterial activity of six identified compounds (1; *α*-mangostin; 2, *γ*-mangostin; 3, smeathxanthone A; 4, mangostenol; 5, garcimangosxanthone H; 6, garcimangosxanthone I) was evaluated against *Ralstonia solanacearum*. Only *γ*-mangostin inhibited *Ralstonia solanacearum* growth in an agar medium, and α-mangostin had modest antibacterial activity, whereas the other compounds were inactive. In the OPLS-DA model, the VIP value of *γ*-mangostin (14.48) was the greatest, indicating that it was the most active compound in the active fractions.

4.7 Antiviral activity of *Garcinia mangostana*

Some studies show *α*-mangostin has antiviral properties as it can inhibit the production of dengue virus (DENV) in hepatocellular carcinoma HepG2 and Huh-7 cell lines and the expression of cytokine/chemokine in HepG2 cells. Dengue virus consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), and it was circulated and transmitted by *Aedes* mosquitoes and quickly passed the mosquito-borne disease to the human population (Quispe-Tintaya, 2017). As a broad spectrum of clinical phenotypes ranging from mild manifestation (dengue fever, DF) to the more severe spectrum (dengue hemorrhagic fever, DHF and dengue shock syndrome, DSS), DENV infection in a susceptible human host can be described as a capillary leakage, coagulopathy and organ impairment syndrome and partially thought to be immune-mediated.

Regarding Sugiyanto *et al.* (2019), *α*-mangostin has been reported to have the potential as antiviral as it is effective against dengue virus (DENV) infection in human peripheral blood mononuclear cells (PBMC) by determination of virus titer and tumour necrosis factor-alpha (TNF-*α*) and interferon-gamma (IFN-*γ*) cytokines concentration after infection. TNF-*α* and IFN-*γ* were used to demonstrate the expression profiles of two cytokines involved in the inflammatory immune response to DENV infection and to identify the antiviral activity of α-mangostin. The cytokines/chemokines gene expression profiles were observed in patients during the dengue disease process. As a result, increased concentration of inhibited viral replication of α-mangostin and decreased production of inflammatory cytokines at 24 and 48 hours post-infection. The use of *α*-mangostin as an antiviral drug to inhibit the transmission of DENV is of valuable benefit, particularly in endemic countries where both circulating and secondary infections with DENV serotypes lead to a more severe form of the disease are inevitable. The intervention of *α*-mangostin in dengue patients during the acute phase of the disease can decrease the severity of the disease by raising the viral load and activating the immune response of the host by interfering with the function of the DENV NS5 protein necessary for the replication of DENV and reducing the transcriptional response of cytokines (Tarasuk *et al.*, 2017).

5. Bioassay-guided approach of *Garcinia mangostana*

Apart from investigating antibacterial, antifungal and antioxidant activities, the extract is also subjected to quantitative and qualitative phytochemical analysis. The composition of the crude extract of *G. mangostana* renders various bioactive compounds bound with impurities, where the

bioassay-guided approach on the crude extract separates. It purifies the bioactive compounds before further studies. Bioassay-guided approach fractionates components of crude extract based on differences in their physicochemical properties assessment of biological activity. Then, the fractionated components undergo another round of separation and assay to obtain a pure bioactive compound of natural origin, i.e. *G. mangostana* (Malviya & Malviya, 2017).

For this bioassay-guided approach, Vacuum Liquid Chromatography (VLC) incorporated with Radial Chromatography (RC) is the common technique to separate and purify secondary metabolites (Gartanin compounds) from *G. mangostana* pericarp. Both VLC and RC employ physical separation techniques where the fractionation of components of crude extract occurs between the mobile and stationary phases (Oetari *et al.*, 2019). In the chromatography principle, molecules in a mixture are placed on the stationary face's surface, and the mobile phase is injected to pass the mixture to be separated onto the solid phase. The essential factors effective in this separation process are molecular properties associated with adsorption (liquid-solid), partition (liquid-solid), and affinity or variations among their molecular weights. As a result of these discrepancies, certain components in the mixture spend a long time in the stationary phase and travel slowly through the chromatographic system, whereas others exit the system quickly (Sayed, 2021). Figure 6 shows the method to obtain the gartanin. To obtain pure gartanin compound from *G. mangostana* pericarp, the crude extract of *G. mangostana* undergoes fractionation via VLC to produce subfraction, followed by RC to obtain subfraction, which is anticipated to be a purified gartanin. To confirm obtaining the purified gartanin, the subfraction is subjected to Thin Layer Chromatography (TLC) to produce a single stain with a representative retention factor (RF). Meepagala & Schrader (2018) utilised silica gel to obtain and mark the stain with a fluorescent indicator. Subfractions of the *G. mangostana* with similar RF are combined, and the pure gartanin is subjected to confirmatory techniques such as spectrophotometry and mass spectrometry (Sani *et al.*, 2021).

In other studies by Meepagala & Schrader (2018), the bioassay-guided approach has been used to isolate and identify α-mangostin, γ-mangostin and (-)-epicatechin from *G. mangostana* pericarp extracted by ethyl acetate (EA) and methanol (ME) against the bacterium *Flavobacterium columnare* to help catfish aquaculture industry. Column chromatography fractions and purified compounds were analysed using silica gel thin-layer chromatography plates with fluorescent indicators. The step used is quite the same compared to (Oetari *et al.*, 2019). An isolated compound has been used to test the antibacterial assay against *F.columnare*. Isolation and identification of further fractionation of ethyl acetate and methanol extract, α-mangostin, γ-mangostin and (-)-epicatechin has been carried out via spectroscopic techniques. (-)-epicatechin showed the lowest activity while γ-mangostin showed the most significant activity compared to other purified compounds though α-mangostin is the major xanthone constituent in *G. mangostana* pericarp.

6. Bioactive compounds of *Garcinia mangostana*

Xanthone, tannins and flavonoids (isoflavones) are bioactive compounds found in *G. mangostana* pericarp extract. Xanthone is a group of yellow pigments and has been seen as the main polyphenolic compound in the *G. mangostana* pericarp. They are primarily polar compounds and can be dissolved in organic or hot water (Chhouk *et al.*, 2016; Pratiwi *et al.*, 2017). More than 50 xanthone derivatives were isolated from the pericarp of *G. mangostana*. However, from the xanthones that have been identified, *α*-, *β*-, *γ*-mangostins, garcinone E, 8-deoxygartanin, and gartanin are the most investigated (Ramesh *et al.*, 2017).

G. mangostana pericarp extract contains numerous bioactive chemicals, including prenylated and oxygenated xanthones and xanthones. The chemical structure of xanthones is unique, consisting of a tricyclic system (C6-C3-C6). The xanthones α and *γ*-mangostin are the most plentiful in *G. mangostana* pericarp. Garcinones C, D, 8-deoxygartanin, mangostin, garcinones A, B and E, mangostinone, 9-hydroxycalabaxanthone, and isomangostin are among the other xanthones found in *G. mangostana* pericarp (Gondokesumo *et al.*, 2019). Garcinone E has a significant cytotoxic impact against hepatocellular carcinoma and lung cancer cell lines. Garcinone E has an antiproliferative effect on gastric cancer cell lines and has been discovered to have a wide range of dose- and time-dependent cytotoxic effects on various cancer cell lines (Ramesh *et al.*, 2017).

The concentration of *α*-mangostin, the main xanthone, is substantially associated with most *G. mangostana*'s biological activities. Alpha mangostin has been isolated from dried *G. mangostana* extract and has been confirmed to have a wide range of functional properties, including antioxidant, antitumor, anti-inflammatory, antiallergy, antibacterial, antifungal, anticancer, antivirus and cytotoxicity activities (Chhouk *et al.*, 2016; Ghasemzadeh *et al.*, 2018).

G. mangostana pericarp extract is very popular in ancient medicine to treat abdominal pain, diarrhoea, infection and chronic ulcers. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HTLC) are two modern techniques used to trace the quantitative analysis possible and determine *α*-Mangostin in *G. mangostana* pericarp. The TLC method was precise and exact (Pratiwi *et al.*, 2017). Based on (Muchtaridi *et al.*, 2017), *α*-Mangostin, *γ*–Mangostin, gartanin and 3-isomangostin can be isolated from *G. mangostana* pericarp extract. These isolated xanthones can provide various functions, especially for pharmacological uses, including antimicrobial, antimalarial, antioxidant, and anti-inflammatory. Many methods can be used to analyse the presence of bioactive compounds. High-Performance Liquid Chromatography (HPLC) validly analyses bioactive compounds such as α-mangostin, *γ*–mangostin and gartanin in *G. mangostana* pericarp extract. *G. mangostana* was purchased from different places in West Java, Indonesia (Bogor, Purwakarta, Tasikmalaya and Subang). The highest among other samples was the α-mangostin, *γ*–mangostin, and gartanin levels of *G. mangostana* pericarp from Bogor.

In addition, anthocyanin is also one of the bioactive compounds found in the *G. mangostana* fruit. Anthocyanin is a water-soluble flavonoid pigment in many flowers, fruits, and vegetables responsible for reddish, bluish, and purple hues. Anthocyanins have sparked a surge of interest in natural colourants because of their wide variety of colours and the many health benefits they have been linked to, including anti-inflammatory, antimicrobial, anti-carcinogenic, and anti-diabetic properties. Data suggests that anthocyanins are beneficial therapeutically and are also non-toxic and non-mutagenic. However, anthocyanin is highly unstable and quickly degraded in its isolated form, and its stability is greatly influenced by factors such as pH, temperature, oxygen, and light (Ramasamy *et al.*, 2016).

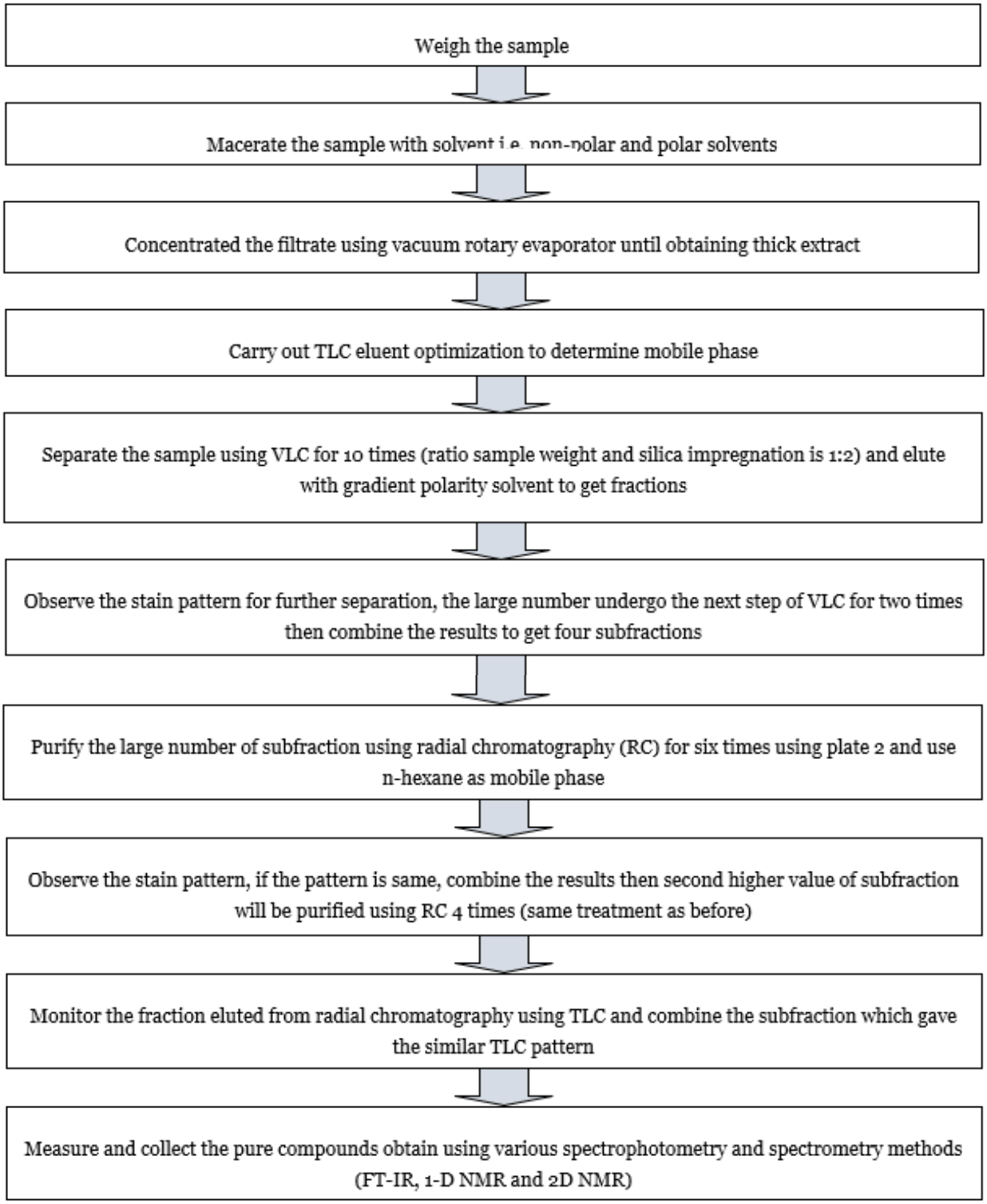


Figure 6: Chromatography techniques to obtain bioactive compounds.

7. Toxicity of Garcinia mangostana

When discussing the issue of toxicity, halal and *toyyiban* products constitute a significant cause of concern, particularly among Muslims. Halal is an Arabic term that implies 'permitted,' 'lawful,' 'approved,' and 'legal.' The *Qur'anic* phrase 'halal' is used to designate the permissible elements. Haram is the polar opposite of halal (forbidden or prohibited). Between halal and haram, there is a clear distinction (Khattak *et al.*, 2011). *Toyyiban* can be summed up as pure, good and wholesome. Technically, the words 'halal' and '*toyyiban*' have two different connotations. Halal also denotes adherence to the fundamental principles of *Shari'ah*. Yet, the *toyyiban* goes above and beyond these requirements to incorporate improved elements that make something fine, pure, and safe (Abdul Rahman & Sahari, 2022). Nowadays, many products contain

an extract from a questionable source, putting consumers at risk. Even though from halal sources, Muslims are concerned about this issue because if the product is unsafe to use, it will affect consumers in the future. As a result, this laboratory and toxicity test are critical to guarantee that the manufactured product is safe and halal. Plants and the substances derived from them are halal if they are not contaminated with haram nor contain intoxicants (Khattak *et al.*, 2011). The toxicity of an extract used from various sources is a significant concern, especially in cosmetic, medical and food products. Therefore, laboratory and toxicity tests should be conducted to ensure that a product is safe to use, especially for humans.

Determination of the toxicological profile of a substance is an essential prerequisite for guaranteeing public health. Toxicity is the degree to which animals or human beings are affected by a compound, and it may be acute, subchronic or chronic. Acute toxicity causes adverse effects from a single or short-term exposure in an organism. Subchronic toxicity means results that occur for more than a year due to the capability of toxic substances but less than the shelf life of the exposed organism. Chronic toxicity is the power of a substance or a mixture to cause adverse effects, usually after prolonged or continuous exposure, over an extended period (V *et al.*, 2010).

Many rodents and nonrodents, such as rats, pigs, monkeys, rabbits, guinea pigs and dogs, are also used in toxicity studies. Rodents have been the most commonly used species in research due to their low acquisition and maintenance costs (housing and food), standardised hygienic environment, high ethical acceptance, rapid reproductive biology, effective and well-established genetic modification techniques, large-scale standardised phenotyping protocols and access to an extensive database of reference information. Apart from rodents, domestic rabbits (Oryctolagus cuniculus) are the second most frequently used experimental animals because of their low maintenance costs, good reproductive characteristics, and importance as model organisms for translational medicine, even though they have many significant differences from humans like gastrointestinal tract physiology. Other than that, Old World monkeys also used experimental animals such as cynomolgus monkeys *(Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*) as they are phylogenetically much more similar to primates than humans. Nevertheless, they have significant limitations as animal models due to inadequate ethical recognition, and a long generation interval is among them. Pigs have become standard animal models because they are anatomically, metabolically, physiologically, and pathophysiologically identical to humans, have sound reproduction and are ethically appropriate. A growing number of well-established techniques for genetically modifying pigs, such as lentiviral transgenesis, nuclear transfer from gene-targeted cells, inducible transgene expression, and site-directed nuclease gene editing, now make it easier to create customised broad animal models for diabetes research and other translational medicine applications. While inbreeding may achieve genetic standardisation, as seen in the Massachusetts General Hospital miniature pig, outbred pigs can more closely match human genetic variation. The small size of rodents and rabbits saves money on maintenance and reduces the amount of test compound necessary. Still, it restricts the sample material available per animal, especially in rodents. Minipigs (Göttingen minipig) are a cross between rodents and domestic pigs and are commonly used in drug effectiveness and safety testing. Pigs are the only animals that can approximate the size and weight of humans across a wide variety of developmental stages. Domestic pigs cover infancy and adolescence due to their rapid development as they are ideal for short and medium terms studies which can take up to several months, while minipig breeds cover adulthood as they are suitable for long-term studies, which can go up to years of the period after they have reached their limit of the growth phase. Medical devices such as bioartificial pancreas, surgical procedures (bariatric surgery) and percutaneous catheter treatments for revascularisation, or noninvasive imaging techniques (ultrasonography, computed tomography, and magnetic resonance imaging) can also be directly used transferred from laboratory studies to clinical use in human patients due to pig size. Unlike pigs, noninvasive imaging techniques in rodents have resolution limitations due to their limited scale, and even ultrasonography necessitates anaesthesia.

Furthermore, noninvasive imaging methods for quantifying islet/beta-cell mass can be evaluated in human-sized pig models, such as decreased beta-cell mass or diet-induced obesity and then checked using quantitative stereological pancreas analyses. Pigs have a large blood volume, which allows for accurate metabolic tests such as glucose/insulin tolerance tests and clamp trials and regular blood sample recovery large enough to conduct complex hormone and metabolite profiling in each sample. Blood samples from pig foetuses or neonatal piglets can be easily obtained. However, assessing stimulated insulin secretion is different in rodents, especially mice, because it is difficult as the sample material limitations restrict the resolution achieved (Renner *et al.*, 2016).

Regarding Shaik & Reddy (2017), in dental treatment, the use of opioid agents helps clean the root canal between appointments and reduce discomfort between appointments. The dentinal tubules are required to penetrate these medications, enter the root canal and inhibit the growth of the bacteria inside the root canal. Irreversible pulpitis is an inflammatory dental pulp disease requiring root canal treatment to clear the microbial infection from the root canal system and the periradicular zone. Treatment of the root canal comprises three steps: preparation of the root canal, which involves cleaning and forming the root canal system; disinfection or sterilization, and obturation of the root canal. Root canal disinfection products appear to induce side effects because they fall into the therapeutic agent group, consist of active chemical agents, and are usually toxic. The herbal-based agent is used as an alternative to lessen the dependence on chemical agents, as almost all countries have also accepted it. The use of traditional medicine in public health care is also advocated by the World Health Organisation (WHO), especially for the prevention and treatment of chronic diseases, degenerative diseases and cases of cancer. It has been proved by previous studies that show the usage of herbal medicine is less toxic as it has comparatively limited side effects than modern medicine/chemical synthesis.

G. mangostana pericarp has been proven by phytochemical research to have the most active ingredients, such as xanthones, flavonoids, saponins and tannins. Pharmacological effects produced by xanthones are antibacterial, antifungal, and anti-inflammatory, and it has been proven non-toxic to mice as it is orally administrated at 100 mg/kg of body weight for seven days. Other research also determined that α-mangostin was non-toxic to human gingival fibroblasts for 480 mins at specific dosages. Tannins are primarily found in several plant species' bark, stems, leaves and fruit and play vital roles in cell defence and growth control. Tannins are also believed to have several elements of chemical activities such as apoptosis, antitumor, antibacterial and antiplasmin. However, tannin can

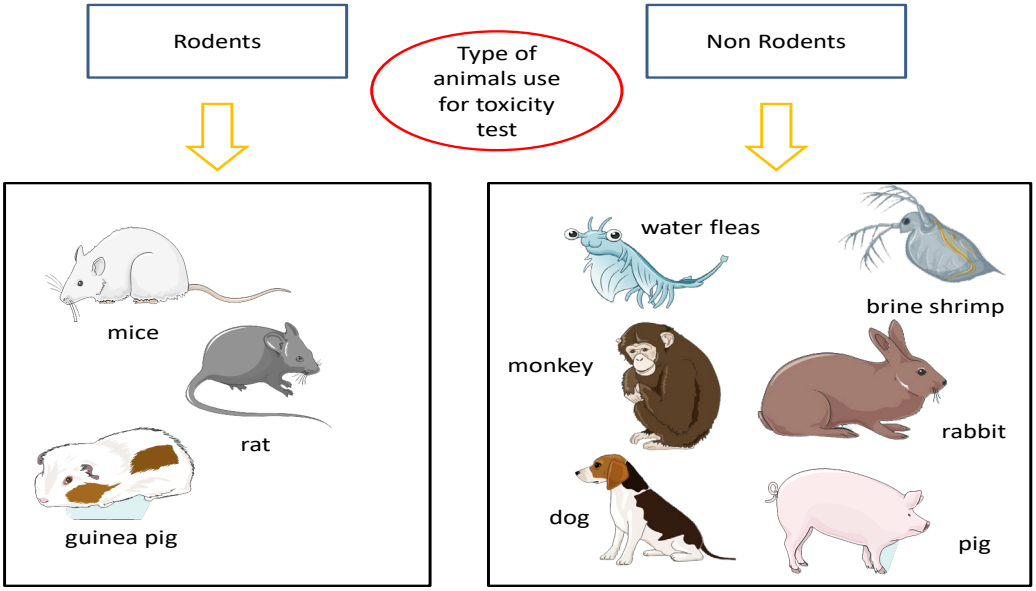


Figure 7: Type of animal used for toxicity test.

cause mucous membrane irritation at high concentrations. These active compounds of *G. mangostana* pericarp extract provide an encouraging potential to promote root canal treatment effectiveness, but all dentistry products must meet biocompatibility criteria. During the initial step of a substance biocompatibility assessment, the toxicity assay is carried out and forms part of the dental material evaluation, and this is one of the procedures needed for standard screening. The toxicity of xanthones and tannins from *G. mangostana* pericarps were determined and contrasted via BHK-21 fibroblast cell culture (Baby Hamster Kidney-21) assay since both xanthone and tannin are the most active ingredients and encourage antimicrobial activities. As a result, it can be concluded that 3.98% of xanthones and 2.2% of tannins were toxic to the culture of BHK-21 fibroblast cells. It is proposed that tannins are more toxic when compared to xanthones.

For nonrodents, daphnia was used to test the toxicity of *G. mangostana* extract. According to Sonawane & George (2018), daphnia is a freshwater filter-feeding crustacean used in ecotoxicity studies because it is a highly susceptible organism. It serves as a model organism for the standard testing protocols of the US Environmental Protection Agency (EPA), the Organization for Economic Cooperation and Development (OECD), and the International Standards Organization (ISO). The *G. mangostana* crude extracts obtained from three different extraction methods (cold extract, Soxhlet extract and microwave-assisted extract) were used to test their toxicity efficacy against *Daphinia magna*. The cytostatic medication 5-fluorouracil has an EC50 value of 15 ppm against *daphnia magna*. Interestingly, some unicellular flagellate organisms grew even at the maximum concentration of extracts (20 ppm). This difference in toxicity begs to be investigated, suggesting that it could be extrapolated to human normal and cancer cell lines.

8. Conclusion

In conclusion, several researchers have described and investigated various bioactivities of *G. mangostana* fruit since long ago. Each of the bioactive components found in *G. mangostana* parts demonstrated a variety of bioactivities that give multiple benefits for human health and wellbeing. The selection of a proper extraction method plays an essential and crucial role in determining the final result and outcome of the study. In addition, each source that will be used on humans must be subjected to a toxicity test to ensure its safety before use.

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Conflict of interest statement

We declare no conflict of interest.

References

Abdul Rahman, F. A., & Sahari, N. H. (2022). Halalan Toyyiban Concept in Food Truck Business. Journal of Contemporary Islamic Studies, 8(2), 49–60. https://doi.org/10.24191/jcis.v8i2.4

Ahmad, M. I., Keach, J. E., Behl, T., & Panichayupakaranant, P. (2019). Synergistic effect of α-mangostin on antibacterial activity of tetracycline, erythromycin, and clindamycin against acne involved bacteria. Chinese Herbal Medicines, 11(4), 412–416. https://doi.org/10.1016/j.chmed.2019.03.013

Aizat, W. M., Jamil, I. N., Ahmad-Hashim, F. H., & Noor, N. M. (2019). Recent updates on metabolite composition and medicinal benefits of mangosteen plant. PeerJ, 7. https://doi.org/10.7717/peerj.6324

Article, F. (2021). Mangosteen. In Britannica Online Encyclopedia (pp. 1–2).

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, a., Sahena, F., Jahurul, M. H. a., Ghafoor, K., Norulaini, N. a. N., & Omar, a. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Boonmak, N., Niyompanich, J., Chuysinuan, P., Niamlang, P., Ekabutr, P., & Supaphol, P. (2018). Preparation of mangosteen extract-loaded poly(vinyl acetate) for use as an antibacterial spray-on dressing. Journal of Drug Delivery Science and Technology, 46, 322–329. https://doi.org/10.1016/j.jddst.2018.05.033

Bundeesomchok, K., Filly, A., Rakotomanomana, N., Panichayupakaranant, P., & Chemat, F. (2016). Extraction of α-mangostin from Garcinia mangostana L. using alternative solvents: Computational predictive and experimental studies. LWT - Food Science and Technology, 65, 297–303. https://doi.org/10.1016/j.lwt.2015.08.036

Carvalho-Silva, R., Pereira, A. C. F., Santos Alves, R. P. Dos, Guecheva, T. N., Henriques, J. A. P., Brendel, M., Pungartnik, C., & Rios-Santos, F. (2016). DNA protection against oxidative damage using the hydroalcoholic extract of garcinia mangostana and alpha-mangostin. Evidence-Based Complementary and Alternative Medicine, 2016. https://doi.org/10.1155/2016/3430405

Chaovanalikit, A., Mingmuang, A., Kitbunluewit, T., Choldumrongkool, N., Sondee, J., & Chupratum, S. (2012). Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of Mangosteen products. International Food Research Journal, 19(3), 1047–1053.

Chhouk, K., Quitain, A. T., Gaspillo, P. A. D., Maridable, J. B., Sasaki, M., Shimoyama, Y., & Goto, M. (2016a). Supercritical carbon dioxide-mediated hydrothermal extraction of bioactive compounds from Garcinia Mangostana pericarp. Journal of Supercritical Fluids, 110, 167–175. https://doi.org/10.1016/j.supflu.2015.11.016

Chhouk, K., Quitain, A. T., Gaspillo, P. A. D., Maridable, J. B., Sasaki, M., Shimoyama, Y., & Goto, M. (2016b). Supercritical carbon dioxide-mediated hydrothermal extraction of bioactive compounds from Garcinia Mangostana pericarp. Journal of Supercritical Fluids, 110, 167–175. https://doi.org/10.1016/j.supflu.2015.11.016

Datta, N., Pal, M., Roy, U., Mitra, R., & Pradhan, A. (2014). World Journal of Pharmaceutical Research. Infection, 13(March), 15. https://doi.org/10.20959/wjpr20169-7046

Espirito Santo, B. L. S. do, Santana, L. F., Kato Junior, W. H., de Araújo, F. de O., Bogo, D., Freitas, K. de C., Guimarães, R. de C. A., Hiane, P. A., Pott, A., Filiú, W. F. de O., Arakaki Asato, M., Figueiredo, P. de O., & Bastos, P. R. H. de O. (2020). Medicinal Potential of Garcinia Species and Their Compounds. Molecules (Basel, Switzerland), 25(19), 1–30. https://doi.org/10.3390/molecules25194513

FAOSTAT. (2019). Mangosteen production 2019. Food and Agricultural Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC

Freiesleben, S. H., Soelberg, J., Nyberg, N. T., & Jäger, A. K. (2017). Determination of the wound healing potentials of medicinal plants historically used in Ghana. Evidence-Based Complementary and Alternative Medicine, 2017. https://doi.org/10.1155/2017/9480791

Ghasemzadeh, A., Jaafar, H. Z. E., Baghdadi, A., & Tayebi-Meigooni, A. (2018a). Alpha-mangostin-rich extracts from mangosteen pericarp: Optimisation of green extraction protocol and evaluation of biological activity. Molecules, 23(8), 1–16. https://doi.org/10.3390/molecules23081852

Ghasemzadeh, A., Jaafar, H. Z. E., Baghdadi, A., & Tayebi-Meigooni, A. (2018b). Alpha-mangostin-rich extracts from mangosteen pericarp: Optimisation of green extraction protocol and evaluation of biological activity. Molecules, 23(8), 1–16. https://doi.org/10.3390/molecules23081852

Gondokesumo, M. E., Pardjianto, B., Sumitro, S. B., Widowati, W., & Handono, K. (2019a). Xanthones analysis and antioxidant activity analysis (Applying ESR) of six different maturity levels of mangosteen rind extract (Garcinia mangostana Linn.). Pharmacognosy Journal, 11(2), 369–373. https://doi.org/10.5530/pj.2019.11.56

Gondokesumo, M. E., Pardjianto, B., Sumitro, S. B., Widowati, W., & Handono, K. (2019b). Xanthones analysis and antioxidant activity analysis (Applying ESR) of six different maturity levels of mangosteen rind extract (Garcinia mangostana Linn.). Pharmacognosy Journal, 11(2), 369–373. https://doi.org/10.5530/pj.2019.11.56

Guerrero-Pepinosa, N. Y., Cardona-Trujillo, M. C., Garzón-Castaño, S. C., Veloza, L. A., & Sepúlveda-Arias, J. C. (2021). Antiproliferative activity of thiazole and oxazole derivatives: A systematic review of in vitro and in vivo studies. In Biomedicine and Pharmacotherapy (Vol. 138). Elsevier Masson s.r.l. https://doi.org/10.1016/j.biopha.2021.111495

Gutierrez-Orozco, F., & Failla, M. L. (2013a). Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients, 5(8), 3163–3183. https://doi.org/10.3390/nu5083163

Gutierrez-Orozco, F., & Failla, M. L. (2013b). Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients, 5(8), 3163–3183. https://doi.org/10.3390/nu5083163

Hendiani, I., Hadidjah, D., Susanto, A., & Setia Pribadi, I. M. (2017). The effectiveness of mangosteen rind extract as additional therapy on chronic periodontitis (Clinical trials). Padjadjaran Journal of Dentistry, 29(1), 64–70. https://doi.org/10.24198/pjd.vol29no1.12986

Khattak, J. Z. K., Mir, A., Anwar, Z., Wahedi, H. M., Abbas, G., Khattak, H. Z. K., & Ismatullah, H. (2011). Concept of Halal food and biotechnology. Advance Journal of Food Science and Technology, 3(5), 385–389. https://doi.org/10.1017/CBO9781107415324.004

Kurniawan, Y. S., Priyangga, K. T. A., Jumina, Pranowo, H. D., Sholikhah, E. N., Zulkarnain, A. K., Fatimi, H. A., & Julianus, J. (2021). An update on the anticancer activity of xanthone derivatives: A review. In Pharmaceuticals (Vol. 14, Issue 11). MDPI. https://doi.org/10.3390/ph14111144

Kusmayadi, A., Adriani, L., Abun, A., Muchtaridi, M., & Tanuwiria, U. H. (2018). The effect of solvents and extraction time on total xanthone and antioxidant yields of mangosteen peel (Garcinia mangostana L.) extract. Drug Invention Today, 10(12), 2572–2576.

Li, P., Yang, Z., Tang, B., Zhang, Q., Chen, Z., Zhang, J., Wei, J., Sun, L., & Yan, J. (2020). Identification of Xanthones from the Mangosteen Pericarp that Inhibit the Growth of Ralstonia solanacearum. ACS Omega, 5(1), 334–343. https://doi.org/10.1021/acsomega.9b02746

López-Romero, D., Izquierdo-Vega, J. A., Morales-González, J. A., Madrigal-Bujaidar, E., Chamorro-Cevallos, G., Sánchez-Gutiérrez, M., Betanzos-Cabrera, G., Alvarez-Gonzalez, I., Morales-González, Á., & Madrigal-Santillán, E. (2018). Evidence of some natural products with antigenotoxic effects. Part 2: Plants, vegetables, and natural resin. In Nutrients (Vol. 10, Issue 12). MDPI AG. https://doi.org/10.3390/nu10121954

Lourith, N., & Kanlayavattanakul, M. (2011). Biological activity and stability of mangosteen as a potential natural colour. Bioscience, Biotechnology and Biochemistry, 75(11), 2257–2259. https://doi.org/10.1271/bbb.110521

Malviya, N., & Malviya, S. (2017). Bioassay guided fractionation-an emerging technique influence the isolation, identification and characterisation of lead phytomolecules. International Journal of Hospital Pharmacy, August. https://doi.org/10.28933/ijhp-2017-07-0901

Markowicz, J., Uram, Ł., Sobich, J., Mangiardi, L., Maj, P., & Rode, W. (2019). Antitumor and anti-nematode activities of α-mangostin. European Journal of Pharmacology, 863(July). https://doi.org/10.1016/j.ejphar.2019.172678

Meepagala, K. M., & Schrader, K. K. (2018). Antibacterial Activity of Constituents from Mangosteen Garcinia mangostana Fruit Pericarp against Several Channel Catfish Pathogens. Journal of Aquatic Animal Health, 30(3), 179–184. https://doi.org/10.1002/aah.10021

Mohammad, N. A., Abang Zaidel, D. N., Muhamad, I. I., Abdul Hamid, M., Yaakob, H., & Mohd Jusoh, Y. M. (2019a). Optimisation of the antioxidant-rich xanthone extract from mangosteen (Garcinia mangostana L.) pericarp via microwave-assisted extraction. In Heliyon (Vol. 5, Issue 10). https://doi.org/10.1016/j.heliyon.2019.e02571

Mohammad, N. A., Abang Zaidel, D. N., Muhamad, I. I., Abdul Hamid, M., Yaakob, H., & Mohd Jusoh, Y. M. (2019b). Optimisation of the antioxidant-rich xanthone extract from mangosteen (Garcinia mangostana L.) pericarp via microwave-assisted extraction. Heliyon, 5(10), e02571. https://doi.org/10.1016/j.heliyon.2019.e02571

Moopayak, W. (2020a). Mangosteen peel and seed as antimicrobial and drug delivery in rubber products. Journal of Applied Polymer Science, December 2019, 1–14. https://doi.org/10.1002/app.49119

Moopayak, W. (2020b). Mangosteen peel and seed as antimicrobial and drug delivery in rubber products. Journal of Applied Polymer Science, December 2019, 1–14. https://doi.org/10.1002/app.49119

Muchtaridi, M., Puteri, N. A., Milanda, T., & Musfiroh, I. (2017). Validation analysis methods of α-mangostin, γ-mangostin and gartanin mixture in Mangosteen (Garcinia mangostana L.) fruit rind extract from west java with HPLC. Journal of Applied Pharmaceutical Science, 7(10), 125–130. https://doi.org/10.7324/JAPS.2017.71018

Oetari, R. A., Hasriyani, H., Prayitno, A., & Sahidin, S. (2019). Gartanin Compounds from Extract Ethanol Pericarp Mangosteen (Garcinia mangostana Linn.). Open Access Macedonian Journal of Medical Sciences, 7(22), 3891–3895. https://doi.org/10.3889/oamjms.2019.527

Ovalle-Magallanes, B., Eugenio-Pérez, D., & Pedraza-Chaverri, J. (2017). Medicinal properties of mangosteen (Garcinia mangostana L.): A comprehensive update. Food and Chemical Toxicology, 109, 102–122. https://doi.org/10.1016/j.fct.2017.08.021

Palapol, Y., Ketsa, S., Stevenson, D., Cooney, J. M., Allan, A. C., & Ferguson, I. B. (2009). Colour development and quality of mangosteen (Garcinia mangostana L.) fruit during ripening and after harvest. Postharvest Biology and Technology, 51(3), 349–353. https://doi.org/10.1016/j.postharvbio.2008.08.003

Panawes, S., Ekabutr, P., Niamlang, P., Pavasant, P., Chuysinuan, P., & Supaphol, P. (2017). Antimicrobial mangosteen extract infused alginate-coated gauze wound dressing. Journal of Drug Delivery Science and Technology, 41, 182–190. https://doi.org/10.1016/j.jddst.2017.06.021

Patel, L. R., Camacho, D. F., Shiozawa, Y., Pienta, K. J., & Taichman, R. S. (2011). Mechanisms of cancer cell metastasis to the bone: A multistep process. In Future Oncology (Vol. 7, Issue 11, pp. 1285–1297). https://doi.org/10.2217/fon.11.112

Pothitirat, W., Chomnawang, M. T., Supabphol, R., & Gritsanapan, W. (2010). Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods. Pharmaceutical Biology, 48(2), 182–186. https://doi.org/10.3109/13880200903062671

Pratiwi, L., Fudholi, A., Martien, R., & Pramono, S. (2017). Development of TLC and HPTLC method for determination α-mangostin in mangosteen peels (Garcinia Mangostana L.,). Available Online on Www.Ijppr.Com International Journal of Pharmacognosy and Phytochemical Research, 9(3), 297–302.

Quispe-Tintaya, W. (2017). 乳鼠心肌提取 HHS Public Access. Physiology & Behavior, 176(3), 139–148. https://doi.org/10.1016/S1473-3099(16)30473-X.Dengue

Ramasamy, S., Mazlan, N. A., Ramli, N. A., Rasidi, W. N. A., & Manickam, S. (2016). Bioactivity and stability studies of anthocyanin-Containing extracts from Garcinia mangostana L. And Etlingera elatior Jack. Sains Malaysiana, 45(4), 559–565.

Ramesh, S., Priya, M., & Prabhu, S. (2017). Isolation of garcinone E from Garcinia mangostana Linn and its cytotoxic effect on sp2/0 cell lines. ~ 67 ~ Journal of Pharmacognosy and Phytochemistry, 6(5).

Renner, S., Dobenecker, B., Blutke, A., Zöls, S., Wanke, R., Ritzmann, M., & Wolf, E. (2016). Comparative aspects of rodent and nonrodent animal models for mechanistic and translational diabetes research. Theriogenology, 86(1), 406–421. https://doi.org/10.1016/j.theriogenology.2016.04.055

Saepudin, A., Natawijaya, D., Hartini, E., & Iskandar, R. (2019). Evaluation of antibacterial activity of mangosteen (Garcinia mangostana L.) pericarp extract against rice leaf blight bacteria (Xanthomonas oryzae pv. oryzae) at various temperatures and durations of fruit storage. IOP Conference Series: Earth and Environmental Science, 250(1). https://doi.org/10.1088/1755-1315/250/1/012026

Sani, M. S. A., Bakar, J., Rahman, R. A., & Abas, F. (2021). Antibacterial composition of bioautographic fractions, characteristics, and stability of Carica papaya seed extract. International Food Research Journal, 28(June), 443–456.

Sayed, M. A. (2021). A review of Chromatography: principles, Classification, Applications A role of nanoparticles in diagnosis and treatment of lung cancer. View project A review of Chromatography: principles, Classification, Applications. https://doi.org/10.13140/RG.2.2.22113.43361

Shafy, G. M., Jassim, A. M. N., & Mohammed, M. T. (2019). Study of phytochemical, antioxidant and anti-inflammatory of mangosteen (G. Mangostana) and its ability to woundhealing. Plant Archives, 19(1), 665–673.

Shan, T., Ma, Q., Guo, K., Liu, J., Li, W., Wang, F., & Wu, E. (2011). Xanthones from Mangosteen Extracts as Natural Chemopreventive Agents: Potential Anticancer Drugs. Current Molecular Medicine, 11(8), 666–677. https://doi.org/10.2174/156652411797536679

Soetikno, J. S., Handayani, R., & Rahayu, R. D. (2016). Assay for Antimicrobial Activity of Mangosteen Rind Extracts. Journal of Innovations in Pharmaceuticals and Biological Sciences (JIPBS), Vol 3 (1)(January), 54–60.

Sombolayuk, H. B., Djawad, K., Wahab, S., Miskad, U. A., Alam, G., & Pattelongi, I. (2019a). The effectivity of topical mangosteen pericarp extract cream on wound healing in Swiss albino mice. Journal of Biological Research (Italy), 92(2). https://doi.org/10.4081/jbr.2019.8150

Sombolayuk, H. B., Djawad, K., Wahab, S., Miskad, U. A., Alam, G., & Pattelongi, I. (2019b). The effectivity of topical mangosteen pericarp extract cream on wound healing in Swiss albino mice. Journal of Biological Research (Italy), 92(2). https://doi.org/10.4081/jbr.2019.8150

Sonawane, V. R., & George, I. A. (2018). the Toxicity of Mangosteen Rind Extracts on Daphnia. International Research Journal Of Pharmacy, 9(3), 103–104. https://doi.org/10.7897/2230-8407.09350

Sugiyanto, Z., Yohan, B., Hadisaputro, S., Dharmana, E., Suharti, C., Winarto, Djamiatun, K., Rahmi, F. L., & Sasmono, R. T. (2019). Inhibitory Effect of Alpha-Mangostin to Dengue Virus Replication and Cytokines Expression in Human Peripheral Blood Mononuclear Cells. Natural Products and Bioprospecting, 9(5), 345–349. https://doi.org/10.1007/s13659-019-00218-z

Suhartati, R., Apriyani, F., Khusnul, Virgianti, D. P., & Fathurohman, M. (2019a). Antimicrobial Activity Test of Mangosteen Leaves Ethanol Extract (Garcinia mangostana Linn) Against Pseudomonas aeruginosa Bacteria. Journal of Physics: Conference Series, 1179(1). https://doi.org/10.1088/1742-6596/1179/1/012167

Suhartati, R., Apriyani, F., Khusnul, Virgianti, D. P., & Fathurohman, M. (2019b). Antimicrobial Activity Test of Mangosteen Leaves Ethanol Extract (Garcinia mangostana Linn) Against Pseudomonas aeruginosa Bacteria. Journal of Physics: Conference Series, 1179(1). https://doi.org/10.1088/1742-6596/1179/1/012167

Sunarjo, L. (2017). The Preliminary Study on Safety of Using Mangosteen Peel Extract as Natural Herbs. Journal of Medical Science and Clinical Research, 5(7), 24851–24856. https://doi.org/10.18535/jmscr/v5i7.92

Suttirak, W., & Manurakchinakorn, S. (2014). In vitro antioxidant properties of mangosteen peel extract. Journal of Food Science and Technology, 51(12), 3546–3558. https://doi.org/10.1007/s13197-012-0887-5

Tarasuk, M., Songprakhon, P., Chimma, P., Sratongno, P., Na-Bangchang, K., & Yenchitsomanus, P. thai. (2017). Alpha-mangostin inhibits both dengue virus production and cytokine/chemokine expression. Virus Research, 240(March), 180–189. https://doi.org/10.1016/j.virusres.2017.08.011

Tatiya-aphiradee, N., Chatuphonprasert, W., & Jarukamjorn, K. (2019). Anti-inflammatory effect of Garcinia mangostana Linn. pericarp extract in methicillin-resistant Staphylococcus aureus-induced superficial skin infection in mice. Biomedicine and Pharmacotherapy, 111(December 2018), 705–713. https://doi.org/10.1016/j.biopha.2018.12.142

V, V. P., Jainu, M., Mohan, S. K., Karthik, B., Saraswathi, P., & S, C. S. G. V. (2010). Toxicity Study of Garcinia Mangostana Linn . Pericarp Extract in Rats. 1(3), 633–637.

Weller, M. G. (2012). A unifying review of bioassay-guided fractionation, effect-directed analysis and related techniques. Sensors (Switzerland), 12(7), 9181–9209. https://doi.org/10.3390/s120709181

Wibawanti, J. M. W., Zulfanita, Z., & Runanto, D. (2019). The Antioxidant Activity of Yogurt Drink by Mangosteen Rind Extract (Garcinia mangostana L.). Journal of Applied Food Technology, 6(1), 15–18. https://doi.org/10.17728/jaft.4267

Widowati, W., Darsono, L., Suherman, J., Fauziah, N., Maesaroh, M., & Erawijantari, P. putu. (2016). Anti-inflammatory effect of mangosteen (Garcinia mangostana l.) peel extract and its compounds in lps-induced raw264.7 cells. Natural Product Sciences, 22(3), 147–153. https://doi.org/10.20307/nps.2016.22.3.147

Yang, R., Li, P., Li, N., Zhang, Q., Bai, X., Wang, L., Xiao, Y., Sun, L., Yang, Q., & Yan, J. (2017). Xanthones from the pericarp of Garcinia mangostana. Molecules, 22(5), 1–10. https://doi.org/10.3390/molecules2205068

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Best Practice in Halal Frozen Meat Products in Brunei Darussalam: A Legal Review

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Abstract

This paper provides a legal review of the best practices in halal frozen meat products in Brunei Darussalam. This paper examines the regulatory framework for halal meat production in Brunei and analyses the country's current state of halal frozen meat products. This paper aims to review the laws and regulations governing importing of halal frozen meat products in Brunei Darussalam. Moreover, this paper also aims to provide recommendations for improving the legal and regulatory framework governing the importation of halal frozen meat products in Brunei Darussalam. This paper has implemented qualitative methods using library research, and all the information for the findings is obtained through secondary sources. Overall, the paper highlights the importance of ensuring that halal frozen meat products comply with Islamic dietary laws, the production process adheres to the strict rules and regulations of the country and ensuring that imported meat products have their certificate and permit to be able to commercially sold and consumed in the country.

1. Introduction

Brunei Darussalam is known as one of the oil and gas producers. Since the first oil production, the economy has run heavily on oil revenues. However, Brunei began to generate more non-oil activities to diversify its economy a decade ago. The halal and agricultural sectors are among those Brunei is now emphasizing, per the country's Eleventh National Development Plan (2018-2023). Recently, Brunei Darussalam has supported agricultural growth and facilitated the outsourcing of farming commodities and food supply to ensure food security (Ministry of Foreign Affairs, n.d). In addition, Borneo Bulletin Yearbook 2022 has reported that Brunei aspires to enhance the supply of its red meat by opening sites for ruminant livestock enterprises for PDS Abattoir Sdn Bhd (PDS). Nonetheless, Brunei imports most of its raw beef primarily from Australia, the United Kingdom, Malaysia, China, and India (Khalid, Haji Masr, Muhammad, and Pang, 2018).

As stated on the website of Brunei Darussalam National Single Window (BDNSW), meat products are regarded as restricted and controlled goods. Thus, halal certification and permits for halal frozen meat products are necessary to ensure that the halal integrity of the supply chain is preserved from any fraudulent exploitation practices. However, numerous illegal frozen meat importation cases have been reported over the past few years. For instance, in 2023 alone, the local newspaper Borneo Bulletin reported several cases of frozen meat without a halal permit and smuggled raw meat seized by the authorities. These reported cases show that the authorities are now proactive in conducting surveillance and inspection.

On the other hand, it is alarming that the number of reported incidents is increasing and that more fraud is occurring, raising serious concerns. Hence, this paper aims to review the laws and regulations governing importing halal frozen meat products in Brunei Darussalam. Moreover, this paper aims to provide recommendations for improving the legal and regulatory framework governing the importation of halal frozen meat products in Brunei Darussalam.

Considering the benefit of this paper, it aims to highlight the best practices in meat importation for the government and industry to pay attention to. When the best practices are met and followed, it will outline the effective, streamlined process and improve overall efficiency. Existing research on meat importation has a limited focus on what is considered the best practices. Hence, this paper adequately adds to the scholarly literature and discussion.

Materials and methods

As for the materials and methods, this paper has implemented qualitative methods using library research. The information for the findings were obtained through secondary sources, mainly from journals, books, newspapers, and electronic media such as Google Scholar, ResearchGate, and other related official websites. This mainly involved around 18 articles and journals from the relevant field.

3. Results and discussion

3.1 Literature review on halal meat products

In Malaysia, particular halal requirements must be met to ensure that halal meat products are *Halalan Toyyiban* following modern practice. MS1500: 2019, Halal Food-General criteria, and Malaysian Protocol for Halal Meat and Poultry Productions are among the Malaysian standard criteria for halal meat products. Both functions are designed to aid in implementing the halal certification process. In addition, the Department of Islamic Development Malaysia (JAKIM) has issued the new Manual Procedure for Malaysia Halal Certification (MPMHC) (Domestic) 2020 and Malaysian Halal Management System (MHMS) 2020 to promote halal integrity in Malaysia's halal certification process. The standards are crucial for supporting Malaysia's halal meat sector development by ensuring Muslims have real access to only halal meat products as well as preventing any misconduct. The government's efforts have demonstrated the need to protect specific important Shariah values, particularly the religion (faith), body, and mind of Malaysia's Muslim society. It involves the notion of *Sadd al-zara'ie* (stopping the means) and the government's accountability as the *'Ulul amr* to guarantee that *Halalan Toyyiban* meat will be enough and accessible in the local market through the adoption of effective halal governance to address halal meat-related concerns (Ruzulan & Ishak, 2021).

Overall, these authorities work together to ensure that halal frozen meat products meet the requirements and standards for halal certification and that the importation and exportation of these products are adequately regulated.

3.4 Issue: illegal meat importation

Since the emergence of COVID-19 in Brunei, it is acknowledged that the price of imported animal products such as beef and lamb has skyrocketed (Musa & Basir, 2021) as cited in (Sulaiman & Hashim, 2022). For instance, beef prices rose from $15.20 per kilogram in September 2020 to $16.14 per kilogram in December 2020. Similarly, by 2020, frozen beef prices had risen from $12.40 to $13.50 per kilogram. Hence, it is not surprising that individuals and businesses are more likely to commit food crimes as the cost of food rises (Sulaiman & Hashim, 2022).

Between the years 2020 and 2023, several cases of illegal meat importation have been recorded. On December 29, 2020, an article titled "Frozen Meat without Halal Permit Seized" was published by Borneo Bulletin. The news reported that a joint operation in Temburong District, conducted by BKMH, RCED, and Narcotics Control Bureau (NCB), seized 1.4 tonnes of smuggled frozen meat.

Whereas on February 11, 2021, an article titled 'Smuggled Raw Meat, Beef Lungs Seized Outside House' was published by Borneo Bulletin. The article stated that RCED and BKMH had discovered 1,120kg of raw meat and 191.2kg of beef lungs outside a residence in Mentiri, which has since been confiscated. According to the report, the absence of a halal logo from MUIB on the raw meat and beef lungs suggests they were likely imported into the country without the necessary halal import permit.

Lastly, on January 9, 2023, Borneo Bulletin published an article about meat smuggling titled 'Meat without Halal Import Permit Confiscated in Raid.' The report detailed a joint operation conducted by the BKMH and the RCED, confiscating six packets of marinated lamb meat, one packet of marinated chicken, and two packets of marinated beef. The meat products were brought into Brunei without a halal import permit, and the raid occurred at a residence in Kampong Mata-Mata, Gadong.

Upon reviewing these published articles, it is evident that all these articles have mentioned that imported halal meat into Brunei must possess a valid halal import permit approved by the relevant authorities. The articles mentioned above have also emphasized the importance of public collaboration in reporting cases of illegal meat importation to the authorities. Nonetheless, the escalating cases of unlawful meat importation have caused apprehension, prompting the authorities and the public to undertake requisite measures and initiatives to mitigate these cases.

3.5 Recommendation

As for recommendations, firstly, the authorities must step up to strengthen the enforcement of HMA and the penalty to intimidate and discourage offenders. Additionally, the authorities should amplify inspections at ports, borders, and the market to ensure that there are no illegal meat import activities. This can be achieved by having two BKMH officers witnessing every shipment or recruiting additional inspectors.

Secondly, reducing individual and corporate ignorance of meat importation legislation is necessary. Hence, it is recommended that the relevant authority be more transparent and open regarding the steps and procedures for Halal import meat (Khalid *et al.*, 2018) by educating the public through any means, such as mass media.

Additionally, acquiring a permit has also been regarded as a challenge for some businesses. They perceived it as burdensome, complicated, lengthy, and expensive. It is suggested that to improve the system's efficiency, recruiting more qualified staff is recommended to quicken the process. Moreover, letting the importing company hire a qualified halal certifier in the country of origin to witness and report to the relevant agency is also a way to improve efficiency. Other than that, the government may appoint local halal certifiers as their representatives in the country of origin.

Finally, it is recommended that the government set up a service or agency with the work scope on halal matters, but primarily to assist and consult companies in applying for certification and permits related to halal. This resolves the 'burdensome, lengthy, and complicated' process of getting a halal certification and permit. This is beneficial for many individuals as it makes it simpler for companies to get halal certification and provides skilled graduates with more significant employment opportunities.

4. Conclusion

As Brunei imports most of its raw beef primarily from foreign countries, halal certification and permits for halal frozen meat products are necessary. This is to ensure that the halal integrity of the supply chain is preserved from any fraudulent and exploitation practices. Consequently, only those with a Halal Import Permit and an export permit from the meat country of origin can import frozen meat products. By highlighting the HMA Section 4[2], the slaughtering house must acquire a permit and certificate to import and distribute the halal frozen

Table 1: Summary of Authorities' Role in Administering and Managing Halal Frozen Meat Products

Ministry	Department/Agency	Role (s)
MORA	MUIB	1. Established HMA and HCHLO. 2. Halal Accreditation Body (approves the issuance of Halal Certificate and Halal Permit). 3. The Religious Council shall be the authority responsible for 4. advising His Majesty, the Sultan and Yang Di-Pertuan on all matters relating to the Islamic Religion. 5. For this Article, His Majesty the Sultan and Yang Di-Pertuan may make laws concerning matters relating to the Islamic Religion after consultation with the Religious Council, but not necessarily in accordance with that Council's advice.
	BKMH	1. To enforce HMA and HCHLO; 2. To control and handle imported halal frozen meat in Brunei. 3. To ensure the imported meat is from the slaughterhouses certified halal by MUIB. 4. To monitor halal slaughterhouses and certificates issued by the Department of Syariah Affairs.
MOH	FSQCD	1. To ensure the importers comply with the Public Health (Food) Act (Chapter 182) and Public Health (Food) Regulation (R1 Chapter 182).
MPRT	DAA	1. Inspecting and certifying frozen meat products to comply with MORA sets' halal requirements. 2. They are working with abattoirs, meat processors, and importers to ensure that frozen meat products meet halal standards. 3. To strengthen traceability through mandatory registration for commercial importers. 4. To inspect and approve applications for authorized quarantine areas. 5. To conduct document and physical inspections at the Brunei Darussalam port of entry. 6. Appointed as the national and international focal point for Sanitary and Phytosanitary (SPS) measures on plant and animal commodities.
MOFE	RCED	1. To administer the regulations governing importing and exporting halal frozen meat products. 2. To ensure frozen meat products meet halal requirements. 3. To ensure all necessary permits and documentation are in place for importation and exportation. 4. To inspect products and their clearance for importation and exportation.

Source: Authors Developed the Table Based on Published Materials.

meat to Brunei Darussalam. In addition, individuals and companies must adhere to Brunei's importation regulation which entails acquiring the necessary licenses and permits to guarantee that the imported goods are safe and conform to Brunei's guidelines. Violating the law may result in severe consequences such as financial penalties, confinement, and other lawful consequences.

In conclusion, each of the authorities has roles and responsibilities to ensure that halal frozen meat products comply with *Shari'ah* law, meet the requirements and standards for halal certification, and that their importation and exportation are appropriately regulated. The researchers have suggested a few recommendations to overcome the illegal smuggling of frozen meat importation cases. This paper has achieved its objective of reviewing the laws and regulations governing the importation of halal frozen meat products in Brunei Darussalam and discovering the role of relevant authorities, as well as addressing the gaps and proposing recommendations to improve the administration and management of the permits for halal frozen meat products in Brunei Darussalam. BKMH, alongside other agencies, will persist in monitoring and conducting operations regularly to ensure compliance with the country's laws and regulations. The public must also follow the halal meat importation regulations established by the agencies and relay information regarding the dubious sale of meat products in the country to BKMH. Lastly, the public is also urged to acknowledge and be aware of the status of the frozen meat products they purchased as lawful and halal in the country to avoid any doubtful matters.

References

Attorney General's Chamber. Custom Order 2006. Retrieved from https://www.agc.gov.bn/AGC%20Images/LOB/Order/ABC/C/Custom%20Order,%202006/Customs%20Order,%202006.pdf (Accessed on March 3, 2023).

Attorney General's Chamber. Halal Meat Act Chapter 183. Retreievd from https://www.agc.gov.bn/AGC%20Images/LAWS/ACT_PDF/H/CHAPTER%20183.pdf

Azalie, I. Z.N. & Samad, N. F. H. (2022). Brunei as a Global Halal Food Hub: Production Network and Strategic Relations in Halal Food Production. Journal Of Contemporary Islamic Studies, Volume 8 (Special Issues). Retrieved from https://jcis.uitm.edu.my/images/journal/volume8/issue3si/1_Brunei_as_a_Global_Halal_Food_Hub_Production_Network_and_Strategic_Relations_in_Halal_Food_Production.pdf

Brunei Darussalam National Single Window. (n.d.). License or Permit. Retrieved from: https://bdnsw.mofe.gov.bn/Pages/ImpExpLicenceorPermit.aspx (Accessed on March 3, 2023).

Brunei Darussalam National Single Window. (n.d.). Prohibited and Restricted Goods. Retrieved from: https://bdnsw.mofe.gov.bn/Pages/ImpExpProh-RestictionGoods.aspx (Accessed on March 3, 2023).

Department of Economic Planning and Statistics, Ministry of Finance and Economy. (n.d). Eleventh National Development Plan (2018-2023) Brunei Darussalam. Retrieved from: https://deps.mofe.gov.bn/DEPD%20Documents%20Library/NDP/NDP11_Eng.pdf (Accessed on March 30, 2023).

Deuraseh, N. (2019). Shariah and Life: *Halalan Toyyiban* Perspective. Brunei Darussalam: UNISSA Press, Universiti Islam Sultan Sharif Ali

Ibrahim, N. H. (2022). Halal Policy in Brunei Darussalam. Journal of Halal Science and Technology, 1(1), 86-91.

Khalid, A. M., Haji Masr, M., Muhammad, N., & Pang, W. L. (2018). Brunei Darussalam: Halal Meat and Meat Products Processing. ERIA, pp.89-117. Retrieved from https://www.eria.org/uploads/media/RURB_2018_Chapter_4_Halal_meat_Brunei.pdf

Ministry of Foreign Affairs. (n.d). Quick Facts on Brunei Darussalam. Retrieved from: https://www.mfa.gov.bn/usa-washington/SitePages/businessinbrunei.aspx (Accessed on March 30, 2023).

Ministry of Health (n.d). Importation of Food & Drinks. Retrieved from: https://www.moh.gov.bn/SitePages/Food%20Safety%20and%20Quality%20Control%20Division.aspx (Accessed on April 1, 2023).

Ministry of Health. (n.d). Importation of Fresh Meat. Retrieved from: https://www.moh.gov.bn/SitePages/Fresh%20Meat%20Import.aspx (Accessed on April 28, 2023)

Ministry of Health. (n.d). Personal Imports, Trade Samples & Seized or Detained or Detained Goods. Retrieved from: https://www.moh.gov.bn/SitePages/Personal%20Import.aspx (Accessed on April 28, 2023).

Ministry of Primary Resources and Tourism. (n.d.). Biosecurity Division, Department of Agriculture and Agrifood; Organizational Structure. Retrieved from: http://www.agriculture.gov.bn/SitePages/Biosecurity.aspx (Accessed on March 3, 2023).

Othman, A. (December 29, 2020). Frozen Meat without Halal Permit Seized. Retrieved from Borneo Bulletin's Website: https://borneobulletin.com.bn/frozen-meat-without-halal-permit-seized-2/ (Accessed on March 3, 2023).

Othman, A. (February 11, 2021). 'Smuggled' Raw Meat, Beef Lungs Seized Outside House. Retrieved from Borneo Bulletin's Website: https://borneobulletin.com.bn/smuggled-raw-meat-beef-lungs-seized-outside-house/ (Accessed on March 3, 2023).

Othman, A. (January 9, 2023). Meat without Halal Import Permit Confiscated in Raid. Retrieved from Borneo Bulletin's Website: https://borneobulletin.com.bn/meat-without-halal-import-permit-confiscated-in-raid-2/ (Accessed on March 3, 2023).

Othman, B., Shaarani, S. M., & Bahron, A. (2016). The Potential of ASEAN in Halal Certification Implementation: A Review. Pertanika Journal of Social Sciences and Humanities, 24(1), 1-24. Retrieved from https://myjurnal.mohe.gov.my/filebank/published_article/44592/1.pdf

Ruzulan, Z., Jamaludin, M. A., & Ishak, A. H. (2021). Meat and meat-based products: Challenges and opportunities in halal food security. IOP Conference Series: Earth and Environmental Science, 756(1). Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/756/1/012017/pdf

Sulaiman, S., & Hashim, N. (2022). The Emergence of Halal Food Crimes amidst COVID-19: An Analysis of Illegal and Frozen Meat Issues in Brunei Darussalam. In Journal of Contemporary Islamic Studies, Volume 8 (Special Issue). Retrieved from https://jcis.uitm.edu.my/images/journal/volume8/issue3si/12_The_Emergence_of_Halal_Food_Crimes_amidst_COVID-19_An_Analysis_of_Illegal_and_Frozen_Meat_Issues_in_Brunei_Darussalam.pdf

HALALSPHERE
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Navigating the Halal Food Ingredients Industry: Exploring the Present Landscape

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Abstract

Due to their religious obligations, Muslims strongly believe that halal food ingredients are crucial to protecting their health and faith. As a result, the halal food ingredient industry is growing very rapidly. This review focuses on the latest trends and advancements involving halal food ingredients. In this review, halal certification and the standards governing the production of halal ingredients are discussed. The latest technological developments for authenticating and tracking halal components are also brought up in this review. It addresses the impact of halal ingredient manufacturing on the environment, promotes social responsibility, and places emphasis on procuring ingredients sustainably and ethically. It also examines customer awareness and preferences and the marketing of halal food ingredient brands. In conclusion, this study analyses the current condition of halal food ingredients and emphasises the importance of continuous improvement and adaptation to fulfil the requirements set out by the customers. By navigating the market for halal food ingredients, those involved should ensure that Muslim customers everywhere have access to items that are authentic and in compliance with relevant standards.

1. Introduction

Halal food ingredients are the components and substances used in producing food products that are considered permissible and in accordance with Islamic dietary laws. The term 'halal' originates from Arabic, which means 'lawful' or 'permissible'. It encompasses a set of guidelines derived from the teachings of the *Qur'an* and the *Hadith*. While halal primarily pertains to the consumption and preparation of meat, its scope extends beyond the absence of pork in food products. It also applies to various food ingredients and additives (Nazaruddin *et al.*, 2023). The significance of halal food ingredients lies in their adherence to Islamic dietary laws, which hold great importance in Muslims' religious and cultural practices. For Muslims, halal food is not just about physical nourishment but also a spiritual practice (Suleman *et al.*, 2021). It is seen as an act of obedience and submission to the commands of Allah as outlined in the *Qur'an* and the *Hadith*.

The halal industry is experiencing rapid global expansion, extending beyond its traditional consumer base of 1.8 billion Muslims (Akram, 2022). It has become a market of interest for non-Muslims as well. Various countries such as China, Thailand, Indonesia, Singapore, Korea, the Philippines, and Australia have recognized the potential of this market and have made significant progress in tapping into it, even in non-Muslim-majority countries. Brazil, Australia, New Zealand, Italy, India, and Germany are among the top ten in the Global Islamic Economy Indicator (GIEI) score for different halal sectors (Azam & Abdullah, 2020). The global demand for halal food products has steadily increased due to the growing Muslim population, globalization, and the rising awareness and preference for halal-certified products among Muslims and non-Muslims. In 2022, the halal food industry experienced remarkable growth, with the global market size reaching US$2,221.3 billion. The IMARC Group predicts continued expansion, with the market expected to reach an impressive US$4,177.3 billion by 2028. This projection signifies a compound annual growth rate (CAGR) of 10.8% from 2023 to 2028, highlighting the positive trajectory of the halal food industry (imarcgroup.com, 2022).

Ensuring Muslim consumers have access to food choices that align with their dietary requirements is crucial, and halal certification plays an important role. It is a reliable confirmation that the food meets the criteria outlined in Islamic law, including ingredients sourcing, preparation and processing methods, and the absence of prohibited substances or additives. Aside from religious obligations, halal food must adhere to safety standards such as Hazard Analysis Critical Control Points (HACCP) and Good Manufacturing Practices (GMP). To ensure effective halal certification, the certifying body must possess technical competence and expertise to verify compliance with religious requirements and food safety standards (Abdallah *et al.*, 2021). Such measures ensure that halal food products are safe, hygienic and free from harmful entities or contaminants. By integrating food safety practices

into the certification process, halal certification reinforces the idea that halal is not just about religious compliance but also about maintaining high-quality and safe food products. This article provides a detailed overview of the halal food ingredients industry, presenting a broad understanding of its present condition. It is a valuable reference for industry experts and researchers who wish to remain informed about the latest advancements and emerging patterns within this thriving field. The information for this review is obtained primarily from secondary sources, including journals, books and electronic media such as Google Scholar, ResearchGate, and other related official websites.

2. Halal certification and regulatory framework in Malaysia

The issue of halal certification is currently a global concern. Halal has been incorporated into governmental regulations in both nations with a majority Muslim population and nations with a small Muslim population. This is due to the fact that halal concerns both religious teachings and people's freedom to practice their religion. Consuming halal food, beverages, medicines, cosmetics, and other products is required for Muslim consumers as it is a religious principle that must be upheld (Kohilavani *et al*., 2021). However, the world of halal is not only limited to consumable items like toiletries, food, pharmaceuticals, cosmetics, and financial services, but it is also crucial in ensuring the status of halal from the beginning of origin and throughout the supply chain process, encompassing logistics activities (Majid *et al*., 2019).

Halal certification has been a valuable tool for Muslim consumers by providing them with the information they need to make an informed decision, giving industries a marketing tool, establishing the supply and sale of more halal-certified products, stimulating the growth of more halal enterprises, and providing regulatory bodies with a means of enforcing the law. Halal certification ensures Muslims can consume the products per the *Shari'ah* law (Al-Teinaz *et al*., 2020; Sulaiman *et al*., 2019). The Malaysian Halal Certification Programme is separated into nine schemes, and manufacturers or businesses can select the plan they want to utilise based on the kind of product or service they have. Food and drink, cosmetics, pharmaceuticals, restaurants or hotels, consumer products, logistics, slaughterhouses, OEM (original equipment manufacturer), and medical devices are among the schemes. As a result, businesses related to the schemes mentioned can seek halal certification for their products or services (Saiman & Yusma, 2022). According to Mokti *et al.* (2022); Saiman & Yusma (2022); and Sulaiman *et al.* (2019), the halal certification procedure typically starts when applicants submit their applications to the Halal Certification Body. Before going through the halal inspection process, the halal Certification Body will evaluate and confirm the application. The application will then be presented to the halal certification panels during a specific meeting, along with all the compliance proof acquired during the halal inspection. The application's approval or rejection status is determined at the halal inspection phase. Afterwards, the relevant applicant receives a halal Certificate and logo after meeting all conditions and having the application for halal certification approved by the halal certification panels. The authority will supervise the holder of the halal certificate during the final stage of certification to ensure they abide by the certification guidelines. The Manual Procedure for Malaysia Halal Certification (MPPHM) (Domestic) 2020 outlines particular regulations that must be followed by all parties participating in the Malaysia halal certification system and provide a description of the halal certification system's procedure. Figure 1 summarises the Malaysia Halal Certification Process flow according to MPPHM (Domestic) 2020. Muslim consumers can be confident that whatever products and services they consume are in line with their religious principles according to the halal certification procedure. It entails a thorough analysis of the procedures, components, and manufacturing processes used by businesses. By becoming certified, businesses may access the expanding Muslim market, win over customers, and support the worldwide halal economy.

Malaysia is acknowledged as a world pioneer in fostering the halal industry. The scheme acknowledges emerging opportunities for halal in a number of different sectors, including logistics and supply chain management, food and restaurant management, tourism, banking, textiles, medical devices, and finance. Malaysia has established JAKIM (Jabatan Agama Kemajuan Islam, Malaysia) and the Halal Development Corporation (HDC) in order to promote the expansion of the halal trade (Majid *et al*., 2019). The halal certification system is strictly regulated and influenced by the Malaysian government. However, multiple halal authorities at the federal and state levels oversee and issue the halal certification. The Halal Certification Body, an Islamic organisation tasked with regulating halal certification in the country, carries out the certification procedure. The Department of Islamic Development Malaysia, or Jabatan Kemajuan Islam Malaysia (JAKIM), regulates halal certification on a federal level. The State Islamic Religious Department (JAIN) and the State Islamic Religious Council (MAIN) are responsible for managing the system at the state level (Sulaiman *et al.*, 2019). The government authorizes them under the Trade Description Act of 1983. To protect, oversee, and uphold Islamic matters in accordance with state constitutions, each state and the federal territories possess their Islamic religious authority. The Sultanates are associated with anything related to Islam throughout the nation, and the states have authority over how the religion is regulated. As a result, the states under Sultan's rule oversee the halal certification system (Sulaiman *et al*., 2019).

Regulatory bodies and certifying bodies crucially support the reliability and integrity of halal certifications. Developing standards, supervising the certification procedure, performing audits, confirming the ingredients, dispensing training, granting certifications, and enforcing legal frameworks are all part of their responsibilities. These organisations work to increase consumer trust in halal goods and services while promoting the expansion and development of the global halal market.

Food products are manufactured and imported all across the world. The integrity of halal food products must be managed and monitored to satisfy customers with genuine halal products because they travel a considerable distance and experience several handlings along the supply chain before reaching the consumers (Mohamed *et al*., 2022). For organisations and regulatory authorities engaged in the halal industry, upholding halal integrity and authenticity is essential since they count as a benchmark for assessing the potential for sustainable business growth (Majid *et al*., 2019). But upholding consistent adherence to halal standards while preventing fraud or deception presents a number of challenges. Muslim consumers heavily depend on the halal certifications or logos that HCBs provide to determine whether food is halal. However, unregulated Halal Certification Bodies (HCB) frequently follow distinct operating methods, and some of these organisations' level of knowledge has frequently been

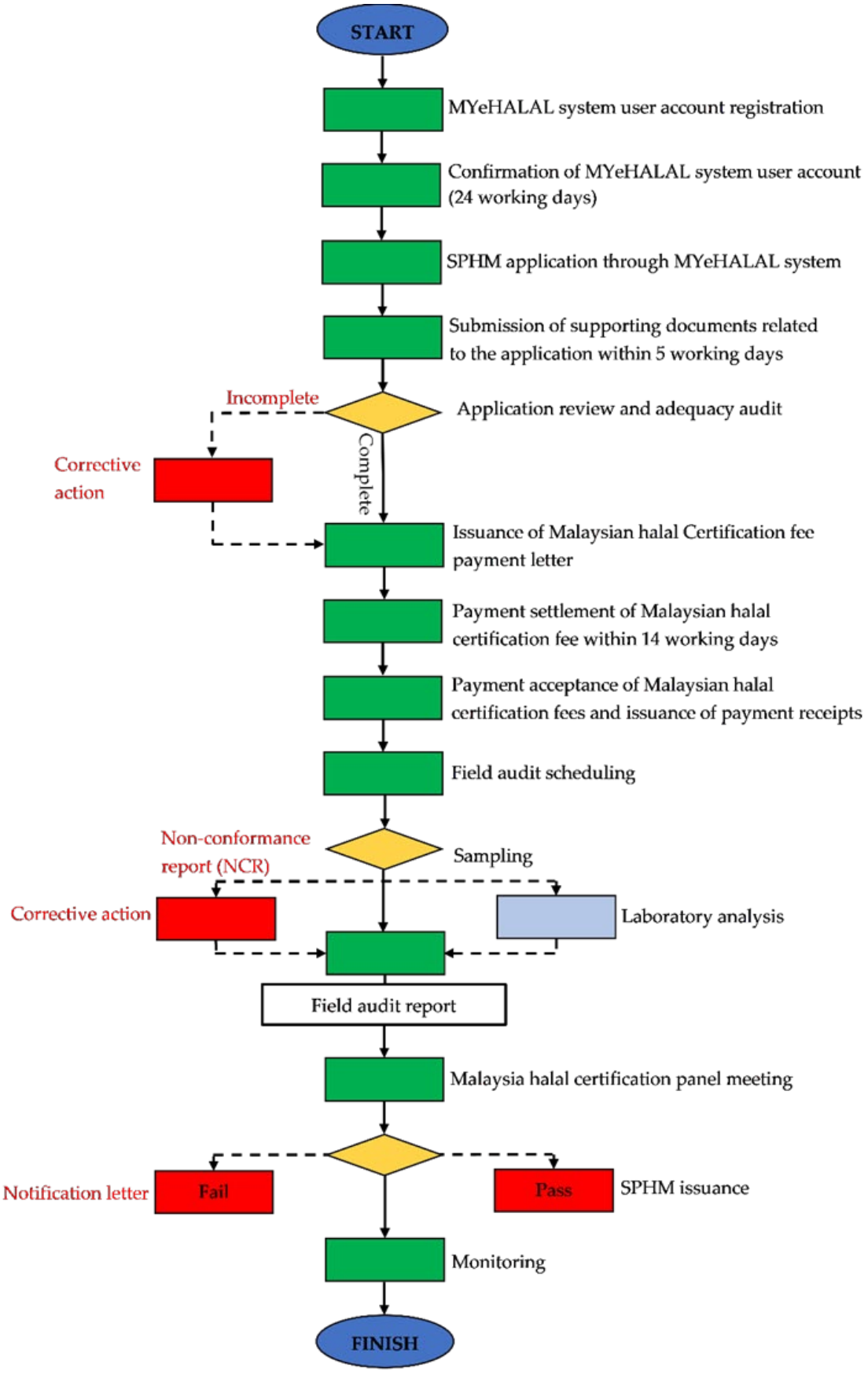

Figure 1: Malaysia Halal Certification process flow chart - MPPHM (Domestic) 2020. (Source: MPPHM (Domestic) 2020)

questioned. Insufficient technical knowledge has prevented some halal-certified establishments from being properly monitored and scrutinised when halal products are cross-contaminated over the course of the supply chain, such as handling halal products alongside non-halal products (Ab Rashid & Bojei, 2020; Masood & Rahim, 2021) halal certification organisations, regulatory authorities, and evaluation systems are susceptible to sabotage and commercially motivated adulteration because to these vulnerabilities. Contamination, tampering, counterfeiting, artificial enhancement, use of undeclared, prohibited, or restricted products, misrepresentation of nutritional content, false claims on labelling, and removal of genuine ingredients are all examples of food fraud (Masood & Rahim, 2021; Tola, 2018).

Managing halal supply chains has become more difficult, especially worldwide. It is crucial to monitor product flows, distribute precise data and information across stakeholders, and integrate a wide range of products to ensure the integrity of the halal supply chain. Integrating blockchain technology with RFID/NFC technologies can reduce supply chain complexity. In order to recognise and monitor tags connected to things that contain microchips and crucial data that include lot numbers, manufacturing dates, and validity dates, radio-frequency identification (RFID) employs electromagnetic fields. Data can be transmitted between devices using Near Field Communication (NFC) technology, a contactless method based on a radio frequency field. NFC technology offers a secure connection to information regarding the place of origin, product certificates, and the supply chain's path. Integrating these two technologies with Blockchain Technology solutions can result in improved security, automation of administrative processes through smart contracts, and increased transparency that is advantageous to all stakeholders, from suppliers to customers (Masood & Rahim, 2021; Tan *et al.*, 2022).

Insufficient awareness may result from a lack of knowledge and comprehension of halal criteria and principles, significantly impacting how customers demand authenticity in halal products (Ali & Ahmad, 2023; Ruslan *et al.*, 2018). Aware consumers are more likely to buy and consume halal items, enabling Muslims and non-Muslims to develop halal goods. Halal awareness for businesses refers to using a halal process in manufacturing products. Entrepreneurs must secure the cooperation and contribution of many stakeholders in the product supply chain to highlight halal as a crucial component. Making decisions as a client requires knowledge. After learning new information and recalling various facts, an individual's level of knowledge might indicate how well they comprehend a subject. One's awareness of halal issues can be increased by understanding halal topics (Akın & Okumuş, 2020; Ali & Ahmad, 2023; Amarul *et al.*, 2019).

Businesses, governing organisations, and customers must work together to maintain halal integrity and authenticity. The continuing development and credibility of the halal business depend on overcoming obstacles relating to ingredient verification, cross-contamination hazards, monitoring compliance, combating fraud, standardisation, consumer awareness, and certification capabilities. Stakeholders can guarantee the constant delivery of authentic halal products and services by addressing these issues, boosting consumer confidence and promoting the growth of the global halal market.

3. Current status of halal food ingredients

Consumer health and wellness consciousness is driving the halal food and beverage industry. Functional food and beverages, which provide health advantages beyond nutrition, are popular. People are growing more health-conscious and seeking goods to improve their diets. The market's future looks promising, supported by the increasing demand for health supplements and dietary products derived from halal sources. Because halal products only utilize natural ingredients, many believe they are healthier.

According to Laluddin *et al.* (2019), the Muslim community in Malaysia confronts obstacles when it comes to following the sunnah of the Holy Prophet (PBUH) and recognizing the health benefits of food consumption. Consequently, the market has witnessed an increase in the production of sunnah food products. Entrepreneurs have capitalized on this opportunity to market these products, utilizing Islamic characteristics to attract Muslim consumers. In the study by Osman *et al.* (2020), these characteristics allow producers to charge higher prices for their products.

3.1 Commonly used halal food ingredients

Muhammad *et al.* (2019) and Mutmainah (2018) underline the need for customer awareness and familiarity with food security, halal certification, and food quality. The advent of contemporary science and technology has enabled food production to combine a variety of substances, some of which may be permissible (halal) or prohibited (haram). However, gelatine has grown contentious, as about 95% of gelatine on the market is obtained from animals prohibited for Muslim consumption, as underlined by (Sin & Sin 2019; Zin *et al.*, 2021). Muslim communities are concerned about consuming food products contaminated with porcine-derived gelatine. Nonetheless, there are alternatives, such as gelatine derived from halal sources such as fish, cattle, chicken, and turkey, as proposed by Rakhmanova *et al.* (2018); Uddin *et al.* (2021); and Zin *et al.* (2021). Adherence to halal guidelines is critical from an Islamic perspective, as even minor deviations might affect the overall halal status of consumed food. There are now processed foods and items that violate Islamic standards, such as the usage of pork oil, khamr-derived substances in cakes, and even brushes made from pork fur for food spreading. Furthermore, Fatmi *et al.* (2020) and Habibie & Donna (2020) discovered that ingredients substantially influenced consumers' views toward halal products. Manufacturers and customers express concern about product ingredients, as they play a significant role in consumer decision-making processes.

3.2 Key considerations in sourcing halal ingredients

The lack of standardization in the halal ingredient industry has sparked concern among consumers pursuing better-quality products. Consumers are preoccupied with safety and quality. Haleem *et al.* (2018) highlighted that government regulations on the standardization of halal ingredients have presented obstacles and impeded business expansion, as noncompliance can result in substantial losses. These standardization requirements have disproportionately impacted the cosmetics, fragrances, and pharmaceutical industries. In addition, the costs of halal ingredients are frequently increased due to increased demand and the specialized preparation techniques required (Ahmed *et al.*, 2019). Due to their one-of-a-kind production processes, these goods are deemed exclusive. Complying with strict safety and standardization regulations is also challenging for the pharmaceutical industry (Hole *et al.*,

2021). Extra procedures necessitated by the need for transparent labelling and uncompromised quality result in marginally higher costs.

3.3 Halal labelling and packaging requirements

According to Zhao *et al.* (2021), labelling is crucial in helping consumers differentiate between similar products and influencing their purchase decisions. Labelling policies can address market inefficiencies and bridge the information gap between firms and consumers by providing relevant information. The use of labels such as 'HALAL,' 'CERTIFIED HALAL,' or similar statements signifies that the product has undergone strict testing and certification, assuring Muslims that it is permissible for consumption, manufacturing, preparation, and sale (Khan & Haleem, 2018; Zin *et al.*, 2021). Halal certification provides a sense of security for Muslim consumers, ensuring that the product meets their dietary requirements. The certification instils confidence in consumers who may have hesitated to consume a product, as it signifies that it has been approved by a halal-certifying institution (Mutmainah, 2018). In Malaysia, the halal label is not required for all products consumed by the public, including ready-to-eat items (Azizah, 2022). On the other hand, businesses can obtain halal certification and label their products accordingly to cater to Muslim clients and ensure that the items follow halal regulations.

Sungit *et al.* (2020) discussed the Halal Product Guarantees In-Law Number 33 of 2014, which explains that halal products have been deemed halal by Islamic law. According to Government Regulation Number 69 of 1999, food labels are any information about food in pictures, texts, or a combination of both. Other forms attached to food, inserted into, fastened to, or part of food packaging are now called labels in government regulations. Meanwhile, according to Halal Product Guarantee Law Number 33 of 2014, a halal label signifies that a product is halal (Badriyah *et al.*, 2021). The following are the indicators of halal labelling, according to Government Regulation Number 69 of 1999 (Millatina *et al.*, 2022):

1. Image: is the result of imitation in shapes or patterns (animals, people, plants) made with writing utensils.
2. Writing: is the result of writing which is expected to be read.
3. Combination of Images and Writings: a combination of the results of images and writings made into one part.
4. Sticking to the packaging can be interpreted as something attached (intentionally or unintentionally) to the packaging (protecting a product).

Producers and customers in the halal market must fully understand how halal works based on Islamic *Shari'ah* law. Determining if food is halal is difficult because you can't just go by how it smells, feels, or tastes. Because of this, certification and labelling are very important for helping Muslim customers determine if food products follow halal rules (Ahmed *et al.*, 2019; Zainalabidin *et al.*, 2019). Concerns about contamination, improper slaughter, and the need to verify food products mean that fast screening methods for halal food must be improved immediately (Rakhmanova *et al.*, 2018). These improvements should make real-time, on-site tracking more sensitive, accurate, portable, and cheap. Muslim and non-Muslim customers can ensure their food is safe using cutting-edge technologies and verification methods (Ng *et al.*, 2022). Traditional methods like physical approaches and electrophoresis have been used to determine what's in food, but they aren't good for screening and finding things in large quantities. Halal packaging ensures product labelling. Food additives, enzymes, emulsifiers, and flavours must be labelled. Traceability also certifies halal compliance and shows consumers the food's origin and composition (Masudin *et al.*, 2022). The certifying authority must label all goods and containers with the correct information, halal label, and logo (Osman *et al.*, 2020; Ruslan *et al.*, 2018). Halal meat should be shipped with a certificate from the certifying organisation. The Muslim supervisor controls halal logos, stamps, and seals.

4. Technological advances in halal ingredients analysis

Technological advancements have been made to meet the increasing demand for halal food ingredients. These technological developments aim to enhance the evaluation procedure's precision, productivity, and transparency, thereby facilitating consumers' and regulatory agencies' effective identification and authentication of halal constituents. Several techniques for halal authentication have been introduced to analyse processed and unprocessed food ingredients. Examples of these techniques include High-Performance Liquid Chromatography (HPLC), Enzyme-Linked Immunosorbent Assays (ELISA), Fourier Transform Infrared Spectroscopy (FTIR), Electronic Nose coupled with Gas Chromatography-Mass Spectrometry (GC-MS), Polymerase Chain Reaction (PCR) assays and Radio Immunoassays (RIA). These methods provide insights into the chemical composition and molecular profiles of the ingredients, helping to determine their halal status accurately. Furthermore, the amalgamation of artificial intelligence (AI), chemometrics, and the Internet of Things (IoT) with these advanced methods can augment the precision, velocity, and potential of multiplexed analyte detection (Ng *et al.*, 2022). For instance, a study by Islam *et al.* (2021) focuses on the fundamental methods of constructing an intelligent fluorescence spectroscope apparatus coupled with a straightforward DNA extraction protocol crucial in detecting pork contamination in food and beverage samples. The spectroscope was utilised to observe various material combinations, including those with and without pork, and subsequently measure their respective light spectra using RSpec software. Upon analysing the spectral patterns of various combinations, it has been proven that the suggested system can recognise the difference between uncontaminated pork, pork blends, and non-pork samples. The commonly employed techniques in the halal food ingredients analysis are discussed below.

Infrared spectroscopy is a technique that analyses the materials that comprise a sample using infrared, allowing for qualitative identification of sample components. When the bonds of the material's atoms vibrate, the frequency of vibrations is recorded as absorption peaks, which form the sample's infrared spectrum. This spectrum is considered the 'fingerprint' of the sample because no two compounds may have the same spectrum due to differences in atom combinations from one compound to the other (Lubis *et al.*, 2016). It has been employed to analyse the molecular composition of food ingredients and identify non-halal components. In 2018, Rahayu *et al.* (2018) showed that combining FTIR spectroscopy with multivariate data analysis can accurately identify and quantify dog meat in beef meatballs. Other than that, a combination of Fourier Transform Infrared Spectroscopy (FTIR) and Attenuated Total Reflectance (ATR)

successfully identified the porcine-based source in 13 types of capsules (Mustafa, 2014).

Other than that, Electronic Nose coupled with Gas Chromatography-Mass Spectrometry (GC-MS) is also commonly applied in detection analysis. The E-nose is a detection system or device that emulates the human olfactory system (Di Rosa *et al.*, 2017; Kadafi & Putra, 2021). It uses semi-selective sensors that undergo a physical change when volatile compounds adsorb on their surface. E-nose is suitable for identifying adulterants, and its ability is significantly enhanced when integrated with Mass Spectroscopy. The advantages of E-nose include its low cost; rapid and accurate qualitative detection, high sensitivity (requires only a small amount of sample), environmental friendliness; simple sample pre-treatment process and the ability to integrate it with other instruments, such as a mass spectrometer (Ng *et al.*, 2022b). A study was carried out to examine seven categories of meat, each made up of a different combination of beef and pork. The optimised support vector machine achieved a 98.10% accuracy in the classification test for detecting beef and pork. This research exemplifies the efficacy of E-nose technology in detecting pork adulteration in beef for halal authentication (Sarno *et al.*, 2020). In another research by Kadafi & Putra (2021), the results demonstrated that the e-nose system developecan efficiently identify food samples containing lard fat across various types of samples based on the output values of its six sensors.

Additionally, PCR analysis is also frequently utilised, particularly for meat products. A specific DNA sequence of the animal species is amplified using species-specific primers during species-specific PCR DNA amplification. The PCR results are then analysed using agarose gel electrophoresis to determine species distinction. The benefits of PCR analysis include its sensitivity, ability to analyse large quantities of samples, and potential to save time and money. Using various PCR analytical techniques, porcine DNA has been detected in raw and processed food. A study by Cahyadi *et al.* (2020) showed that multiplex-PCR with 12S rRNA gene primers uniquely and accurately detect bovine, dog, pig, and rat species on beef meatballs in one reaction. Besides that, the detection limit of the PCR technique for identifying swine and poultry species in meat products under different processing conditions was investigated (Felk *et al.*, 2017). From the results, it can be concluded that PCR is an effective tool that is sufficiently specific and sensitive for identifying animal species in processed meat products.

Other than the absence of pork, halal foods must also be alcohol-free. Alcohol, particularly ethyl alcohol or ethanol, is frequently used in the production of foods or as a by-product of food processing. Therefore, it is equally important to have porcine detection technology as its analytical detection method for alcohol in food products. Alcohol detection in complicated food samples has not been established or developed yet. The ethanol detector formerly only utilised ethanol solution, vapours, or alcoholic beverages like beer. However, these detectors give us useful starting points for creating alcohol biosensors that can be incorporated into food mixtures in the future. Determining whether alcohol is present in food and drink as a result of a natural occurrence or an intentional addition during manufacturing may also be important. Since no tests have yet been able to differentiate between the two, future research in the field of halal analysis could focus on this.

Regardless of the approaches used, a significant improvement has been made in the sensitivity and specificity of analytical procedures for halal ingredient detection. It is important to note that each technique has a unique benefit and application that depends on the needs and complements the others.

5. Sustainability and ethical sourcing of halal ingredients

The importance of sustainable and ethical ingredient sourcing in today's society cannot be emphasized enough. As consumers become more aware of the consequences of their purchasing decisions, there is a rising trend of making thoughtful food choices that consider environmental impact, social values, and economic factors (Chevallier-Chantepie & Batt, 2021). This growing consciousness among both businesses and consumers has led to an increased demand for products that adhere to principles of sustainability and ethic. The shift in perspective has put ingredient-sourcing practices under scrutiny, emphasizing the need for responsible approaches that prioritize the well-being of our planet and its inhabitants. We can uncover its diverse benefits by exploring the importance of sustainable and ethical ingredient sourcing. From preserving ecosystems and ensuring fair treatment of workers to promoting animal welfare and building consumer trust, embracing these practices is an important step towards shaping a more sustainable and ethical future.

Sustainable ingredient sourcing is critical in protecting the environment and its delicate ecosystems (Ojo *et al.*, 2018). Businesses can play a vital role in preserving biodiversity, maintaining soil quality, and conserving water resources by adopting methods that minimize negative impacts. For example, embracing organic farming practices can significantly reduce the use of synthetic chemicals, thus minimizing pollution and soil degradation (Chandini *et al.*, 2019). Similarly, responsible fishing techniques combined with a scientific approach can support marine ecosystems' long-term health and resilience. Bolin *et al.* (2021) reported on the prediction metrics of body condition and nutritional quality in commercially valuable wild-caught fish. This gives insight into the overall health of fish populations, guides sustainable fishing practices, supports conservation efforts, and ensures the availability of nutritious seafood for human consumption. Additionally, companies can mitigate climate change, an urgent global concern, by reducing their carbon footprint through sustainable sourcing practices (Qian *et al.*, 2022; Skärin *et al.*, 2022; Yuan *et al.*, 2022). These efforts are essential for safeguarding the environment and promoting a more sustainable future.

On the other hand, ethical practices strongly focus on social responsibility, ensuring that farmers, labourers, and suppliers involved in the supply chain are treated fairly and provided with safe working conditions. In recent years, a notable shift in consumer behaviour has shifted towards being more informed and conscientious about their shopping habits. Consumers are dedicating more time and effort to understanding the origins and manufacturing processes behind their products, seeking to align their purchases with their values and contribute to a more sustainable and equitable world. Ethical sourcing also extends to animal welfare, recognizing the inherent value of animals and emphasizing the need for compassionate treatment by those who benefit from their existence. The issue of safeguarding the welfare of farmed animals has gained significant traction in public policy across a growing number of countries. Robust regulations, both from the public and private sectors, have been implemented to govern the treatment and well-being of animals in our care. However, it is worth noting that several countries, including some of the largest animal-

producing nations such as Brazil, India, and China, still lack of comprehensive formal legislation dedicated to animal welfare (Buller *et al.*, 2018). From the Islamic perspective, integrating religious adherence with a commitment to sustainability and ethicality in the sourcing process allows the production of halal ingredients to embody responsible and conscientious business practices. This approach meets Muslim consumers' religious requirements and contributes to broader societal and environmental objectives. It reflects the industry's dedication to delivering high-quality products while upholding environmental and ethical standards, positively impacting the Muslim community and society.

5.1 Impact of halal ingredient production on the environment

Recently, the demand for halal products has been rising, driven by the growing Muslim population globally. What was once a market primarily confined to Muslim-majority countries has also expanded to non-Muslim countries. In these non-Muslim countries, halal food represents a new benchmark for ensuring safe, clean, and hygienic food consumption practices (Rahman, 2021). Halal production and processing of products can indeed contribute to addressing sustainability issues in various processes and procedures. The core principle of producing halal products centres around eliminating substances that can harm human health and the environment (Mabkhot, 2023). Like any other sector, the halal industry prioritises responsible sourcing practices that consider the environmental impact of ingredients. By doing so, it can contribute to sustainable development and help address global environmental challenges. According to the *Qur'an*, environmental conservation is a religious and social obligation. It is considered a matter of necessity rather than choice. The *Qur'an* emphasises the responsibility of protecting and preserving natural resources while emphasizing the direct connection between sustainable resource use and our accountability for their maintenance. This important message is captured in verse translated as "And do no mischief on the earth after it has been set in order: that will be best for you if ye have Faith" (*Qur'an* 7:85). The environmental impact of halal ingredient production is not exclusive to the halal industry. Similar considerations apply to the wider food industry as a whole. Adopting sustainable and environmentally friendly practices throughout the supply chain can minimise the impact, contributing to a more sustainable and responsible approach to halal ingredient production (Figure 2).

Halal principles encourage the humane treatment of animals and emphasize their well-being (Khan *et al.*, 2018). This includes the responsible sourcing of ingredients from suppliers that prioritize animal welfare. Emphasizing animal welfare within halal principles can also positively affect the environment. By prioritizing the humane treatment of animals, halal practices contribute to the overall well-being of the ecosystem. The treatment, rearing, and fattening of livestock are given utmost consideration to protect animal health and ensure compliance with halal guidelines. By adhering to these principles, halal supply chains aim to safeguard animal welfare while meeting consumers' nutritional and organic requirements (Azhar & Tu, 2021).

Halal principles promote organic and sustainable agricultural practices that reduce reliance on synthetic fertilizers, pesticides, and genetically modified organisms (GMOs). These practices aim to prevent environmental pollution and maintain soil health. According to halal principles, the use of pesticides is acceptable when applied in appropriate dosages and carefully

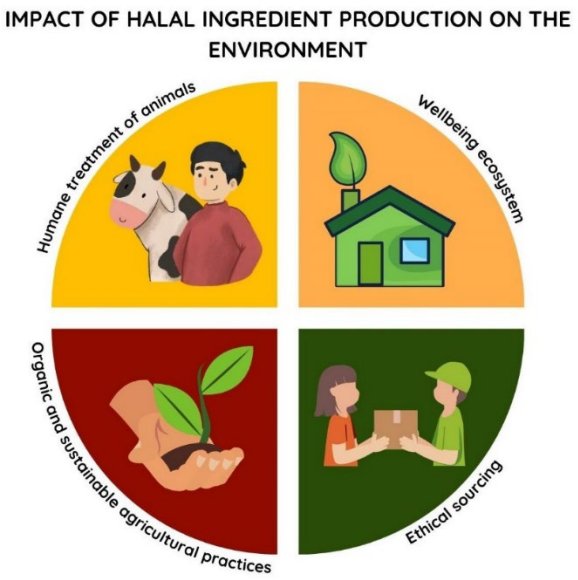

Figure 2: Environmental implications of halal ingredient production.

selected. However, the use of pesticides that cause significant harm to crops, the environment, or human life is considered non-halal (Alzeer *et al.*, 2020). In line with the concept of Tayyib, any food contaminated with pathogenic microbes or potentially toxic ingredients that endanger human health is categorized as non-*toyyib*. Consequently, such food is deemed non-halal and should not be consumed (Kurniadi & Frediansyah, 2016). The principle of *toyyib* emphasizes the importance of ensuring food safety and purity, aligning with the core values of halal consumption (Yahaya & Ruzulan, 2020).

It is important to note that the positive impact of halal ingredient production on the environment is not solely determined by halal requirements alone. The environmental benefits depend on adopting sustainable practices, responsible sourcing, and implementing eco-friendly approaches at every stage of the production chain. While halal requirements can have positive implications for animal welfare and farming practices, addressing broader environmental goals necessitates a comprehensive and holistic approach that encompasses sustainable practices throughout the production process.

5.2 Social Responsibility and fair-trade practices in the halal food industry

Social responsibility and fair-trade practices are essential in the halal food industry, just as in any other sector. Social responsibility is important to the halal industry as it encompasses the entire supply chain, from carefully selecting raw materials to producing the final product. It is important for everyone in society, particularly Muslim consumers, to take part in the efforts to acquire halal-certified food. This responsibility extends to non-government organizations, the private sector, entrepreneurs, suppliers, and consumers (Figure 3).

According to Mohd Riza *et al.* (2022), the government must establish a law concerning halal food that benefits all parties, including the industry and consumers. This law should emphasize the importance of stakeholders recognizing their responsibilities and contributions to the country rather than

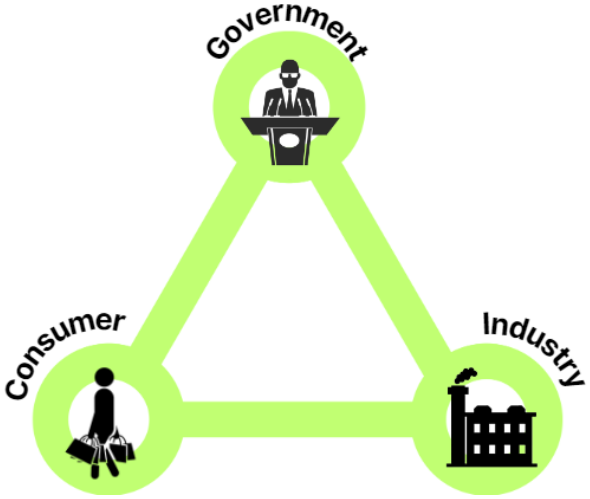

Figure 3: Fostering social responsibility through government, consumers, and industry collaboration.
(Adapted from Mohd Riza *et al.*, 2022)

prioritising profit maximization. Social responsibility in guaranteeing halal food can be viewed from various perspectives, where the focus extends beyond profit-making activities. It encompasses efforts to protect the environment, ensure the well-being of employees, engage in ethical trading practices, and other similar considerations.

Halal and fair trade are closely tied because both concepts promote ethical practices, social justice, and sustainable development (Mustun, 2021). While halal certification ensures that products meet the religious requirements of Muslims, fair trade certification ensures that the products are produced under fair and equitable conditions. Fair-trade practices in the halal food industry involve adopting ethical and transparent business approaches that promote fairness and social responsibility across the entire supply chain of halal food products. Embracing and implementing halal practices can offer several advantages for companies regarding sustainability and corporate social responsibility (CSR). Firstly, companies can enhance their CSR profiles and programs by adhering to the principles of good deeds embedded in halal, such as transparency, fairness, and community. Secondly, by positioning halal as a health and wellness initiative, firms can avoid being perceived solely as a Muslim-exclusive company by global consumers. These efforts allow halal products to attract a broader audience, including non-Muslims, similar to how some non-Jewish consumers embrace kosher items as pure and ethical alternatives (Izberk-Bilgin & Nakata, 2016). Integrating fair-trade practices in the halal food industry aligns with halal principles and helps promote a more sustainable and socially responsible business environment.

6. Market and consumer's purchase intention

The halal ingredients market size was valued at USD 325.2 Billion in 2021 and is projected to reach USD 435.5 Billion by 2030, growing at a CAGR of 3.2% from 2023 to 2030 (Verified Market Research, 2023). The expansion of the halal ingredients market has been driven by the increasing demand for halal food and ingredients, which can be attributed to the growing Muslim population. In addition to its religious connotations, halal has acquired considerable importance in commercial and economic spheres. The advocacy pertains to providing food products that are healthy, safe, and fresh to consumers, thereby representing cleanliness, superior quality, and food safety (Alzeer *et al.*, 2018). The halal ingredients market is anticipated to experience significant expansion due to the contemporary consumer's increasing inclination towards halal ingredients. Assessing the purchase intention of halal foods would help better understand consumers' needs, expectations and perceptions (Shaari & Arifin, 2009). Thus, it is necessary to analyse and understand the motivating factor influencing Muslim consumers' purchase of halal food products (Khan *et al.*, 2020).

Knowledge of halal, halal logo and labelling, economic factors, food safety concerns, and religiosity of customers (Figure 4) are among the factors that affect the purchase intention of halal food ingredients (Khan *et al.*, 2020; Shaari *et al.*, 2021; Shaari & Arifin, 2009).

The level of religious knowledge is vital in determining the extent of engagement in halal consumption. Insufficient education and understanding of halal rules and guidelines can result in a lack of awareness among individuals. This can be addressed by implementing educational programs and initiatives to enhance knowledge and familiarity with halal practices. Without marketing or branding efforts for halal items, awareness and information about halal products would not reach the broader consumer base.

Figure 4: Factors affecting customers' purchase intention of halal food ingredients.

Besides that, the halal logo and labelling also impact the purchasing intention of buyers. It is generally agreed that the halal logo is the most important and influential component of halal products and services. According to Ismail *et al.* (2016), the halal Malaysia logo is the most prevalent graphic presentation, and it indicates that the product or service in question has been approved by JAKIM (Figure 5). When a product or service bears the halal logo or label, the relevant government agency has determined that it complies with the principles of Islamic values. halal logo itself is perceived as an important factor because consumers consider that if a product has halal labelling, it means that the product is safe and hygienic.

Economic factors have the potential to influence consumers' purchasing intention, particularly concerning the accessibility and affordability of halal products. The limited availability and comparatively higher cost of halal products may present

obstacles for adherents of halal dietary restrictions to sustain a halal-compliant diet and remain informed of the range of halal offerings on the market. Muslim consumers might not consider a product's prices because they already understand that the prices can be higher due to many factors, but the economic reason might affect the purchase intention of non-Muslim buyers (Syukur & Nimsai, 2018).

Figure 5: Halal logo by JAKIM.

People's perception of food safety has noticed a noticeable shift in the past few years. In recent years, consumers have increasingly regarded food hazards as significant(Ali & Ahmad, 2023). Several incidents associated with consumers' increasing lack of confidence regarding food safety matters have been reported. The concerns above are primarily associated with the increased utilization of chemical inputs in conjunction with food production and processing. Halal food ingredients' safety and health benefit significantly impact health-conscious consumers' preferences (Rani *et al.*, 2016).

Religiosity refers to recognising, respecting and dedicatingne's religious beliefs, practices, values, and symbols influenced by spiritual forces (Mutmainah, 2018). This dedication will be evident in their attitudes and behaviour. The level of religiosity exhibited by an individual can affect their consumer behaviour. Consumers tend to purchase or utilise products that align with their philosophies and beliefs (Ali & Ahmad, 2023). The role of religiosity is to control customer behaviour on purchase intention of halal food. This indicates that if a person is more obedient to religion, the halal food ingredients purchase intention is higher. Thus, this is also an important factor influencing the customers' purchasing intention.

7. Conclusion

Muslims firmly believe that halal food ingredients are crucial for preserving their health and faith, driving the increasing demand for these ingredients. The halal food industry is experiencing significant growth, providing numerous opportunities for businesses and consumers. This growth is influenced by factors such as the rising demand for halal products, the expanding Muslim population, and the globalization of halal standards. Understanding the industry's current status is crucial due to the growing demand for diverse and high-quality halal products, both locally and globally. To seize market opportunities and remain competitive, businesses must stay updated on emerging trends, consumer preferences, and new ingredient offerings. Technological advancements in halal ingredient analysis are transforming the industry by offering more efficient and accurate testing methods and verifying halal compliance. Leveraging these advancements can enhance ingredient analysis processes, improve product quality, and meet consumer expectations for transparency and authenticity. Additionally, sustainability and ethical sourcing play a significant role in the industry. Prioritizing responsible sourcing practices, promoting sustainable agriculture, and ensuring ethical treatment throughout the supply chain is essential to meet the demands of socially conscious consumers. Understanding the market and consumer purchasing intentions is crucial for navigating the halal food industry. Conducting market research and gaining consumer insights provide valuable information about preferences, buying behaviour, and emerging trends. This knowledge enables businesses to develop targeted marketing strategies, innovate product offerings, and build strong brand loyalty among their target audience. By addressing these key aspects, businesses can effectively navigate the halal food industry, tap into the growing market, build consumer trust, contribute to a sustainable and ethical industry, and position themselves for long-term success.

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References

Ab Rashid, N., & Bojei, J. (2020). The relationship between halal traceability system adoption and environmental factors on halal food supply chain integrity in Malaysia. Journal of Islamic Marketing, 11(1), 117–142. https://doi.org/10.1108/JIMA-01-2018-0016

Ab Talib, M. S., Bakar, A. H. A., Zulfakar, M. H., & Jeeva, A. S. (2014). halal logistics PEST Analysis: The Malaysia perspectives. Asian Social Science, 10(14), 119–131. https://doi.org/10.5539/ass.v10n14p119

Abdallah, A., Rahem, M. A., & Pasqualone, A. (2021). The multiplicity of halal standards: a case study of application to slaughterhouses. Journal of Ethnic Foods, 8(1). https://doi.org/10.1186/s42779-021-00084-6

Ahmed, W., Najmi, A., Faizan, H. M., & Ahmed, S. (2019). Consumer behaviour towards willingness to pay for halal products: An assessment of demand for halal certification in a Muslim country. British Food Journal, 121(2), 492–504. https://doi.org/10.1108/BFJ-02-2018-0085

Akın, M. S., & Okumuş, A. (2020). Shaping the consumers' attitudes towards halal food products in Turkey. Journal of Islamic Marketing, 12(6), 1081–1096. https://doi.org/10.1108/JIMA-08-2019-0167

Ali, M. Q., & Ahmad, N. (2023). The Factors that affect halal Food and Food Products Awareness and Different Challenges: an overview. Journal of Agribusiness Marketing, 10(1). https://doi.org/10.56527/fama.jabm.10.1.6

Al-Teinaz, Y. R., Spear, S., & Abd El-Rahim, I. H. A. (2020). The halal food handbook.

Alzeer, J., Rieder, U., & Hadeed, K. A. (2018). Rational and practical aspects of halal and Tayyib in the context of food safety. Trends in Food Science and Technology, 71, 264–267. https://doi.org/10.1016/j.tifs.2017.10.020

Alzeer, J., Rieder, U., & Hadeed, K. A. (2020). Good agricultural practices and its compatibility with halal standards. Trends in Food Science and Technology, 102, 237–241. https://doi.org/10.1016/j.tifs.2020.02.025

Amarul, A., Sukirno, S., & Kurnia, D. (2019). Understanding the Awareness of the Importance of Halal Labels to Business Actors Based on Range of Long Business Variations. Business and Management Research, 8(1), 17. https://doi.org/10.5430/bmr.v8n1p17

Azam, M. S. E., & Abdullah, M. A. (2020). Global halal Industry: Realities and Opportunities. International Journal of Islamic Business Ethics, 5(1), 47. https://doi.org/10.30659/ijibe.5.1.47-59

Azhar, A., & Tu, Y. T. (2021). The investigation of key factors for successful implementation of sustainable. Food Research, 5, 221–228.

Bolin, J. A., Schoeman, D. S., Evans, K. J., Cummins, S. F., & Scales, K. L. (2021). Achieving sustainable and climate-resilient fisheries requires marine ecosystem forecasts to include fish condition. Fish and Fisheries, 22(5), 1067–1084. https://doi.org/10.1111/faf.12569

Buller, H., Blokhuis, H., Jensen, P., & Keeling, L. (2018). Towards farm animal welfare and sustainability. Animals, 8(6), 1–13. https://doi.org/10.3390/ani8060081

Cahyadi, M., Wibowo, T., Pramono, A., & Abdurrahman, Z. H. (2020). A Novel Multiplex-PCR Assay to Detect Three Non-halal Meats Contained in Meatball using Mitochondrial 12S rRNA Gene. Food Science of Animal Resources, 40(4), 628. https://doi.org/10.5851/KOSFA.2020.E40

Chandini, Kumar, R., Kumar, R., & Prakash, O. (2019). The Impact of Chemical Fertilizers on our Environment and Ecosystem. Research Trends in Environmental Science, 4, 69–86.

Chevallier-Chantepie, A., & Batt, P. J. (2021). Sustainable Purchasing of Fresh Food by Restaurants and Cafes in France. Agronomy, 11(11), 1–13. https://doi.org/10.3390/agronomy11112357

Di Rosa, A. R., Leone, F., Cheli, F., & Chiofalo, V. (2017). Fusion of electronic nose, electronic tongue and computer vision for animal source food authentication and quality assessment – A review. Journal of Food Engineering, 210, 62–75. https://doi.org/10.1016/J.JFOODENG.2017.04.024

Dusuki, A. W., & Abdullah, N. I. (2007). Maqasid al-Shari`ah, Maslahah, and Corporate Social Responsibility. The American Journal of Islamic Social Sciences, 24(1).

Fatmi, F. O., Ahmad, A. N., & Kartika, B. (2020). Determinants affecting purchase intention of halal products: an article review. Journal of Halal Product and Research, 3(2), 63. https://doi.org/10.20473/jhpr.vol.3-issue.2.63-79

Felk, G. S., Marinho, R. S., Montanhini, M. T. M., Rodrigues, S. A., & Bittencourt, J. V. (2017). Detection limit of polymerase chain reaction technique for species authentication in meat products. International Food Research Journal, 24(3), 1353–1356.

Habibie, H. M. H., & Donna, D. R. (2020). Factors that Influence the Intention to Purchase Halal Food Products (Case Study of Universitas Gadjah Mada Students). BENEFIT Jurnal Manajemen Dan Bisnis, 5(1), 83–95.

Haleem, D. A., Khan, M. M. I., Khan, M. S., & Ngah, D. A. H. (2018). Assessing Barriers to Adopting and Implementing Halal Practices in Logistics Operations. IOP Conference Series: Materials Science and Engineering, 404(1). https://doi.org/10.1088/1757-899X/404/1/012012

imarcgroup.com. (2022). halal Food Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023-2028. https://www.imarcgroup.com/halal-food-market

Islam, K. Z., Ahasan, Md. A. Al, Hossain, Md. S., Rahman, M. H., Mousumi, U. S., Asaduzzaman, Md., Islam, K. Z., Ahasan, Md. A. Al, Hossain, Md. S., Rahman, M. H., Mousumi, U. S., & Asaduzzaman, Md. (2021). A Smart Fluorescent Light Spectroscope to Identify the Pork Adulteration for halal authentication. Food and Nutrition Sciences, 12(1), 73–89. https://doi.org/10.4236/FNS.2021.121007

Ismail, W. R. B. W., Othman, M., Rahman, R. A., Kamarulzaman, N. H., & Rahman, S. Ab. (2016). Halal Malaysia Logo or Brand: The Hidden Gap. Procedia Economics and Finance, 37(16), 254–261. https://doi.org/10.1016/s2212-5671(16)30122-8

Izberk-Bilgin, E., & Nakata, C. C. (2016). A new look at faith-based marketing: The global halal market. Business Horizons, 59(3), 285–292. https://doi.org/10.1016/j.bushor.2016.01.005

Kadafi, M., & Putra, R. A. (2021). Electronic Nose (E-Nose) Design for Arduino Nano-Based Halal Haram Identification. Jurnal Neutrino, 13(1), 8–12. https://doi.org/10.18860/neu.v13i1.8903

Khan, M. I., & Haleem, A. (n.d.). Saudi Journal of Business and Management Studies Understanding "halal" and "halal Certification & Accreditation System"-A Brief Review. Saudi J. Bus. Manag. Stud, 1(1), 32–42. http://scholarsmepub.com/

Khan, R., Guo, H., Raza, S. H. A., Rahman, A., Ayaz, M., & Linsen, Z. (2018). Halal slaughtering, welfare, and empathy in farm animals: a review. Tropical Animal Health and Production, 50(8), 1733–1738. https://doi.org/10.1007/s11250-018-1644-1

Khan, W., Akhtar, A., Ansari, S. A., & Dhamija, A. (2020). Enablers of halal food purchase among Muslim consumers in an emerging economy: an interpretive structural modelling approach. British Food Journal, 122(7), 2273–2287. https://doi.org/10.1108/BFJ-08-2018-0528

Kohilavani, Wan Abdullah, W. N., Yang, T. A., Sifat, S. A. din, & Zzaman, W. (2021). Development of Safe Halal Food Management System (SHFMS). Food Control, 127. https://doi.org/10.1016/j.foodcont.2021.108137

Kurniadi, M., & Frediansyah, A. (2016). Perspektif halal Produk Pangan Berbasis Bioproses Mikrobia. Jurnal Universiitas Dipenogoro, 16(3), 147–160.

Laluddin, H., Sikandar, S., Haneef, S., Saad, N. M., & Khalid, H. (2019). The Scope, Opportunities and Challenges of Halal Industry: Some Reflections. In International Journal of Economics (Vol. 27, Issue 2).

Lubis, H. N., Mohd-Naim, N. F., Alizul, N. N., & Ahmed, M. U. (2016). From market to food plate: Current trusted technology and innovations in halal food analysis. Trends in Food Science

and Technology, 58, 55–68. https://doi.org/10.1016/j.tifs.2016.10.024

Mabkhot, H. (2023). Factors Affecting the Sustainability of Halal Product Performance: Malaysian Evidence. Sustainability (Switzerland), 15(3). https://doi.org/10.3390/su15031850

Majid, Z. A., Kamarulzaman, N. H., Rahman, A. A., Suzana Jaafar, H., Rahman, A. A., & Mohammad, M. F. (2019). Halal Integrity from Logistics Service Provider Perspective. In Int. J Sup. Chain. Mgt (Vol. 8, Issue 5). http://excelingtech.co.uk/

Masood, A., & Rahim, A. A. (2021). 3rd International halal Management Conference (3rd IHMC 2021).

Mohamed, Y. H., Binti Ma'aram, A., Rahman, A., Rahim, A., Abdullah, A. S., Abbas, M. S., Adis, A.-A. A., & Ismail, M. (2022). The Moderating Effect of halal Assurance System on the Relationship between halal Food Supply Chain Management and halal Integrity Assurance. In International Journal of Mechanical Engineering (Vol. 7).

Mohd Riza, N. S., Md Ariffin, M. F., Hamdan, M. N., & Ramli, N. (2022). halal food: a social responsibility on cartel meat issue in Malaysia. Food Research, 6(3), 92–100. https://doi.org/10.26656/fr.2017.6(3).277

Mokti, H. A., Kamri, N. 'Azzah, & Mohd Balwi, M. A. W. F. (2022). Halal Food Quality: An Analysis of Relevant Guidelines And Regulations In Malaysia. Journal of Fatwa Management and Research, 27(2-SE), 37–55. https://doi.org/10.33102/jfatwa.vol27no2-se.482

Mustafa, R. R. (2014). Identification of Porcine-Based Source in Capsule by Using FTIR-ATR and Awareness of Muslim Consumers about Halal Authentication. Universiti Teknologi Malaysia.

Mustun, Z. K. (2021). Halal food certification and business excellence: A conceptual paper. Journal of Emerging Economies and Islamic Research, 9(3), 98. https://doi.org/10.24191/jeeir.v9i3.13987

Mutmainah, L. (2018). The Role of Religiosity, halal Awareness, halal Certification, and Food Ingredients on Purchase Intention of halal Food. Ihtifaz: Journal of Islamic Economics, Finance, and Banking, 1(1), 33. https://doi.org/10.12928/ijiefb.v1i1.284

Ng, P. C., Ahmad Ruslan, N. A. S., Chin, L. X., Ahmad, M., Abu Hanifah, S., Abdullah, Z., & Khor, S. M. (2022). Recent advances in halal food authentication: Challenges and strategies. Journal of Food Science, 87(1), 8–35. https://doi.org/10.1111/1750-3841.15998

Ojo, O. O., Shah, S., & Coutroubis, A. D. (2018). An overview of sustainable practices in food processing supply chain environments. IEEE International Conference on Industrial Engineering and Engineering Management, 2017-Decem, 1792–1796. https://doi.org/10.1109/IEEM.2017.8290200

Osman, S., Ab. Rahman, S., & Jalil, A. (2020). Issues in halal food product labelling: A conceptual paper. Food Research, 4, 214–219. https://doi.org/10.26656/fr.2017.4(S1).S15

Qian, D., Dargusch, P., & Hill, G. (2022). Carbon Management behind the Ambitious Pledge of Net Zero Carbon Emission—A Case Study of PepsiCo. Sustainability (Switzerland), 14(4). https://doi.org/10.3390/su14042171

Rahayu, W. S., Rohman, A., Martono, S., & Sudjadi. (2018). Application of FTIR spectroscopy and chemometrics for halal authentication of beef meatball adulterated with dog meat. Indonesian Journal of Chemistry, 18(2), 376–381. https://doi.org/10.22146/IJC.27159

Rani, M. D. M., Umar, N. S., Ramli, S., Rahman, Z. A., Abdullah, M. Y., & Salleh, N. M. (2016). Knowledge of Prophetic Food Consumption in Malaysia: halal, Health Benefits and Practices. In Contemporary Issues and Development in the Global Halal Industry (pp. 3–12). Springer Singapore. https://doi.org/10.1007/978-981-10-1452-9_1

Ruslan, A. A. A., Kamarulzaman, N. H., & Sanny, M. (2018). Muslim consumers' awareness and perception of halal food fraud. In International Food Research Journal (Vol. 25).

Saiman, M. Z., & Yusma, N. S. (2022). Issues And Challenges of Promotion Halal Certification for Muslim Entrepreneurs in Malaysia. Al-Qanatir: International Journal of Islamic Studies, 28(2), 116–128.

Sarno, R., Triyana, K., Sabilla, S. I., Wijaya, D. R., Sunaryono, D., & Fatichah, C. (2020). Detecting Pork Adulteration in Beef for Halal Authentication using an Optimized Electronic Nose System. IEEE Access, 8, 25–30. https://doi.org/10.1109/ACCESS.2020.3043394

Shaari, J. A. N. Bin, Khalique, M., & Ottot, H. Bin. (2021). halal logo and the Confidence Level of Malaysian Customers on Indonesian halal Certified Products. Academy of Entrepreneurship Journal, 27(5), 1–8.

Shaari, J. A. N., & Arifin, N. (2009). Dimension of Halal Purchase Intention: A Preliminary Study.

Sin, K. Y., & Sin, M. C. (2019). Distinguished identification of halal and non-halal animal-fat gelatin using microwave dielectric sensing system. Cogent Engineering, 6(1). https://doi.org/10.1080/23311916.2019.1599149

Skärin, F., Rösiö, C., & Andersen, A. L. (2022). An Explorative Study of Circularity Practices in Swedish Manufacturing Companies. Sustainability (Switzerland), 14(12). https://doi.org/10.3390/su14127246

Sulaiman, M. Z. M., Noordin, N., Noor, N. L. M., Suhaimi, A. I. H., & Isa, W. A. R. W. M. (2019). A comparison of halal inspection process between federal and state halal certification body in Malaysia. International Journal of Digital Enterprise Technology, 1(4), 349. https://doi.org/10.1504/ijdet.2019.103359

Suleman, S., Sibghatullah, A., & Azam, M. (2021). Religiosity, halal food consumption, and physical well-being:An extension of the TPB. Cogent Business and Management, 8(1). https://doi.org/10.1080/23311975.2020.1860385

Sungit, F., Isa Mohd Deni, M., Abdullah, M., Abd Ghani, N., & Syahwahid, F. (n.d.). Concepts And Practices Of Halalan Tayyiban In The Islamic Manufacturing Practice (Imp): An Analysis From Syariah Perspective. www.muslimconsumer.org.my

Syukur, M., & Nimsai, S. (2018). Factors influencing the purchase intention of halal packaged food in Thailand. International Journal of Supply Chain Management, 7(4), 1–6.

Tan, A., Gligor, D., & Ngah, A. (2022). Applying Blockchain for halal food traceability. International Journal of Logistics Research and Applications, 25(6), 947–964. https://doi.org/10.1080/13675567.2020.1825653

Tola, A. (2018). Global Food Fraud Trends and Their Mitigation Strategies: The Case of Some Dairy Products: A Review. 77. www.iiste.org
Verified Market Research. (2023). halal Ingredients Market Size, Share, Opportunities, Trends & Forecast.

Yahaya, A. M., & Ruzulan, Z. (2020). Quranic Concept of Halalan Tayyiban and its Application in Food and Beverages of Hospitality Sector in Malaysia. Journal of Contemporary Islamic Studies, 6(1), 61–76. https://doi.org/10.24191/jcis.v6i1.4

Yuan, Y., Zhang, B., Wang, L., & Wang, L. (2022). Low-Carbon Strategies Considering Corporate Environmental Responsibility: Based on Carbon Trading and Carbon Reduction Technology Investment. Sustainability (Switzerland), 14(11). https://doi.org/10.3390/su14116683

Zhao, H., Yao, X., Liu, Z., & Yang, Q. (2021). Impact of Pricing and Product Information on Consumer Buying Behavior With Customer Satisfaction in a Mediating Role. Frontiers in Psychology, 12. https://doi.org/10.3389/fpsyg.2021.720151

Zin, Z. M., Sarbon, N. M., Zainol, M. K., Nur', S., Jaafar, A., Shukri, M. M., Hj, A., & Rahman, A. (2021). Halal and Non-halal Gelatine as a Potential Animal By-Products in Food Systems: Prospects and Challenges for Muslim Community. Proceedings of the First International Conference on Science, Technology, Engineering and Industrial Revolution (ICSTEIR 2020), 536(Icsteir 2020), 530–540. https://doi.org/10.2991/assehr.k.210312.086

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A Bibliometric Analysis of Halal Cosmetics and Halal Pharmaceuticals

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Abstract

This review presents a bibliometric analysis to shed light on the development of halal cosmetics and pharmaceutical domains, addressing their increasing popularity. The study assessed 248 entries retrieved from the Scopus database, discussing the types of papers available, publication years, contributors, and geographical distribution of the published documents in these fields. The analysis revealed frequently used phrases such as halal, halal cosmetics, gelatine, religiosity, attitude, and purchase intention. However, the study acknowledges limitations related to Scopus potentially overlooking certain articles, leading to possible omissions. Nevertheless, this research contributes to the relatively scarce literature on network analysis and bibliometric approaches in halal cosmetics and pharmaceuticals.

1. Introduction

Food and pharmaceutical items (drugs, cosmetics, and personal care products) have been among the first human requirements from the origin of civilisation. According to a 2006 survey by the Environmental Study Group, the average individual utilises up to 25 personal care items daily (Wan Jusoh & Kit Teng, 2013). Examples of such items include bath treatments, oral care products, skin care products, personal hygiene products, fragrances, deodorants, and cosmetics (Hunter, 2012). These kinds of products are currently acknowledged as fundamental not just for females but also for males.

Manufacturers must utilise halal raw ingredients so halal products are safer (Ahmad *et al.*, 2015). Conversely, *Allah* the Almighty orders Muslims to consume only halal products, which are both religiously lawful and beneficial (Mursyidi, 2013). Halal products must include characteristics such as safety, dependability, handling, production appliances, manufacturing aids, packaging, storage, transportation, distribution, retailing, and components (Muhamad Yunus *et al.*, 2014).

'Halal' is an Arabic term that refers to everything permitted under Islamic law. It is commonly used to indicate what Muslims are allowed to partake in, such as eating, drinking, or using. The antonym of halal is haram, which in Arabic translates to anything banned by Islamic law. These commandments govern the lives of Muslims worldwide and must be followed (Mursyidi, 2013).

The global halal cosmetics industry is predicted to expand significantly from USD 16.32 billion in 2015 to USD 53.81 billion in 2025. Likewise, the worldwide halal pharmaceutical market is anticipated to flourish from US 94 billion in 2019 to USD 174.59 billion by 2025, led by the preventive care sector (Communications, 2020). Global halal cosmetics consumption is estimated to reach US$74.7 billion in 2020. This is due to a rise in desire and interest in halal cosmetics among the Muslim community, which supports the industry. Because halal product consumption is expanding, the younger generations, such as Generation X and Y, are emerging as adaptive and educated customers (Mohamed Elias *et al.*, 2016). Among the cosmetic company exist are Avon, COSWAY and Maybelline; for the mass market, Estee Lauder, Clinique and Shiseido; for prestige brands, and also Body Shop, Sasa and Roche; for franchise chains (Ahmad *et al.*, 2015).

The growing halal awareness and understanding are one of the reasons influencing the rising interest in halal cosmetics and pharmaceuticals (Rahim *et al.*, 2015). Halal cosmetics and pharmaceuticals are well-known for being hygienic, secure, and of exceptional quality. Halal cosmetics and pharmaceuticals vary from other products in that they do not include substances derived from pigs or their derivatives and alcohol (Mohezar *et al.*, 2016). In regard to compliance with the Islamic faith standards, several consumers in many studies claimed that they use halal cosmetics and halal pharmaceutical products because their components are considered safe. As a result,

consumers are no longer afraid to utilise halal cosmetics and pharmaceuticals.

2. Methods

This review emphasises the bibliometric analysis performed to analyse and categorise the publications presented in the halal cosmetics and halal pharmaceuticals areas. The goal of the bibliometric study was to statistically evaluate the halal cosmetics and halal pharmaceutical research sectors to understand how the domains have grown over the last twenty years.

Figure 1 illustrates the methodological approaches indicated by this review. The first stage in carrying out this analysis was to define the topic of the analysis by proposing three research questions. How many papers have been published relating to halal cosmetics and halal pharmaceuticals? From 2000 to 2022, which nation generated the most articles on halal cosmetics and halal pharmaceuticals? Which organisation publishes publications on halal cosmetics and halal pharmaceuticals? The second stage was to define keywords and a period for the review. In this review, the keywords identified were "halal," "cosmetics," and "pharmaceuticals," while the time frame was established from 2000 to 2022, with the respective years sought to be the active years of halal cosmetics and halal pharmaceutical articles being produced.

The next stage was to choose a data surface, and the Scopus database was adopted for this assessment. The fourth stage was data retrieval, which involved exporting all data from the Scopus database in CSV format for detailed and deeper analysis.

The fifth phase included data analysis, which resulted in summary tables and bibliometric measurements. The final stage in this analysis was verifying the outcomes by referencing other publications for verification and reviewing the outcomes for precise and more accurate comprehension.

3. Bibliometric analysis and discussions

The Scopus database of 248 documents pertaining to halal cosmetics and halal pharmaceuticals was retrieved in a .csv (comma-separated value) file format to meet the criteria of VOSviewer, the software tool utilised for data analysis, for this review. The authors' names, affiliations, keywords, and nations that contributed to the publications are all extracted from the downloaded SCOPUS database. The same halal cosmetics and pharmaceutical information from the Scopus database is also stored in Excel format to generate graphs and tables to assess database trends over time, such as keyword frequency.

According to the analysis, halal as a comprehensive research topic has tremendous potential because the demand for halal rises with each year. Other halal research aspects, such as halal cosmetics, are narrow because consumers identify halal with food rather than a way of life. This is consistent with a bibliometric review by Haleem *et al.* (2020), which said that academics are more focused on the food element of halal and concluded that halal is solely for Muslims. Malaysia is the most active country in halal cosmetics publications, followed by Indonesia. This statement is consistent with both nations' growing halal cosmetics markets. Malaysia is also one of the leading Malaysian export markets for halal goods, especially compared to its Southeast Asian neighbours. Though the halal domain is being pursued, the development of halal cosmetics is still in its early stages. As a result, there is a significant need to disseminate the halal idea globally, not just in Muslim nations. This is where academics from all around the world should help with the halal cosmetics industry.

3.1 Document type

Excel is used to determine the document type. According to Table 1, the most common document types for halal cosmetics are articles (73.39%), followed by conference papers (9.27%), reviews (8.87%), book chapters (4.84%), short surveys (1.61%), book (0.81%), erratum, conference review and note (0.40%) each.

Table 1: Document type

Document Type	Frequency	% (N=248)
Article	182	73.39
Conference Paper	23	09.27
Review	22	08.87
Book Chapter	12	04.84
Short Survey	04	01.61
Book	02	00.81
Erratum	01	00.40
Conference Review	01	00.40
Note	01	00.40
Total	**248**	**100.00**

3.2 Year of publications - evolution of published studies

Table 2 shows the published studies of halal cosmetics and pharmaceuticals yearly. Figures 2 and 3 depict the graph for documents published yearly for halal pharmaceuticals and halal cosmetics, respectively. The paper shows that the first Scopus-indexed paper on halal cosmetics and halal pharmaceuticals was posted in 2006, while the second one took a long time in 2011. Although there was less enthusiasm and engagement in both fields in the early years of publishing, articles continued to be added each year, with 2020 being the most popular year for writers to submit their papers. Because millennial Muslim customers are increasingly religiously dedicated, they attempt to implement halal in all facets of their existence. As a result, new possibilities in the cosmetics and personal care fields are emerging. Research on halal cosmetics may have been conducted in-depth a few years prior, resulting in a surge of publications published in 2020.

3.3 Keywords analysis

Figure 4 and Table 3 were generated after utilising VOSviewer and Excel. The connections in Figure 3 show that the majority of the terms are correlated with one another. The scale of the term indicates the frequency with which words linked to halal cosmetics and halal pharmaceuticals are utilised. For instance, halal has the highest number and corresponds to the occurrence of author keywords in Table 3. The same shade of the words indicates that the topic shares similar terms. The grey shade in Figure 3 demonstrates this statement. "Halal" is frequently associated with other phrases like "halal cosmetics" and "religiosity".

Table 3 demonstrates that the most frequently used phrases are halal (around 40 occurrences), followed by halal cosmetics,

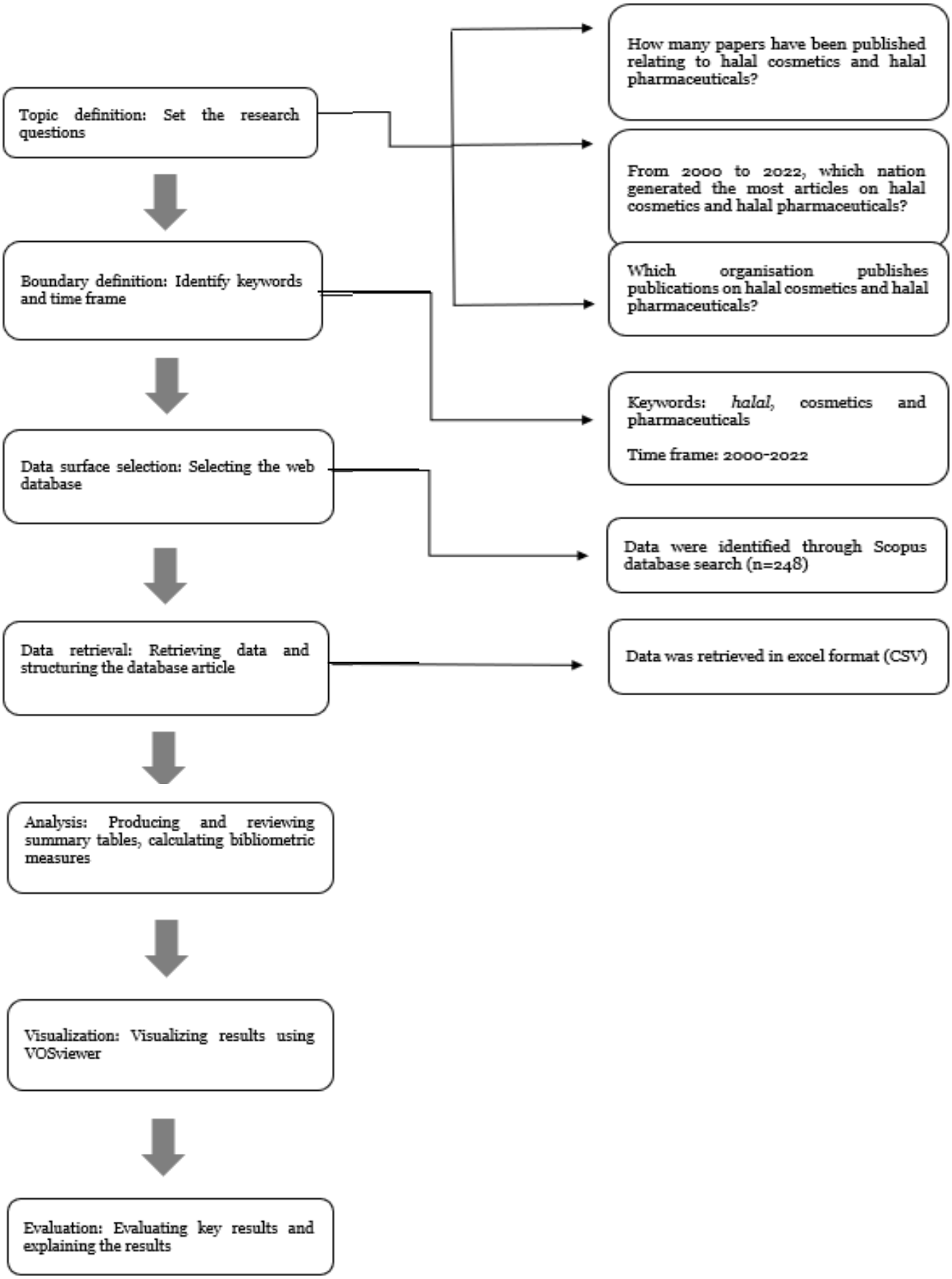

Figure 1: Research methodology adopted in the bibliometric analysis on halal cosmetics over twenty years.

gelatine, religiosity, attitude, and purchase intention. Aside from that, terms like halal medications and cosmetics also rank prominently on the list. Phrases like Islamic marketing, knowledge, and halal certification suggest that some academics are pursuing these areas.

There are also science-related terms, such as chemometrics, indicating that science-based research has been conducted, but to a smaller extent than research on halal cosmetics. In conclusion, academics might maintain their research on existing elements or begin new studies on brand-new topics based on the occurrence of the author's keywords assessed in this study.

Table 2: Year of publications

Year	Frequency	% (N=248)	Cumulative Percent
2006	02	00.81	00.81
2011	02	00.81	01.62
2012	12	04.84	06.46
2013	10	04.03	10.49
2014	13	05.24	15.73
2015	13	05.24	20.97
2016	15	06.05	27.02
2017	18	07.26	34.28
2018	30	12.10	46.38
2019	25	10.08	56.46
2020	50	20.16	76.62
2021	33	13.31	89.93
2022	25	10.08	100.00
Total	**248**	**100.00**	

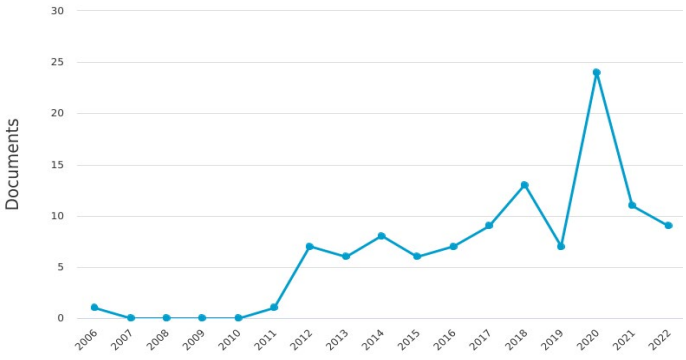

Figure 2: Documents for halal pharmaceuticals by year.

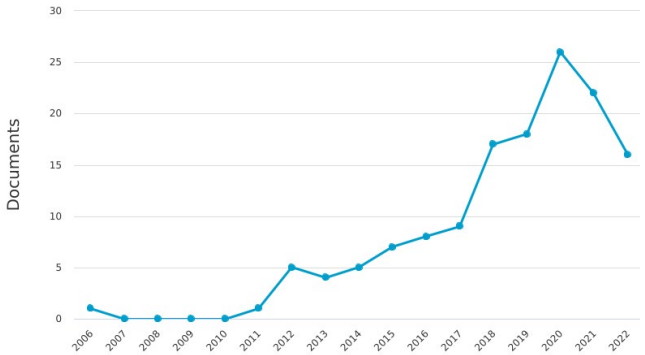

Figure 3: Documents for halal cosmetics by year.

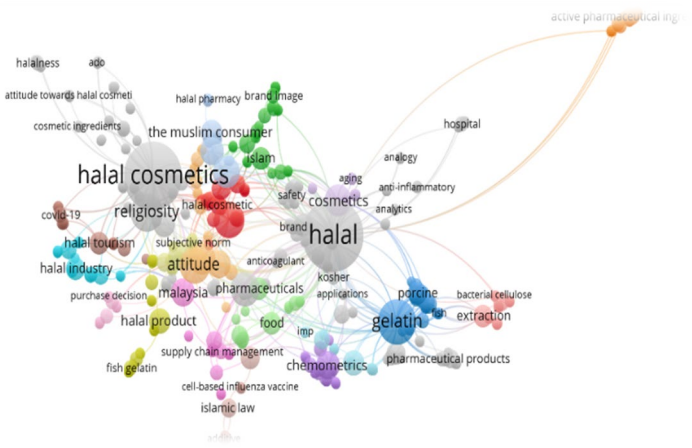

Figure 4: Network visualisation map of the author keywords.

Table 3: Top keywords

Author Keywords	Frequency	Percent
halal	40	19.05
halal cosmetics	36	17.14
gelatine	16	07.62
religiosity	11	05.24
attitude	10	04.76
purchase intention	10	04.76
halal pharmaceuticals	09	04.29
cosmetics	08	03.81
Islamic marketing	08	03.81
knowledge	08	03.81
halal certification	06	02.86
chemometrics	06	02.86
perception	06	02.86
Malaysia	06	02.86
halal market	06	02.86
halal product	05	02.38
halal authentication	05	02.38
halal food	05	02.38
the Muslim consumer	05	02.38
halal industry	04	01.90
Total	**210**	**100.00**

3.4 Geographical distribution of publications - most influential countries

Table 4 shows the top ten nations that contributed the most to publications about halal cosmetics and halal pharmaceuticals between 2000 and 2022. Malaysia generated the most articles, publishing over 109 publications, followed by Indonesia and India, which published 55 and 12 papers, respectively. The large difference in halal cosmetics and halal pharmaceuticals publications may be analysed in two ways: top halal cosmetics and halal pharmaceuticals producing nations and halal cosmetics and halal pharmaceuticals awareness in the countries. According to a survey done in Malaysia by Mohezar *et al.* (2016), the majority of Muslim and non-Muslim customers choose halal cosmetics and halal pharmaceutical products owing to their trust in safe operation, purity, and reliability.

Table 4: Top 10 countries contributed to the publications

Country	Frequency	% (N=217)
Malaysia	109	50.23
Indonesia	55	25.35
India	12	05.53
United States	07	03.23
Turkey	06	02.76
Iran	06	02.76
Canada	06	02.76
United Arab Emirates	06	02.76
Australia	05	02.30
China	05	02.30
Total	**217**	**100.00**

3.5 Authorship

Table 5 displays the number of authors for each published document and paper; meanwhile, Table 6 illustrates the top four most productive contributors regarding the number of papers they published. The biggest number of writers per document reported was four, with a regularity of 07, accounting for 36.84% of the total. The smallest number of writers per document observed was seven, with a regularity of one, accounting for 5.26% of the total. Rohman A. was the most productive author, with nine documents published, accounting for 39.13% of the total. The other two authors registered five published documents for each of them.

Table 5: Number of author(s) per document

Author Count	Frequency	% (N=19)
2	03	15.79
3	02	10.53
4	07	36.84
5	04	21.05
6	02	10.53
7	01	05.26
Total	**19**	**100.00**

Table 6: Top 4 most productive authors

Author's Name	No. of Documents	Percentage (%)
Rohman A.	09	39.13
Erwanto Y.	05	21.74
Othman R.	05	21.74
Ngah A. H.	04	17.39
Total	**23**	**100.00**

The network visualisation map of co-authorship created using VOSviewer is shown in Figures 5, 6, and 7. Figure 5 displays the link between authors who published at least two papers or documents and those who recorded zero citations. It can be observed that ten writers fulfilled the qualifications, and they were linked to each other. Figure 6 illustrates the organisations that collaborated with the authors of published publications on at least one document. Figure 7 demonstrates the association between nations and the writers who contributed to halal cosmetics and pharmaceutical publications. It revealed that Malaysia has a favourable relationship with other nations such as Indonesia, India, the United States, and Turkey. This is because Malaysia's halal cosmetic and halal pharmaceutical businesses have been expanding for an extended time, and the majority of halal cosmetics and halal pharmaceutical goods have been marketed to nations such as the Middle East and ASEAN, allowing collaborative research to be undertaken inside these countries (Mohezar *et al.*, 2016).

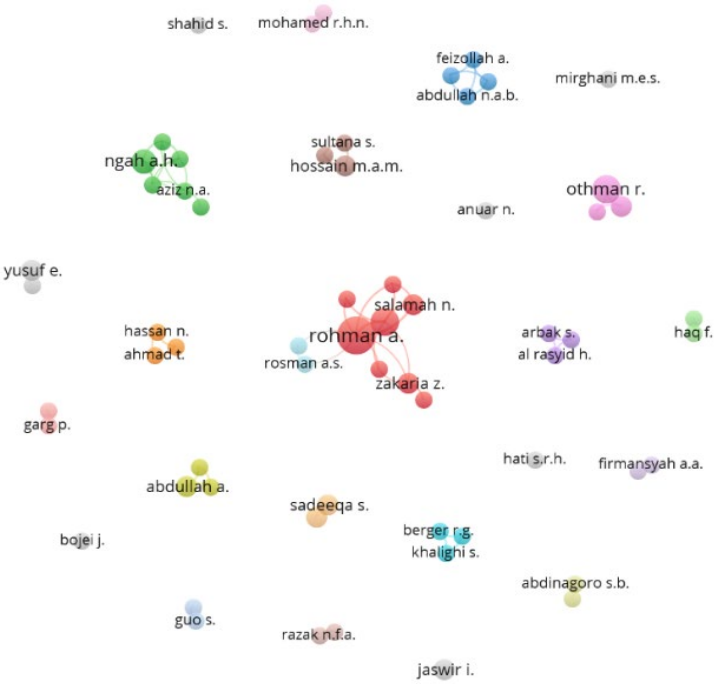

Figure 5: Network visualisation map of the co-authorship.

Unit of analysis: Authors
Maximum number of authors per document: 25
Minimum number of documents of an author: 2
Minimum number of citations of an author: 0

3.6 Most influential organisation

Table 7 highlights the top ten most productive and influential institutions with at least one publication. It can be observed that International Islamic University Malaysia and Universiti Malaya produced the most papers (22), accounting for 15.94% of each of all publications. These universities have their own halal institution, which may slightly influence the journal produced on halal cosmetics and halal pharmaceuticals. Furthermore, we discovered that the most productive organisations appear to be from Malaysia, as there are only a few Indonesian institutions or organisations that have produced publications on halal topics because their research may not be focused on halal cosmetics or halal pharmaceuticals but on other topics such as halal food or logistics.

3.7 Citation analysis

Table 8 displays the citation metrics of halal cosmetics and halal pharmaceutical papers. The top ten most impactful articles are shown in Table 9. The articles were arranged from the most cited to the least cited, with a minimum of thirty citations, starting with the most cited. With 234 citations since its publication in 2012, the most referenced article was Arshia Mukhtar and Mohsin Muhammad Butt's Intention to Choose Halal Products: The Role of Religiosity. With 32 citations, Ali Feizollah, Sulaiman Ainin, Nor Badrul Anuar, Nor Aniza Abdullah, and Hazim Hanif's Halal Products on Twitter: Data

Extraction and Sentiment Analysis Using Stack of Deep Learning Algorithms was the least referenced paper.

Figure 6: Network visualisation map of the most influential organisations.

Unit of analysis = Organisations
Maximum number of organisations per document: 25
Minimum number of documents of an organisation: 1
Minimum number of citations of an organisation: 0

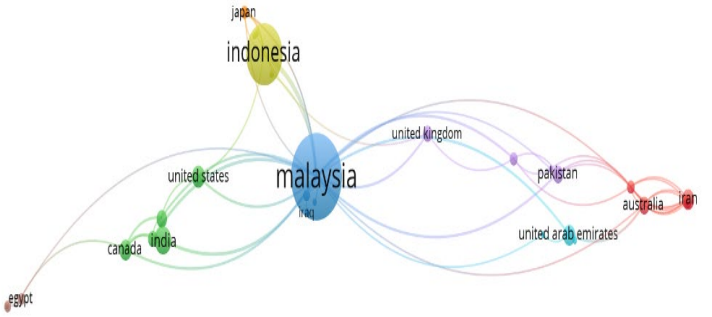

Figure 7: Network visualisation map of active countries in publishing journals in halal cosmetics and halal pharmaceuticals.

Unit of analysis: countries
Maximum number of countries per document: 25
Minimum number of documents of a country: 1
Minimum number of citations of a country: 0

Table 7: Top 10 most influential institutions with at least one publication

Country	Frequency	% (N=138)
International Islamic University Malaysia	22	15.94
Universiti Malaya	22	15.94
Universiti Putra Malaysia	16	11.59
Universiti Kebangsaan Malaysia	15	10.87
Universitas Gadjah Mada	14	10.14
Universiti Teknologi Mara	14	10.14
Universiti Sains Malaysia	12	08.70
Universiti Sains Islam Malaysia	10	07.25
Universitas Airlangga	08	05.80
Universiti Teknologi Malaysia	05	03.62
Total	**138**	**100.00**

Table 8: Citations metrics

Metrics	Data
Publication years	2006-2022
Citation years	16 (2006-2022)
Papers	248

VOSviewer was used to generate a network visualisation map of citations pertaining to documents and countries in Figures 8 and 9. The map in Figure 7 can be used to verify the highly referenced publications or articles in Table 9.

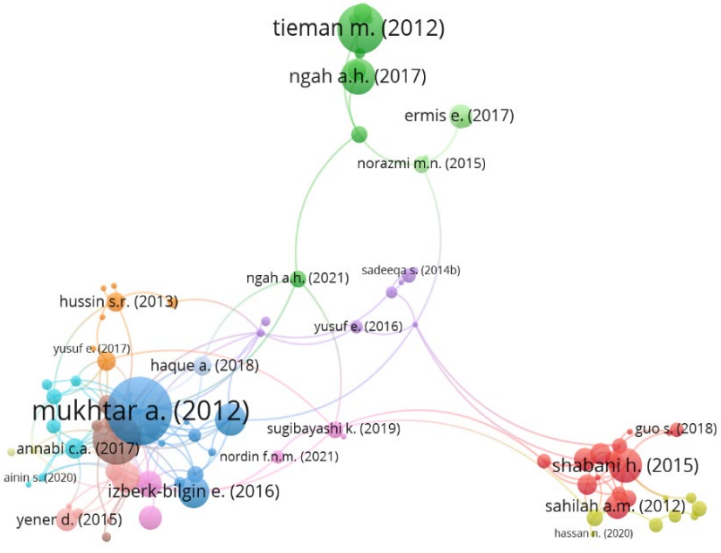

Figure 8: Network visualisation map of the citation.

Unit of analysis: Documents
Minimum number of citations of a document = 1

Table 9: Highly cited articles - most influential papers

No.	Authors	Title	Year	Cites	Cites per Year
1	Arshia Mukhtar, and Mohsin Muhammad Butt	Intention to Choose Halal Products: The Role of Religiosity	2012	234	39
2	Azmawani Abd Rahman, Ebrahim Asrarhaghighi and Suhaimi Ab Rahman	Consumers and Halal Cosmetic Products: Knowledge, Religiosity, Attitude and Intention	2015	131	18.72
3	Marco Tieman, Jack Van der Vorst and Maznah Che Ghazali	Principles in Halal Supply Chain Management	2012	127	21.17
4	Abdul Hafaz Ngah, Yuserrie Zainuddin, and T.Ramayah	Applying the TOE Framework in the Halal Warehouse Adoption Study	2017	67	11.17
5	Hessam Shabani, Mehrangiz Mehdizadeh, Mohammad Mousavi, and Ehsan Ansari Dezfouli	Halal Authenticity of Gelatine using Species-specific PCR	2015	63	10.50
6	Elif Izberk-Bilgin and Cheryl Nakata	A New Look at Faith-Based Marketing: The Global Halal Market	2016	55	09.17
7	Prerna Garg, and Richa Joshi	Purchase Intention of "Halal" Brands in India: The Mediating Effect of Attitude	2018	54	13.50
8	Vita Briliana and Nurwanti Mursito	Exploring Antecedents and Consequences of Indonesian Muslim Youths' Attitude towards Halal Cosmetic Products: A Case Study in Jakarta	2017	48	09.60
9	Isabelle Aoun and Laurent Tournois	Building Holistic Brands: An Exploratory Study of Halal Cosmetics	2015	37	06.17
10	Ali Feizollah, Sulaiman Ainin, Nor Badrul Anuar, Nor Aniza Abdullah, and Hazim Hanif	Halal Products on Twitter: Data Extraction and Sentiment Analysis Using Stack of Deep Learning Algorithms	2019	32	08.00

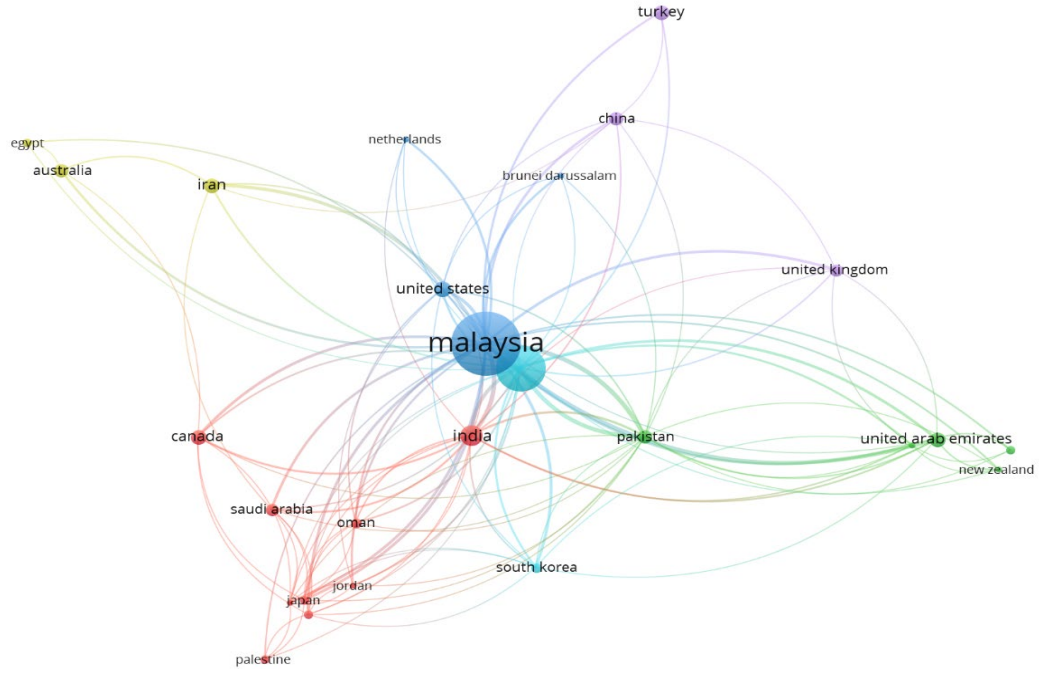

Figure 9: Network visualisation map of countries published the most.

Unit of analysis: countries
Maximum number of countries per document: 25
Minimum number of documents of a country: 1
Minimum number of citations of country: 1

4. Conclusion

This bibliometric analysis demonstrated a rising interest among academics and authors in the halal matter, particularly in the cosmetics and pharmaceutical industries, where publishing in these fields is expanding yearly and transcends many nations. This highlighted how awareness and understanding of halal goods' benefits are spreading worldwide. This analysis provides readers access to the most recent papers on halal cosmetics and halal pharmaceuticals and the writers who contributed to the articles. Although halal issue publications are rising, only 248 articles have been released to date, indicating that many subjects are linked to halal cosmetics and halal pharmaceuticals that have not yet been covered. Nonetheless, this analysis has some limitations because the systematic review and bibliometric analysis were conducted solely on the Scopus database.

Furthermore, the phrases chosen may be inaccurate and imprecise. Aside from that, the analysis demonstrates that the first publication on the issue of halal cosmetics and halal pharmaceuticals was in 2006, implying that this analysis has a restricted time range. Thus, future studies should be undertaken with a greater emphasis on the halal problem to provide outstanding references for readers. Finally, this analysis may assist researchers in identifying the halal cosmetics and halal pharmaceutical fields' gap and expanding their research in these sectors.

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References

Ahmad, A., Abd Rahman, A., & Ab Rahman, S. (2015). Assessing Knowledge and Religiosity on Consumer Behavior towards Halal Food and Cosmetic Products. International Journal of Social Science and Humanity. Vol. 5, No 1: 10-14.

Communications, M. M. A. S. T. (2020). Halal Pharmaceuticals Market 2020 forecasts Muslim consumer market. Bio Spectrum Asia Edition. Retrieved from https://www.biospectrumasia.com/news/25/15307/halal-pharmaceuticals-market-2020-forecasts-muslim-consumer-market.html

Haleem, A., Khan, M. I., Jami, A. R., & Khan, S. (2020). Research Status in Halal: A Review and Bibliometric Analysis. Modern Supply Chain Research & Application. Vol. 2: 23- 41.

Hunter, M. (2012). The emerging Halal cosmetic and personal care market. Personal Care, UK, 37- 41.

Mohamed Elias, E., Othman, S. N., & Mohd Saifudin, A. (2016). Halal awareness and knowledge among Muslim's student entrepreneurship program: A preliminary study. UUM Repository. Vol. 2: 1- 8

Mohezar, S., Zailani, S., & Zainuddin, Z. (2016). Halal Cosmetics Adoption among Young Muslim Consumers in Malaysia: Religiosity Concern. Global Journal Al-Thaqafah. Vol.6, No 1: 47-59.

Muhamad Yunus, N. S. N., Wan Rashid, W. E., Mohd Ariffin, N., & Mohd Rashid, N. (2014). Muslim's Purchase Intention Towards Non-Muslim's Halal Packaged Food Manufacturer. Procedia - Social and Behavioral Sciences, 145-154.

Mursyidi, A. (2013). The Role of Chemical Analysis in the Halal Authentication of Food and Pharmaceutical Products. Journal of Food and Pharmaceutical Sciences. Vol.1: 1- 4.

Rahim, N. @ F., Shafii, Z., & Shahwan, S. (2015). Awareness and Perception of Muslim Consumers on Halal Cosmetics and Personal Care Products. International Journal of Business, Economics and Management. Vol.2, No 1: 1-14.

Wan Jusoh, W. J., & Kit Teng, P. (2013). Investigating Students Awareness and Usage Intention towards Halal Labelled Cosmetics and Personal Care Products in Malaysia. Academia.edu, 1-10.

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Life Cycle Analysis (LCA) of Halal Authentication Approaches on Industrial Food Waste for Gelatine Production: A Mini Review

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Halalan toyyiban, Food waste, Gelatine, Protein analysis and OpenLCA

Abstract

Industrial food waste is a major issue that affects numerous countries. One of the techniques to reduce the generation of food waste is to recycle it for other purposes. Particularly, food waste from livestock industries can be utilised for gelatine preparation. However, the study on halal authentication of industrial food waste is still low. This review is conducted to identify the available protein analysis methods utilised in halal authentication of industrial food waste. It is also to compare and acknowledge the most reliable method. This review is performed in three steps: the planning phase, the conducting phase and the analysing phase. Reliable methods such as polymerase chain reaction (PCR), multiple reaction monitoring (MRM), fluorescent molecularly imprinted polymer nanogel (F-MIP-NG), lateral flow devices (LFDs) and loop-mediated isothermal amplification (LAMP) are identified and discussed. Their sensitivity limits are also recognised and compared.

1. Introduction

Waste is any material or substance that is no longer needed and has no further use or value in its current state. It can take many forms, including solid, liquid or gaseous. It can be divided into many categories according to its composition or origin (Jamal, 2020). According to their origins, waste can be sorted into commercial, institutional, municipal, and industrial categories. The industrial waste comes from various industrial factories; the livestock industry is one of them. Livestock industries generate a variety of food waste, consisting of meat, poultry and fish. According to Harvard (2023), food waste refers to edible food that is intentionally disposed of during the stages of retail or consumption, despite being suitable for eating. Food waste can be utilised and profited in numerous ways: biochemical processing, sewage treatment, and composting (US Environmental Protection Agency, 2018). As reported by Aksun Tümerkan (2021), food waste can also be processed into halal gelatine. This type of product life cycle applied in livestock industries is categorised as cradle-to-cradle (C2C); it is a sustainable framework which creates regenerative products that can be reused or recycled indefinitely. Nevertheless, every process in a product life cycle has its environmental impacts, which can be evaluated by the method of life cycle assessment (LCA) using an open-source software tool called OpenLCA.

Halal is an Arabic term that means 'permissible' or 'lawful'. *Halalan toyyiban* is an Islamic principle that emphasises the importance of consuming halal (lawful) and *toyyib* (good) food (Islamic Religious Council of Singapore, 2023). The term is derived from the *Qur'anic* verse that states,

> *"O mankind, eat from whatever is on Earth [that is] lawful and good"* (*Al-Baqarah* 2:168)

The principle encompasses the method of slaughtering animals and the measures in handling, storing, preparing and processing the ingredients used in food production (Abd Rahman & Abu Dardak, 2021). In Malaysia, a standard called MS 1500:2009 was specifically developed by the Technical Committee on Halal Food and Islamic Consumer Goods for halal food production. The importance of *halalan toyyiban* is recognised by Muslims worldwide, and it has become a significant part of their lifestyle as it highlights the holistic nature of Islam, which stresses the importance of physical, mental, and spiritual well-being.

As previously mentioned, halal gelatine can be produced from food waste. Gelatine is typically derived from animal collagen, which can be extracted from various collagen-rich sources, including bones, skin, and cartilage (Richter, 2021). In many cases, these sources of collagen are considered waste or by-products of the meat, poultry, and seafood industries. Depending on the collagen source, gelatine is either porcine, bovine or piscine. Halal gelatine is a type of gelatine that is permissible according to Islamic law. It is derived from sources that comply with Islamic dietary laws, prohibiting pork and un-slaughtered meat consumption. The use of halal gelatine has become increasingly important in the food and pharmaceutical industries as the Muslim population continues to grow

worldwide. According to Desilver & Masci (2017), the Muslim population is expected to account for 29.7% of the world's population by 2050. This population presents a significant market opportunity for halal products, including halal gelatine. In order to produce halal gelatine from food waste, animal origin needs to be identified. This production requires careful sourcing, in which several techniques can be utilised.

This mini-review paper is conducted to identify the available protein analysis methods utilised in halal authentication of industrial food waste. It is also to compare these methods to recognise the most reliable and efficient technique to be used.

2. Methodology

For this review, three main steps are involved: the planning phase, the conducting phase and the analysing phase. The methodology flow can be seen in Figure 1 below.

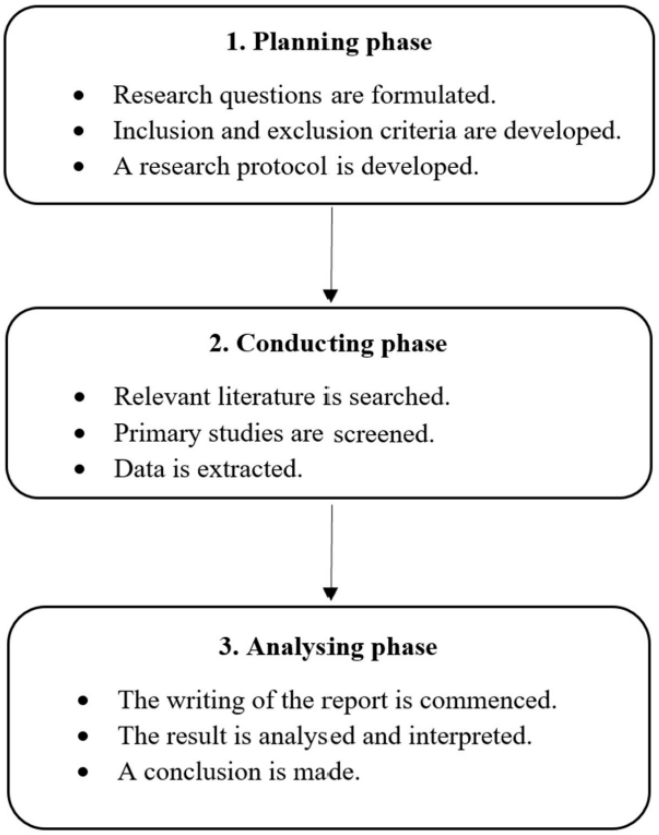

Figure 1: Methodology flow of the mini-review.

2.1 Planning phase

The first step, the planning phase, involves formulating a research question from a PICOS tool short for Population, Intervention, Comparison, Outcomes and Study.

Table 1: PICOS table for the review

Element	Keyword
Population	Industrial food waste
Intervention	Protein analysis methods
Comparison	None
Outcome	Halal authentication
Study	Scientific report papers

After the keywords are set as shown in Table 1, the research question can be put together: "What is known from the scientific report papers about protein analysis methods in halal authentication of industrial food waste?". The inclusion-exclusion criteria can then be determined, as shown in Table 2.

Table 2: Inclusion-exclusion criteria for the review

Factor	Inclusion	Exclusion
Timeline	Reports within 2013-2023	Reports before 2013
Subject area	Industrial food waste	Other types of waste
Publication type	Scientific report	Other types of publication
Language	English articles	Non-English articles

2.2 Conducting phase

The conducting phase involves searching for relevant articles using keywords on well-known search engines such as Google Scholar and PubMed and from backward referencing on obtained review papers. After title and abstract screening, a total of 10 articles investigating industrial food waste in the English language from the last decade (from 2013 until 2023) were acquired for this study. This mini-review targets key data in the articles, such as the objectives, analysis method, result and the industrial food waste investigated. The data is extracted and then synthesised in one table (Table 3) to be analysed. The articles are categorised based on the protein analysis methods utilised, in which the number of articles per method is shown in Figure 2.

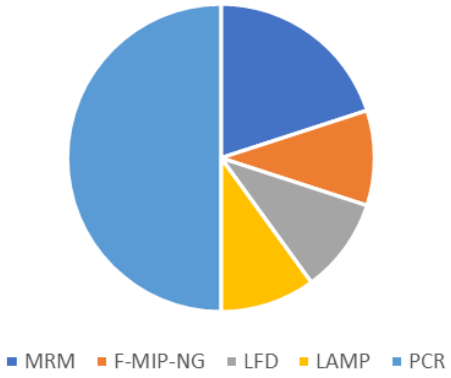

Figure 2: Percentage of studies based on methods utilised.

2.3 Analysing phase

The final step is the analysing phase. The extracted data from the selected articles are analysed, interpreted and summarised to answer the research question and accomplish the main objectives of this review. After the protein analysis methods are identified, the methods are compared to determine the most efficient technique to be utilised in halal authentication of industrial food waste.

3. Results and discussion

In the studies under review, different components were utilised to identify animal species, including meat extracts, processed meat and gelatine; these components are categorised as food waste, a type of waste purposely thrown out despite its edibility. Although different components were tested, all 10 studies investigated the protein level, allowing species differentiation.

Table 3: Extracted data from the reviewed articles

No	Food waste	Title	Method	Objective	Result	Author & year
1.	Processed meat	An SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products	PCR (qPCR using SYBR Green Supermix)	To detect and quantify pork meat in processed poultry meat products.	The developed qPCR assay allowed the detection and quantification of pork meat in the linear dynamic range of 0.1 – 25% with high correlation and PCR efficiency. The proposed SYBR Green I qPCR method proved to be a powerful and simple technique, highly specific and sensitive for pork species identification without requiring any post-PCR treatment.	Soares *et al.* (2013)
2.	Gelatine capsule shell	Analysis of Porcine Gelatine DNA in a Commercial Capsule Shell Using Real-Time Polymerase Chain Reaction for Halal Authentication	PCR (qPCR using SYBR Green Supermix)	To develop a specific primer from mitochondrial D-loop capable of amplifying DNA from porcine gelatine in commercial capsule shells.	From two primers that have been designed specifically, only primer D-Loop108 had the capability to identify the presence of porcine DNA in fresh tissue and gelatine. The lowest concentration of porcine DNA in gelatine capsule shells is 5 pg.	Sudjadi *et al.* (2016)
3.	Skin crackers	Species-specific polymerase chain reaction (PCR) assay for identification of pig (Sus domesticus) skin in "Rambak" crackers	PCR (qPCR using DreamTaq Green Master Mix)	To develop the pig species-specific primer for identification of specific pig DNA in 'Rambak' cracker.	Analysis of experimental mixture meat demonstrated that 0.1% of pig tissues could be detected using a specific primer. The specificity of pig-specific PCR provides a valuable tool for the identification of pig skin and for avoiding its fraudulent substitution and adulteration.	Erwanto *et al.* (2016)
4.	Gelatine	Development of a new and sensitive method for the detection of pork adulteration in gelatine and other highly processed food products	PCR (qPCR using Tubigel)	To detect the presence of porcine DNA in commercial gelatine and processed foods.	The TübiGel method was found to have a detection limit of 0.01% porcine gelatine, whilst the Biotecon method had 0.1%, and the R-Biopharm method detected >5% porcine gelatine. qPCR of TübiGel method was also found to detect porcine DNA better than qPCR of commercial kits.	Yayla & Ekinci Doğan (2021)
5.	Processed meat	Detection of pork adulteration by highly-specific PCR assay of mitochondrial D-loop	PCR (standard PCR)	To perform authentic identification of pork in other species' meat.	The sensitivity of the detection of pork in other species of meat using unique pig-specific PCR was established to be at 0.1%. The technique is cheaper than qPCR. It can be used for the authentication of raw, processed and adulterated pork and products under the circumstances of food adulteration-related disputes or forensic detection of the origin of pig species.	Karabasanavar *et al.* (2014)
6.	Processed meat	New Sensitive High-Performance Liquid Chromatography–Tandem Mass Spectrometry Method for the Detection of Horse and Pork in Halal Beef	MRM (MRM and MRM^3)	To detect trace contaminations of horse meat and pork in halal beef.	Able to detect down to 0.13% pork contamination in beef. Rapid and sensitive mass spectrometrical method for the detection of horse and pork by use of MRM and MRM^3.	Von Bargen *et al.* (2013)

7.	Processed meat	Meat authentication: a new HPLC-MS/MS-based method for the fast and sensitive detection of horse and pork in highly processed food	MRM (MRM and MRM^3)	To develop an optimised method for the detection of horse and pork in different processed meat.	Identified marker peptides were sufficiently stable to resist the thermal processing of different meat products and thus allow the sensitive and specific detection of pork or horse in processed food down to 0.24% in a beef matrix system. Specific and sensitive detection of horse and pork meat in different processed food matrices using MRM-based detection of marker peptides.	Von Bargen *et al.* (2014)
8.	Meat extract	Molecularly imprinted polymer nanogel-based fluorescence sensing of pork contamination in halal meat extracts	F-MIP-NG	To investigate pork contamination in halal meat extracts.	A detection limit for pork contamination was as low as 0.1% in the pork-contaminated beef extract samples was also achieved. Under optimal conditions, the F-MIP-NG-based sensors exhibited high sensitivity, a detection limit of 40 pM, a linear range of 0.25 – 5 nM, and excellent affinity and selectivity towards PSA, compared to potentially interfering proteins. It was more efficient to detect beef contamination in 1 wt% pork contamination compared to the real-time polymerase chain reaction.	Cheubong *et al.* (2021)
9.	Processed meat	Development and validation of a rapid test system for the detection of pork meat and collagen residues	LFD	To develop a detection system capable of rapidly (~35min) identifying porcine residues.	A detection system was developed based on a lateral flow device (LFD) assay format capable of rapidly (~35 min) identifying porcine residues derived from raw meat, cooked meat, and gelatine down to 0.01%, 1.0%, and 2.5% contamination, respectively. The LFD tests are suitable alternatives in that they do not require any machinery to operate, and report outcomes in ~35 min with the same or better level of sensitivity without any additional processing.	Masiri *et al.* (2016)
10.	Processed meat	Detection of porcine-derived ingredients from adulterated meat based on real-time loop-mediated isothermal amplification	LAMP (real-time LAMP)	To develop an efficient and rapid assay for detecting a porcine gene in meat products.	The amplification showed no cross-reactivity with 11 other meats. The established method required 20 min with an initial amplification curve of approximately 10 min and demonstrated a detection limit of 1.76 pg/µL porcine DNA, which equals to 0.0001%. This method meets specificity, rapidness, robustness, and sensitivity criteria; its practical application will greatly aid in battling adulteration in the food industry.	Cai *et al.* (2020)

Available protein analysis methods utilised in halal authentication of industrial food waste are as listed below:

1. Polymerase chain reaction (PCR)
2. Multiple reaction monitoring (MRM)
3. Fluorescent molecularly imprinted polymer nanogel (F-MIP-NG)
4. Lateral flow devices (LFDs)
5. Loop-mediated isothermal amplification (LAMP)

3.1 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a widely used molecular biology technique that amplifies specific DNA sequences in vitro. In accordance with the National Library of Medicine (2017), PCR is a method based on the ability of the DNA polymerase enzyme to extend primers annealed to a template DNA strand. It involves three basic steps: denaturation, annealing, and extension (Khan Academy, 2016). In the denaturation step, the DNA is heated to a high temperature to separate the double-stranded DNA into two single strands. In the annealing step, primers complementary to the target DNA sequences anneal to the single-stranded DNA template by the cooling process. DNA polymerase extends the primers in the extension step, producing a copy of the original DNA sequence. These three steps are repeated for multiple cycles, resulting in exponential amplification of the target DNA sequence. PCR is a molecular biology technique that can rapidly and efficiently amplify specific DNA sequences from small or degraded samples.

There exist multiple varieties of PCR. Real-time PCR (qPCR) allows for real-time DNA quantification as the reaction occurs. This type of PCR uses fluorescent dyes or probes to detect DNA amplification as it happens. Over time, several types of qPCR-ready mixes have been developed. Soares *et al.* (2013) utilised an SYBR Green Supermix, a twice-concentrated mix with real-time fluorescence enhanced for dye-based qPCR. It consists of iTaq DNA polymerase, an antibody-mediated hot-start polymerase (Bio-Rad Laboratories, 2023b). In the study, PCR was conducted to detect and quantify pork meat in processed poultry. The assay detected pork meat in the range of 0.1 - 25%. Furthermore, Sudjadi *et al.* (2016) also utilised SYBR Green Supermix to develop a highly specific primer from the mitochondrial D-loop for DNA amplification of porcine gelatine in commercial capsule shells. Correspondingly, primer D-loop 108 detected the presence of porcine DNA in gelatine capsule shells at 5 pg. Both studies prove that SYBR Green for the method of qPCR is a powerful yet simple method of identifying pork meat.

Besides SYBR Green, DreamTaq Green PCR Master Mix (2X) is another ready-to-use mix utilised for qPCR. It contains DreamTaq DNA Polymerase, an optimised thermostable DNA polymerase with high sensitivity and yield for DNA synthesisation (Thermo Fisher Scientific, 2023). It was applied by Erwanto *et al.* (2016) to develop pig species-specific primers for identifying specific pig DNA in the 'Rambak' cracker. Their study proved that 0.1% of porcine DNA could be identified with the DreamTaq Green. It demonstrated that DreamTaq Green is as powerful as SYBR Green in pork DNA identification.

DNA detection kits were also developed for conducting qPCR. Yayla & Ekinci Doğan (2021) developed a method called TübiGel to detect the presence of porcine DNA in commercial gelatine and processed foods. It was proven that TübiGel has a porcine gelatine detection sensitivity of 0.01%, while commercial detection kits such as Biotecon and R-Biopharm only have a sensitivity of 0.1% and >5%, respectively. This showed that the newly designed kit, TübiGel, is more sensitive to detecting porcine DNA in processed foods than commercial detection kits and ready-to-use mixes.

Apart from qPCR, another form of PCR commonly used in research is standard PCR. In contrast with qPCR, which allows real-time quantification, standard PCR detects the product only at the end-point (Bio-Rad Laboratories, 2023a). Karabasanavar *et al.* (2014) applied standard PCR in designing new highly specific primers to conduct genuine identification of pork in the meat of other species. As a result, a detection sensitivity of down to 0.1% was achieved. Although standard PCR is proven to be less sensitive than qPCR, it is also less costly.

3.2 Multiple reaction monitoring (MRM)

Multiple reaction monitoring (MRM) is an analytical technique used in mass spectrometry for the targeted quantification of proteins (You *et al.*, 2013). According to Meng & Veenstra (2013), MRM is a type of mass spectrometry that involves selecting a specific precursor ion in the first stage, fragmenting it to produce multiple product ions in the second stage and monitoring the product ions within the third stage. The precursor and product ions selection is based on the specific mass-to-charge ratio (m/z) of the analyte and its fragments. The precursor and product ions are chosen based on their unique m/z values, which allows for specific detection of the analyte of interest. As stated by Rai & Satija (2021), MRM is a powerful method that provides higher selectivity than PCR for detecting low-abundance analytes in complex samples.

MRM can also be conducted with multistage fragmentation, known as MRM^3. This technique enhances the sensitivity, specificity and selectivity of the method. Von Bargen *et al.* (2013) employed the method of MRM and MRM^3 to find trace contaminations of pork meat in halal beef. The results showed that MRM could detect 0.55% contamination, while MRM^3 detected down to 0.13% of porcine DNA. A year later, Von Bargen *et al.* (2014) established an optimised method for identifying pork in other species' processed meat. The MRM and MRM^3 detection were down to 0.24%, demonstrating a rapid 2-minute extraction process. This result proved that MRM and MRM^3 are quick yet sensitive techniques for DNA identification.

3.3 Fluorescent molecularly imprinted polymer nanogel (F-MIP-NG)

Fluorescent molecularly imprinted polymer nanogels (F-MIP-NG) are a relatively new material class that has gained increasing interest in nanotechnology. These nanogels are made up of a network of cross-linked polymers designed to selectively recognise and bind specific target molecules through molecular imprinting (Xu *et al.*, 2021). F-MIP-NG are distinguished from other molecularly imprinted polymers by their ability to emit light when exposed to a specific wavelength of light. According to Huang *et al.* (2018), the fluorescent molecules serve as a signal transducer and allow for detecting the binding event between the nanogel and the target molecule. The signal produced by the nanogel can be easily detected and quantified using a fluorescence spectrometer, allowing for rapid and accurate analysis.

The method of F-MIP-NG was utilised by Cheubong *et al.* (2021) to investigate pork contamination in extracts of halal meat. In the study, a sensitive F-MIP-NG-based sensor was imprinted and established for rapid porcine serum albumin (PSA), lowering the porcine DNA detection sensitivity to 0.1%. The F-MIP-NG sensors revealed high sensitivity, affinity and

selectivity at optimal conditions, proving it more efficient than qPCR.

3.4 Lateral flow devices (LFDs)

Lateral flow devices (LFDs), also known as lateral flow assays, are paper-based diagnostic tests that use an immunoassay to detect the presence or absence of a specific analyte in a sample (Abingdon Health, 2022). LFDs detect and capture a target analyte in a sample using binding agents immobilised on a nitrocellulose membrane (Sadeghi *et al.*, 2021). The sample is typically applied to a sample pad at one end of the device, and the sample flows along the membrane due to capillary action. If the target analyte is present in the sample, it binds to the immobilised binding agent, resulting in a visible signal, such as a coloured line or dot, at the test line on the membrane. A control line containing a different immobilised binding agent that should always produce a signal if the device is working properly is also included to ensure the test is valid.

LFDs can be applied in numerous fields, including porcine DNA identification. Masiri *et al.* (2016) utilised LFDs to establish an identification system capable of rapidly detecting porcine residues. The system could detect down to 0.01%, 1.0%, and 2.5% contamination derived from raw pork, cooked pork and porcine gelatine, respectively. LFDs are simple alternatives for porcine detection; anyone can perform them as it does not involve complicated steps. Results are also processed within 35 minutes, proving LFDs to be rapid.

3.5 Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a highly sensitive and specific nucleic acid amplification technique that has become increasingly popular due to its ability to amplify DNA under isothermal conditions (Wong *et al.*, 2018). Unlike traditional PCR, LAMP does not require thermal cycling, which makes it particularly useful in resource-limited settings. LAMP amplifies target DNA using a strand-displacing DNA polymerase and a set of four to six primers recognising six or eight regions on the target DNA sequence. These primers are designed to recognise a target sequence and initiate a process of strand displacement and amplification in the presence of a constant temperature of 60 - 65°C (Marroki & Bousmaha-Marroki, 2022). According to Soroka *et al.* (2021), LAMP amplifies DNA faster and more efficiently than PCR; the former can amplify up to a billion copies in less than an hour, compared to a million copies by the latter.

Cai *et al.* (2020) established a rapid real-time LAMP method for porcine DNA detection in meat products. The method has a reaction time of 20 minutes and an initial amplification curve of 10 minutes with an identification limit of 1.76 pg/µL contamination, which equals 0.0001%. Compared with other DNA identification techniques, LAMP demonstrated itself to be a quick yet highly specific and sensitive method in porcine DNA detection.

4. Conclusion

There are various techniques to be used in DNA identification, including PCR, MRM, F-MIP-NG, LFDs and LAMP. Each technique was demonstrated to be highly specific, with a detection sensitivity of lower than 5%. Compared to other methods investigated, LAMP proved to be the most sensitive method in performing porcine DNA identification. With a rapid 20-minute reaction time and 10-minute amplification, LAMP detected down to 0.0001% porcine contamination in processed meat products of other species. As LAMP demonstrated to be the most sensitive method, MRM proved to be the least sensitive. By the multistage fragmentation method known as MRM^3, the lowest contamination percentage it could detect was down to 0.13%.

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References

Abd Rahman, R., & Abu Dardak, R. (2021, April 30). Halal Principles as one of Food Safety Measurements | FFTC Agricultural Policy Platform (FFTC-AP). FFTC-AP. https://ap.fftc.org.tw/article/2731

Abingdon Health. (2022). What is a lateral flow test and how does the technology work? Abingdon Health. https://www.abingdonhealth.com/services/what-is-lateral-flow-immunoassay/

Aksun Tümerkan, E. T. (2021). Sustainable utilisation of gelatin from animal-based agri–food waste for the food industry and pharmacology. Valorisation of Agri-Food Wastes and By-Products: Recent Trends, Innovations and Sustainability Challenges, 425–442. https://doi.org/10.1016/B978-0-12-824044-1.00041-6

Bio-Rad Laboratories. (2023a). What is Real-Time PCR (qPCR)? Bio-Rad Laboratories, Inc. https://www.bio-rad.com/en-my/applications-technologies/what-real-time-pcr-qpcr?ID=LUSO4W8UU

Bio-Rad Laboratories, I. (2023b). iTaq Universal SYBR Green Supermix Bio-Rad. Bio-Rad Laboratories, Inc. https://www.bio-rad.com/en-my/product/itaq-universal-sybr-green-supermix?ID=M87FTF8UU

Cai, S., Kong, F., & Xu, S. (2020). Detection of porcine-derived ingredients from adulterated meat based on real-time loop-mediated isothermal amplification. Molecular and Cellular Probes, 53. https://doi.org/10.1016/J.MCP.2020.101609

Cheubong, C., Takano, E., Kitayama, Y., Sunayama, H., Minamoto, K., Takeuchi, R., Furutani, S., & Takeuchi, T. (2021). Molecularly imprinted polymer nanogel-based fluorescence sensing of pork contamination in halal meat extracts. Biosensors & Bioelectronics, 172. https://doi.org/10.1016/J.BIOS.2020.112775

Desilver, D., & Masci, D. (2017, January 31). World Muslim population more widespread than you might think. Pew Research Center. https://www.pewresearch.org/fact-tank/2017/01/31/worlds-muslim-population-more-widespread-than-you-might-think/

Erwanto, Y., Yuliatmo, R., Sugiyono, & Abdul Rohman. (2016). (PDF) Species specific polymerase chain reaction (PCR) assay for identification of pig (Sus domesticus) skin in " Rambak " crackers. International Conference on Tropical Animal Science and Production. https://www.researchgate.net/publication/313441858_Species_specific_polymerase_chain_reaction_PCR_assay_for_identification_of_pig_Sus_domesticus_skin_in_Rambak_crackers

Harvard. (2023). Food Waste | The Nutrition Source . Harvard T.H. Chan School of Public Health. https://www.hsph.harvard.edu/nutritionsource/sustainability/food-waste/

Huang, X., Song, J., Yung, B. C., Huang, X., Xiong, Y., & Chen, X. (2018). Ratiometric optical nanoprobes enable accurate molecular detection and imaging. Chemical Society Reviews, 47(8), 2873. https://doi.org/10.1039/C7CS00612H

Islamic Religious Council of Singapore. (2023). The Concept of *Halalan Toyyiban* in Our Food Consumption. Islamic Religious Council of Singapore. https://www.muis.gov.sg/officeofthemufti/Khutbah/Tamil/2021-Aug-06

Jamal, H. (2020, January 16). Types of Solid Wastes - Based on Origin and Composition. About Civil Engineering. https://www.aboutcivil.org/types-of-solid-wastes

Karabasanavar, N. S., Singh, S. P., Kumar, D., & Shebannavar, S. N. (2014). Detection of pork adulteration by highly-specific PCR assay of mitochondrial D-loop. Food Chemistry, 145, 530–534. https://doi.org/10.1016/J.FOODCHEM.2013.08.084

Khan Academy. (2016). Polymerase chain reaction (PCR) (article) | Khan Academy. Khan Academy. https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/polymerase-chain-reaction-pcr

Marroki, A., & Bousmaha-Marroki, L. (2022). Antibiotic Resistance Diagnostic Methods for Pathogenic Bacteria. Encyclopedia of Infection and Immunity, 320–341. https://doi.org/10.1016/B978-0-12-818731-9.00133-6

Masiri, J., Benoit, L., Barrios-Lopez, B., Thienes, C., Meshgi, M., Agapov, A., Dobritsa, A., Nadala, C., & Samadpour, M. (2016). Development and validation of a rapid test system for detection of pork meat and collagen residues. Meat Science, 121, 397–402. https://doi.org/10.1016/J.MEATSCI.2016.07.006

Meng, Z., & Veenstra, T. D. (2013). Mass Spectrometry–Based Approach for Protein Biomarker Verification. Proteomic and Metabolomic Approaches to Biomarker Discovery, 407–424. https://doi.org/10.1016/B978-0-12-394446-7.00026-1

National Library of Medicine. (2017). Polymerase Chain Reaction (PCR). National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/

Rai, A. K., & Satija, N. K. (2021). Importance of targeted therapies in acute myeloid leukemia. Translational Biotechnology: A Journey from Laboratory to Clinics, 107–133. https://doi.org/10.1016/B978-0-12-821972-0.00017-4

Richter, A. (2021, December 13). Gelatin: What it is made of, health benefits, nutrition, and more. Medical News Today. https://www.medicalnewstoday.com/articles/319124

Sadeghi, P., Sohrabi, H., Hejazi, M., Jahanban-Esfahlan, A., Baradaran, B., Tohidast, M., Majidi, M. R., Mokhtarzadeh, A., Tavangar, S. M., & de la Guardia, M. (2021). Lateral flow assays (LFA) as an alternative medical diagnosis method for detection of virus species: The intertwine of nanotechnology with sensing strategies. Trends in Analytical Chemistry, 145, 116460. https://doi.org/10.1016/J.TRAC.2021.116460

Soares, S., Amaral, J. S., Oliveira, M. B. P. P., & Mafra, I. (2013). A SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products. Meat Science, 94(1), 115–120. https://doi.org/10.1016/J.MEATSCI.2012.12.012

Soroka, M., Wasowicz, B., & Rymaszewska, A. (2021). Loop-Mediated Isothermal Amplification (LAMP): The Better Sibling of PCR? Cells, 10(8). https://doi.org/10.3390/CELLS10081931

Sudjadi, Wardani, H. S., Sepminarti, T., & Rohman, A. (2016). Analysis of Porcine Gelatin DNA in a Commercial Capsule Shell Using Real-Time Polymerase Chain Reaction for Halal Authentication. Https://Doi.Org/10.1080/10942912.2015.1110164, 19(9), 2127–2134. https://doi.org/10.1080/10942912.2015.1110164

Thermo Fisher Scientific. (2023). DreamTaq PCR Master Mix (2X). Thermo Fisher Scientific Inc. https://www.thermofisher.com/order/catalog/product/K1071

US Environmental Protection Agency. (2018). Wasted Food Report Estimates of generation and management of wasted food in the United States in 2018.

Von Bargen, C., Brockmeyer, J., & Humpf, H. U. (2014). Meat authentication: a new HPLC-MS/MS based method for the fast and sensitive detection of horse and pork in highly processed food. Journal of Agricultural and Food Chemistry, 62(39), 9428–9435. https://doi.org/10.1021/JF503468T

Von Bargen, C., Dojahn, J., Waidelich, D., Humpf, H. U., & Brockmeyer, J. (2013). New sensitive high-performance liquid chromatography-tandem mass spectrometry method for the detection of horse and pork in halal beef. Journal of Agricultural and Food Chemistry, 61(49), 11986–11994. https://doi.org/10.1021/JF404121B/SUPPL_FILE/JF404121B_SI_001.PDF

Wong, Y. P., Othman, S., Lau, Y. L., Radu, S., & Chee, H. Y. (2018). Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. Journal of Applied Microbiology, 124(3), 626. https://doi.org/10.1111/JAM.13647

Xu, S., Wang, L., & Liu, Z. (2021). Molecularly Imprinted Polymer Nanoparticles: An Emerging Versatile Platform for Cancer Therapy. Angewandte Chemie (International Ed. in English), 60(8), 3858. https://doi.org/10.1002/ANIE.202005309

Yayla, M. E. A., & Ekinci Doğan, C. (2021). Development of a new and sensitive method for the detection of pork adulteration in gelatin and other highly processed food products. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 38(6), 881–891. https://doi.org/10.1080/19440049.2021.1902574

You, J., Willcox, M. D., Madigan, M. C., Wasinger, V., Schiller, B., Walsh, B. J., Graham, P. H., Kearsley, J. H., & Li, Y. (2013). Tear Fluid Protein Biomarkers. Advances in Clinical Chemistry, 62, 151–196. https://doi.org/10.1016/B978-0-12-800096-0.00004-4

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Halal Fermented Functional Food in Indonesia: A Review

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Abstract

Nowadays, people prefer to consume foods with better nutrition to enjoy life more healthily. However, Muslims must also eat halal food following the Islamic way of life; eating nutritious food alone is insufficient. Indonesia, the largest Muslim population in the world, presents a market potential for halal food manufacturers and consumers worldwide. Several varieties of regional fermented foods were listed on the halal-positive food list and free from the requirement of halal certification, as per Indonesian Halal rules. The review of Indonesian fermented foods listed in the category of positive halal foods is the main topic of this essay. The procedures used to review the chosen themes include finding the literature in online sources, screening for topic inclusion, and evaluating, extracting, and discussing the accessible data found in the publications. The potential functional local fermented food varieties are included on the positive halal list: T*ape* (sticky rice and cassava), *Dadih,* and *Tempeh*. These fermented foods' microbiological and chemical characteristics were discussed. Therefore, the functional value of these fermented foods is rich in phytochemicals, anti-cancer (*β-glucan*), antioxidant compounds (isoflavones) and probiotic components (lactic acid bacteria and yeasts).

1. Introduction

Consumption of halal food in Indonesia has begun to experience a significant increase, in line with the global demand for halal products, which has reached USD 1.8 trillion to 2.1 trillion (GIFR, 2015). The global growth of the Muslim population and increased understanding of halal are two essential factors behind changing consumption patterns among generations of Muslims (Ahmad *et al.*, 2015). In Indonesia, the Government has begun to pay attention to halal consumption patterns, along with the passing of the Halal Product Guarantee Law No. 33 of 2014, which became the initial foundation for changing the halal system in Indonesia. Before this, only a small portion of Indonesia's entire food industry used halal food processing, and halal certification was registered as voluntary. The whole food business is now required to have halal certification due to the passage of the Halal Product Guarantee Law No. 33 of 2014. This law represents a state's responsibility to provide protection and a sense of security in consuming and using products that comply with Islamic law.

Additionally, evolving patterns of dietary needs are encouraged by shifts in the community's worldview regarding the value of health and healthy living (Khoerunisa, 2020). In today's world, functional food is often defined as providing the body with comprehensive nutrition and certain physiological benefits (Amaliah *et al.*, 2019). Health costs, which tend to increase in Indonesia, are one of the drivers of the increasing need for functional food because it is hoped that this type of food can be a solution to minimise disease with essential components (Abbas, 2020). For Muslims, eating functional food is also necessary, but their religious beliefs require halal food. Foods without any non-halal ingredients are necessary for Muslim consumers. However, several compounds frequently used in food production today can originate from non-permissible substances. Using fermentation techniques to create these components offers a substantial advantage in avoiding confusion if the microbial production is carried out in line with halal regulations. Furthermore, the demand for halal microbial products has expanded, giving producers an edge in market rivalry. This is due to the interest in halal-certified food components.

Local food processing in Indonesia also uses conventional biotechnology and fermentation methods (Susanti *et al.*, 2019). Various microorganisms were used in fermentation (Griana & Kinasih, 2020). Several types of fermented local food that are known in Indonesia include fermented milk-based food (Soenarno *et al.*, 2013) tubers based (Utami & Djaafar, 2014), fruit and vegetable-based (Febricia *et al.*, 2020), meat and fish-based (Antika, 2019; Patel & Shah, 2017). However, Muslims cannot immediately consume food derived from fermented products because microorganisms are a critical point of haram in processing (Komisi Majelis Ulama Indonesia, 2010). According to Qaradawi (1994) , Islamic law prohibits the use of substances scientifically shown to be harmful to human health at any step of the fermentation process and stipulates that halal necessitates that human health protection always comes first.

In Indonesia, materials that are exempt from the requirement to obtain halal certification include 1) materials originating from nature in the form of plants or mining materials without going through any processing; 2) materials that are categorised as not at risk of containing proscribed substances; 3)

substances that are not deemed hazardous and do not mix with prohibited substances, according to the Decree of the Minister of Religion of the Republic of Indonesia No. 1360 of 2021 regarding the list of foods that are exempt from this requirement. In addition, the Decree listed several foods originating from local Indonesian fermentations, including *Tape* (sticky rice/cassava), *Dadih,* and *Tempeh*. Indonesia is inhabited by more than 300 ethnic groups, affected by the diversity of traditional fermented foods that reflect the importance of culture in each area, involving microbes of Indonesian biodiversities. The food products such as sticky rice/cassava) *Dadih,* and *Tempeh* is Indonesian indigenous fermented food that has formed an integral part of the diet and can be prepared using relatively simple techniques and equipment (Surono, 2016). This study aims to review the potential of fermented foods with clear halal status as functional foods so that they are safe and permissible for consumption by Muslims. Therefore, the results of this study are expected to shed light on alternative functional foods with clear halal status.

2. Materials and methods

2.1 Research methods

2.1.1 Journal reviews

Analysis was carried out on several articles related to functional food and local fermented food. Reputable search engines and databases such as Science Direct, Scopus, and Google Scholar are used to assist the research process. The following terms were searched: critical halal point, microbial, alcohol, and food industry; apart from that, scans were also carried out to search for articles, including research reports, journal articles, textbooks, and publications from both the Government and the private sector with years of publication between 2005 – 2022. The year 2005 was selected due to the year of forming the Indonesian Halal Product Guarantees Law began.

2.1.2 Data collection and analysis

This research used a literature review. This method was used to review the selected paper from online sources, including Science Direct and Google Scholar, screening for topic inclusion, assessing, extracting, and discussing fermented food based on local food with functional potential. Descriptive methods from several perspectives with elaboration were used in this analysis.

3. Results and discussion

3.1 Halal perspective of fermented food products

The fermentation process is a complex process that involves various components and changes in compounds (Ye *et al.*, 2019). The fermentation process is one way to improve the quality of food ingredients by adding microbes. Adding these microbes can produce taste, aroma, and colour in food (Kusuma *et al.*, 2020). Halal market trade is still very open, reinforced by the Asian market share, which is still the most significant global market (60%) (Alexander, 2018). Many fermented dairy products have been traditionally produced worldwide, generally dominated by fermented milk-based products, such as yoghurt and cheese (Bintsis & Papademas, 2022). These kinds of food products are susceptible to contamination by haram items because of the intricate nature of the fermentation process. The support materials utilised for immobilisation and other methods used in the fermentation process must be halal-approved. However, if the microbial generation complies with halal requirements, modern fermentation technologies significantly minimise interpretation (Yap & Al-Mutairi, 2023).

Fermentation processes are composed of two steps, preparations before fermentation and treatments after fermentation. While doing these steps, some principles should be considered, as the goal is to produce halal products. Based on Kurniadi & Frediansyah (2017) and Karahalil (2020), six main critical points are determining halal perceptions of fermentation-based products 1) microbial source, 2) microbial isolates, 3) growth media, 4) metabolic products, 5) production site (fermentation media), and 6) others added ingredients for a specific purpose. From this explanation, the microbial source in question is the origin of the microbe isolated or taken. Sources of microbes used in the fermentation process can come from various places, such as fruit, milk, and water (Gulitz *et al.*, 2011; Rahmah, 2021; Sujaya *et al.*, 2004; Sukmarini *et al.*, 2014) but can also be taken from animal body parts or animal waste (Azizah *et al.*, 2012; Purba *et al.*, 2022). Fermentations with microbes from animal body parts or isolated from the non-halal source are nonpermissive (Nuraida, 2015). Employing microbial obtained from a halal and hygienic environment in fermentations is also a critical prerequisite to reach the halal product. For example, it has been reported that some lactic acid bacteria strains were isolated from human faeces and meat, and their functionality was investigated using them for fermented products containing various vegetables (Nuraida, 2015). In the food industry, microbes and genetic modification used in the production process must be non-toxic and are only derived from halal-based sources (Estiati & Herman, 2016). Genetic modification of microbes also needs to be evaluated. At the same time, the gene source, the status of the final product and the potential effect on human health is generally discussed to be evaluated from a halal perspective (Karahalil, 2020).

Besides microbial origin, microbial growth media is also at risk of non-permissible contamination. Some microbial growth media were not approved as growth media because they have the potential for non-permissible contamination, including blood-based media (Djannatun *et al.*, 2008; Nurhidayanti, 2019), brain heart infusion whose components come from animal tissue (Liofilchem, 2017) and peptone media obtained from sources of non-permissible contamination. The termed inoculation, which is the addition of the microorganism to the fermentation medium, is another necessary procedure to start a fermentation process, such as tween 80, which needs to be checked carefully.

In addition to growth media, the fermentation media also should be halal-approved. Fermentation media is a controlled environment in which microbial productions are carried out that contain two major groups of nutrients. The major nutrients added to the medium are nitrogen and carbon sources. Their origin must be known because nitrogen and carbon can be obtained from animal sources (Lopes *et al.*, 2018). Nitrogen sources should not be derived from animals that are not halal, non-halal slaughtered and blood. They cannot be used if the aim is to produce halal bioproducts. In recent years, low-cost materials have been used with the potential to enrich the fermentation medium to minimise the fermentation cost. This media enrichment substrate, which has high nitrogen and carbon content, should be derived from a permissible source (Sulaiman *et al.*, 2014).

The results of metabolic processes during fermentation are also critical in determining the halal status of fermented products. One of the metabolic products that need attention is related to the amount of ethanol. Based on *Qur'an*, as Islamic Law, alcohol has been identified as a non-halal (haram, forbidden) substrate, and halal-certified products are usually alcohol-free (Alzeer & Abou Hadeed, 2016). In Indonesia, based on MUI FATWA No. 10 of 2018, it is explained that alcoholic drinks are considered *khamr* when the ethanol content is more than 0.5%. The law on products containing ethanol exceeding the limit set by the MUI, whether a lot or a little, is haram. Both food and drink containing *khamr* are considered haram (Halalmui.org, 2021). High amounts of ethanol in food products due to fermentation can cause these products to become non-halal. Ethanol is said to cause sodium disturbances in the human brain's synapses. There is also a link between alcohol and plasma membrane disturbances (Kurniadi & Frediansyah, 2017). Another process in fermentation where there is potential for permissible contamination is in the production process; one example is the production of yeast produced by beer companies (MUI FATWA No. 4 of 2003).

In addition, other auxiliary materials added for specific purposes in the fermentation process must also be considered. Auxiliary materials that are often used in the fermentation process include the use of skim milk (Juniawati, Sri Usmiati, 2013), alginate coating (Purukan *et al.*, 2020) and the use of whey protein (Tratnik *et al.*, 2006). The auxiliary materials were added to fermentation processes for various functionalities, such as improving the sensory and quality (Alonso, 2016). The food industry develops flavour innovation to reach consumers who enjoy the sensory experience of eating (Bublitz *et al.*, 2013). All auxiliary materials need to be halal-approved to have a final halal product.

Decree of the Minister of Religion of the Republic of Indonesia no 1360 of 2021 regarding the List of Materials Exempted from Obligation for Halal Certification (Positif List) mentions several types of naturally fermented food, such as *Tape* (cassava/glutinous rice)*, Dadih* and *Tempeh*. Based on these regulations, it can be assumed that the naturally fermented products mentioned above are categorised as having no risk of containing prohibited materials and are not classified as dangerous and not in contact with illicit substances. Natural ethanol produced by natural fermentation under aerobic conditions is halal by nature (Alzeer & Abou Hadeed, 2016). Therefore, KMA no 1360 of Decree of the Minister of Religion of the Republic of Indonesia no 1360 of 2021 guarantees that the Indonesian Muslim community can consume naturally fermented food without worrying about halal status. This is undoubtedly a positive value because the obligation to consume halal food is fulfilled. Another benefit that can be obtained from naturally fermented food is the health potential of the food mentioned above, which will indirectly improve the quality of life of Muslim communities and society in general (Suter, 2013). Fermented products included in the positive list are one of the reasons that the ingredients come from nature without processing and without adding other ingredients, including non-permissible materials and additives. This study will discuss the potential of sticky rice *Tape,* cassava, *Dadih,* and *Tempeh* as a halal functional food. The fermented foods from plants are well-developed in Southeast Asia and common in Indonesia (Law *et al.*, 2011). The fermentation process in various solid products can cause changes in taste and simplify complex compounds into simpler ones to be utilised optimally by the human body. Muslims rationalised the benefit concerning halal. The comfortable feeling when food is taken can be achieved by having healthy, safe and pleasant food that complies with our beliefs (Bublitz *et al.*, 2013).

3.2 The potential of locally fermented halal functional food

3.2.1 *Tape* (sticky rice and cassava)

Tape is a fermented carbohydrate product using yeast and bacteria, which belongs to the lactic acid bacteria (LAB) group (Sulistiani & Hidayat, 2020). BAL itself has known as GRAS (generally recognised as safe) microbes; in other words, this type of bacteria is known as a microbe that is not a health risk (Antika, 2019). Tape in Indonesia is predominantly made with essential ingredients of cassava and glutinous rice (Griana & Kinasih, 2020) and differ in any area based on its ingredients. In cassava *Tape* fermentation, the dominant microbes play a role, including *Saccharomyces cerevisiae, Saccharomycopsis fibuligera, Candida tropicalis,* and *Candida guilliermondii* (Cempaka, 2021). In fermented sticky rice, the significant microbes *Lactobacillus plantarum, Lactobacillus curvatus, Lactobacillus fermentum, Pediococcus pentosaceous, Weissella confuse, Weissella paramesenteroides, Weissella kimchi* (Hasanah *et al.*, 2019; Rahayu *et al.*, 2018). The types of microbes that play a role in fermenting cassava *Tape* and sticky rice *Tape* are dominated by lactic acid bacteria and bacteria that can potentially be probiotics. Probiotics are living organisms that can provide health effects on the body by improving the balance of the digestive tract microflora if consumed in sufficient quantities (Antika, 2019).

Various sources show that the functional potential of sticky rice and cassava *Tape* is based on the fermented compounds, as shown in Table 1.

In addition to fermented compounds, microbes that play a role in fermentation also have health functions in the presence of lactic acid bacteria and mould. In addition to maintaining the proper balance of the microflora in the digestive system, lactic acid bacteria also improve health and act as an immunomodulator. (Azizah *et al.*, 2019; Rahmah, 2021). *Saccharomyces cerevisiae*, also used in *Tape*, is known to have benefits because it can synthesise one of the metabolites beneficial to the human body, such as folic acid, which is more easily absorbed by the body (Lazo - Vélez *et al.*, 2018). One of the moulds is *Candida sp.* also can stimulate functional activity in immune cells and encourage antioxidant activity, primarily related to yeast structural polysaccharides (Cempaka, 2021; Sujaya *et al.*, 2004). Sticky rice *Tape* and cassava *Tape* use vegetable ingredients as their main ingredients, such as glutinous rice and cassava. The microbes used in making *Tape* are the fungus *Aspergillus sp.,* yeast, strain *Saccharomyces cerevisiae*, and bacteria, strain *Acetobacter aceti*, which are grown spontaneously in media and used as *Tape* yeast. Although *Tape* yeast contains different microorganisms from one brand to another, the different brands of *Tape* yeast used can affect the taste characteristics of the *Tape* so that the level of consumer preference for *Tape* produced from different yeast brands will be different. Tape based on tubers fermented using yeast consisting of a mixture of bacteria, yeast and mould is preferred by consumers compared to *Tape*, which is fermented with yeast which only contains mould (Muhiddin, Ramlawati, Yanti, & Mun'im, 2019).

One of the critical points for halalness in sticky rice and cassava *Tape* is the use of microbial starters, both of microbial origin and growth media. In yeast media for the growth of starter

Table 1: Functional potential of sticky rice *Tape* and cassava *Tape*

Substance	Function	References
β-glucan	Play a role in the process of inhibition of cancer cell growth.	(Pramesti Griana & Sekar Kinasih, 2020, Sujaya *et al.*, 2004)
β-glucosidase	It helps in increasing the total flavonoid and phenolic content.	(Nuraida, 2015; Febricia *et al.*, 2020; Jiang *et al.*, 2020)

Tape, it is made from rice flour dough with other natural additives (Asri *et al.*, 2021). Based on the Minister of Religion Decree No. 1306 of 2021, sticky rice and cassava *Tape* are included in the halal positive list, meaning all critical contamination points of haram materials can be confirmed as halal. In addition, sticky rice *Tape* and cassava *Tape* microbes can come from wrapping leaves, such as banana leaves and teak leaves (Aýun *et al.*, 2022). *Tape*, based on Indonesian MUI FATWA No. 4 of 2003, is not considered haram as long as they do not contain toxic ingredients. The ethanol content in the *Tape* that had been left for ferment up to five days increased up to 9,2% (Zainuddin *et al.*, 2022), while ethanol that is regarded as toxic has content higher than 15% solution and can be handled for industrial and medicine, but not for drinking purpose (Alzeer & Abou Hadeed, 2016)

3.2.2 *Dadih*

Dadih is a fermented local milk-based food from Minangkabau Sumatra (Sukmarini *et al.*, 2014) that is also included in the positive halal list based on the Decree of the Minister of Religion No. 1306 of 2021. Minangkabau is one of the famous ethnic groups in Indonesia (Arnold *et al.*, 2021). In Minangkabau, customary practice is based on *sharia*, and *sharia* is based on the *Qur'an* (*Adat bersandi Syarak, Syarak Bersandi Kitabullah*) (Siregar *et al.*, 2022). As their local fermented food for consumption, *Dadih* should have halal-clear status.

The milk used in *Dadih* fermentation is buffalo milk (Juniawati, Sri Usmiati, 2013). *Dadih* is obtained by simply fermenting buffalo milk in a bamboo tube, with the microbial inoculant used from nature, and fermented cassava *Tape* play a role in fermentation are dominated by the lactic acid bacteria strain *Lactobacillus plantarum*, which naturally occurs in bamboo segments (Usmiati *et al.*, 2013). In the fermented food market, *Dadih* is still far behind in sales of other fermented kinds of milk, such as yoghurt and kefir. However, regarding functional properties, the *Dadih* is not inferior to other fermented kinds of milk (Chalid & Hartiningsih, 2013). Several studies show that *Dadih* has many functional properties derived from microbial activity and active components resulting from fermentation.

Lactic acid bacteria (LAB) contained in the *Dadih* can potentially be hypocholesterolemic (Azizah & Usman, 2018); besides that, LAB in the *Dadih* is also able to improve the body's immune system (Griana & Kinasih, 2020). Furthermore, fermented *Dadih* compounds also have the potential as antioxidants and antibacterials (Chalid & Hartiningsih, 2013). The antioxidant properties result from breaking down buffalo milk proteins by enzymes produced by microbes when buffalo milk is fermented. In addition, *Dadih* can potentially prevent cancer, in this case, colon cancer, because of its antimutagenic properties, which can reduce and inhibit food-induced mutagenesis. This antimutagenic effect occurs due to a carcinogen with peptidoglycan found in the BAL cell walls in *Dadih* (Usmiati & Risfaheri, 2013). In addition, this fermentation process has a positive impact on the body because it can act as a supplier of good bacteria that function positively for the digestive tract and can also increase the number of microminerals in milk, such as calcium, magnesium, and phosphorus; and able to increase micronutrients such as folic acid, vitamin B12, and biotin.

In the process of making *Dadih*, buffalo milk was added into a glass or tube made of bamboo and covered with banana leaves tied with bamboo rope or rope made of other materials. The microbes that play a role in the processing are originated from buffalo milk or banana leaves used as cover for bamboo tubes (Sunaryanto & Marwoto, 2012). Other regions in Indonesia have also developed natural fermentation of *Dadih*, such as Bali, where the bamboo used for *Dadih* fermentation uses local Balinese *petung* bamboo (Sugitha & Puspawati, 2018). The critical points for the halalness of the *Dadih* are the milk ingredients used and the medium for making the *Dadih*. Buffaloes are animals that are halal both for their meat and milk. In contrast, the media for making *Dadih,* as well as the growth medium for microorganisms for fermenting buffalo milk, comes from nature, such as bamboo and banana leaves, are free of non-permissible substances.

Reflecting on the critical points of microbial products, one of which is growth media, it is inevitable that the microbial growth media used in making *Dadih* all come from nature, such as bamboo and banana leaves. Including *Dadih* products in the positive halal list further strengthens the public to consume them without worrying about their halal status. Apart from that, the *Dadih* also comes from fresh buffalo milk without the addition of any additional ingredients, so it can be ascertained that it is halal, according to the Decree of the Minister of Religion of the Republic of Indonesia number 1360 of 2021, which states that fresh milk is one of the ingredients that is exempt from the obligation to certify halal.

3.2.3 *Tempeh*

Tempeh is a fermented product made from legumes (soybeans) generally consumed by Indonesians (Dinar, 2013). *Tempeh* is an indigenous Indonesian fermented food originating from Java, formerly used as a food used in cultural events and traditional ceremonies (Romulo & Surya, 2021); where *Tempeh* originated from the introduction of soybeans, Chinese traders brought 1000 AD. to the island of Java(Shurtleff & Aoyagi, 2007). The fermentation process in the manufacture of *Tempeh* involves soybeans as the primary ingredient and the fungus Rhizopus sp, with the dominant fungi being *Rhizopus oligosporus* and *Rhizopus oryzae*. *Tempeh* inoculum can be obtained commercially from the fungus Rhizopus sp and rice flour (Surya & Rahayu, 2012). However, it can also be obtained naturally from the leaves of *Tempeh* wrappers, both banana and teak leaves (Aýun *et al.*, 2022). The ingredients used in making *Tempeh* can vary from soybeans and other grains, such as *benguk* beans, kara beans, red beans, *komak* beans, and green beans (Jayanti, 2019). The ingredients used in making *Tempeh* are vegetable ingredients obtained from nature. *Tempeh* undergoes a process of soaking, removing the

epidermis and steaming before finally being fermented with microbes from artificial yeast and natural ingredients.

The process that occurs in the manufacture of *Tempeh* includes the production of protease, lipase and amylase enzymes due to the growth of mould (fungi). The presence of this enzyme will play a role in breaking down proteins, ats, and complex carbohydrates into simpler compounds (Su *et al.*, 2021). *Tempeh* is locally fermented with functional potential due to bioactive components and microbes which, based on research, positively impact health, as shown in Table 2. The growth of mould (fungi). The presence of this enzyme will play a role in breaking down proteins, ats, and complex carbohydrates into simpler compounds (Su *et al.*, 2021). *Tempeh* is locally fermented with functional potential due to bioactive components and microbes which, based on research, positively impact health, as shown in Table 2.

Table 2: Bioactive components of *Tempeh*

Substance	Function	References
Vitamin B12	Being a coenzyme for metabolic processes, playing a role in the process of blood formation and improving nerve function	(Pinasti *et al.*, 2020; Redi Aryanta, 2020; Sine & Soetarto, 2018; Yarlina & Astuti, 2021; Yongsmith *et al.*, 2016)
Folic Acid, Iron	Play a role in the function of blood formation and prevent anaemia.	(Pinasti *et al.*, 2020; Yarlina & Astuti, 2021)
Isoflavones	Assists in the process of inhibiting cancer cell proliferation, acts as an antioxidant, anti-osteoporosis and helps lower cholesterol levels	(Devi *et al.*, 2021; Krisnawati, 2017; Maryam, 2015; Shetty, 2007; Siti *et al.*, 2008; Surya & Rahayu, 2012)

The critical point in making *Tempeh* is in the microbial growth medium, where the microbes used come from rice flour and are fermented by nature to obtain the expected *Tempeh* bacterial culture. The critical point of processing *Tempeh* is washing and removing the epidermis because it can be contaminated with unholy water. *Tempeh* is included in the positive list according to the Decree of the Minister of Religion of the Republic of Indonesia no 1360 of 2021 because the fermentation material did not need any helpers, additives or other ingredients. *Tempeh*'s halal status can be a unique selling point that opens opportunities for *Tempeh* marketing on a global scale because apart from being halal, *Tempeh* is also known as a superfood that has many benefits. Apart from that, *Tempeh* has also been recognised as one of the intangible cultural heritages from Indonesia and sought recognition by UNESCO, so *Tempeh*'s halal status is needed for the *Tempeh* trade on a larger scale. Natural ethanol produced by natural fermentation under aerobic conditions is halal by nature (Alzeer & Abou Hadeed, 2016). Therefore, there is no doubt about the halal status of sticky rice and cassava *Tape*: However, the ethanol content was more than 1% (Ibrahim *et al.*, 2022; Siebenhandl *et al.*, 2001). Moreover, the materials used are obtained from vegetable sources, which are fermented directly without additional ingredients (Ray & Sivakumar, 2009).

All of these fermented foods are, essentially, the common foods that are found and readily available in the market in Indonesia. Based on the ease of the fermentation process and affordability of the community, these fermented foods become popular among Indonesian Muslims. Knowledge about their functional value and halal status is needed. However, it should be noted that if the functional food undergoes further processing into other foods, such as *Tempeh* chips, *Tempeh* brownies, *Tempeh* ice cream, *Tempeh* nuggets and other food derivatives, the functional food is no longer included in the positive list. It is because the product has undergone additional physical processing and the addition of other ingredients. In addition to the processing, if the packaging and serving of functional food are no longer the same as the original processing, for example, using a ceramic-based serving utensil that has the potential to contain non-halal material contamination, then the functional food needs to be reviewed regarding its halal status (out of the positive list).

4. Conclusion

This study aims to review the functional food in Indonesia that has a clear halal status. Indonesia has shown that local fermented food in Indonesia has the potential as a functional food. Apart from having the potential as functional food, some of the fermented foods described can also be ascertained for their halal status because they are included in the list of foods that are exempt from the obligation of halal certification, meaning that the food in question does not contain critical points of haram contamination. Furthermore, technology is needed to optimise the benefits of local fermented food. Besides that, it needs the Government's support in improving production technology to improve product quality and compete with other types of fermented food.

References

Abbas, A. (2020). Potensi Pangan Fungsional Dan Perannya Dalam Meningkatkan Kesehatan Manusia Yang Semakin Rentan—Mini Review. Teknosains: Media Informasi Sains Dan Teknologi, 14(2), 176–186. https://doi.org/10.24252/teknosains.v14i2.14319

Ahmad, A. N., Rahman, A. A., & Rahman, S. A. (2015). Assessing Knowledge and Religiosity on Consumer Behavior towards Halal Food and Cosmetic Products. International Journal of Social Science and Humanity, 5(1), 10–14. https://doi.org/10.7763/IJSSH.2015.V5.413

Alexander, D. K. (2018). Spotlighting Europe's Muslim consumers. Euromonitor. https://www.euromonitor.com/article/spotlighting-europes-muslim-consumers

Alonso, S. (2016). Novel Preservation Techniques for Microbial Cultures. In Food Engineering Series (pp. 7–33). Springer. https://doi.org/10.1007/978-3-319-42457-6_2

Alzeer, J., & Abou Hadeed, K. (2016). Ethanol and its Halal status in food industries. In Trends in Food Science and Technology (Vol. 58, pp. 14–20). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2016.10.018

Amaliah, I., David, W., & Ardiansyah, A. (2019). Perception of Millennial Generation Toward Functional Food in Indonesia. Journal of Functional Food and Nutraceutical, 1(1), 31–40. https://doi.org/10.33555/jffn.v1i1.11

Antika, Y. E. (2019). Isolasi dan Identifikasi Bakteri Asam Laktat yang Berpotensi sebagai Probiotik pada Acar.

Arnold, M., Rajagukguk, Y. V., Gramza-Michałowska, A., Kandylis, P., Solieri, L., Garde-Cerdan, T., Bartkiene, E., & Rocculi, P. (2021). Characterisation of *Dadih*: Traditional Fermented Buffalo Milk of Minangkabau. Beverages 2021, Vol. 7, Page 60, 7(3), 60. https://doi.org/10.3390/BEVERAGES7030060

Asri, A., Minu, I. W., Riska, R., & Rahmat, R. (2021). Fermentasi Tape dan Minas dalam Perspektif Hukum Islam. Bustanul Fuqaha: Jurnal Bidang Hukum Islam, 2(2), 232–250. https://doi.org/10.36701/BUSTANUL.V2I2.378

Aýun, Q., Suryani, S., & Kurnia, C. (2022). Identifikasi Kapang pada Tempe Bungkus Daun Pisang dan Plastik Asal Pengrajin Tempe Jatiasih, Bekasi. Bioed: Jurnal Pendidikan Biologi, 10(2), 45–51. https://doi.org/10.25157/JPB.V10I2.8685

Azizah, N., Astuti, M. K., & Yudhabuntara, D. (2012). Resistensi Isolat Lokal Escherichia Coil Pembawa Gena Vt1 Dan Vt2 Asal Babi dan Domba/Kambing Terhadap 6 Antibiotik = Resistance of Local Isolates of Vt1 And Vt2 Genes-Bearing Escherichia Coil From Sheep/Goat and Swine. Jurnal Sain Veteriner, 20(2). https://doi.org/10.22146/JSV.308

Azizah, N., Suradi, K., & Gumilar, J. (2019). Pengaruh Konsentrasi Bakteri Asam Laktat Lactobacillus Plantarum dan Lactobacillus Casei Terhadap Mutu Mikrobiologi dan Kimia Mayonnaise Probiotik. Jurnal Ilmu Ternak Universitas Padjadjaran, 18(2). https://doi.org/10.24198/JIT.V18I2.19771

Azizah, N., & Usman, K. S. (2018). Pengaruh Konsentrasi Bakteri Asam Laktat Lactobacillus Plantarum Dan Lactobacillus Casei Terhadap Mutu Mikrobiologi Dan Kimia Mayones Probiotik The Effect Of Concentration Lactic Acid Bacteria Lactobacillus Plantarum And Lactobacillus Casei On Microbiolog. 18(2), 17–23.

Bintsis, T., & Papademas, P. (2022). The Evolution of Fermented Milks, from Artisanal to Industrial Products: A Critical Review. Fermentation 2022, Vol. 8, Page 679, 8(12), 679. https://doi.org/10.3390/FERMENTATION8120679

Bublitz, M. G., Peracchio, L. A., Andreasen, A. R., Kees, J., Kidwell, B., Miller, E. G., Motley, C. M., Peter, P. C., Rajagopal, P., Scott, M. L., & Vallen, B. (2013). Promoting positive change: Advancing the food well-being paradigm. Journal of Business Research, 66(8), 1211–1218. https://doi.org/10.1016/J.JBUSRES.2012.08.014

Cempaka, L. (2021). Peuyeum: fermented cassava from Bandung, West Java, Indonesia. Journal of Ethnic Foods, 8(1). https://doi.org/10.1186/S42779-021-00079-3

Chalid, S. Y., & Hartiningsih, F. (2013). Potensi *Dadih* Susu Kerbau Fermentasi Sebagai Antioksidan dan Antibakteri. Prosiding Semirata FMIPA Universitas Lampung, 369–375.

Devi, A. F., Muzdalifah, D., Athaillah, Z. A., Lioe, H. N., & Artanti, N. (2021). Isoflavones and Bioactivities in Over-fermented *Tempeh* Extracts. Jurnal Kimia Sains Dan Aplikasi, 24(7), 244–251. https://doi.org/10.14710/jksa.24.7.244-251

Dinar, F. (2013). Manfaat Tempe Terhadap Kesehatan Tubuh. Pengabdian Kepada Masyarakat, 19, 1–10.

Djannatun, T., Rochani, J. T., Wikaningrum, R., & Widiyanti, D. (2008). Pemanfaatan darah manusia yang kadaluarsa sebagai pengganti darah domba dalam pembuatan media Agar Darah Plat (ADP) The use of expired human blood as substitution of the sheep blood in preparation of Blood Agar Media (BAM). Jurnal Kedokteran Yarsi, 16(2).

Estiati, A., & Herman, M. (2016). Regulasi Keamanan Hayati Produk Rekayasa Genetik di Indonesia. Analisis Kebijakan Pertanian, 13(2), 129. https://doi.org/10.21082/akp.v13n2.2015.129-146

Febricia, G. P., Nociantiri, K. A., & Pratiwi, I. D. P. K. (2020). Pengaruh lama fermentasi terhadap karakteristik minuman probiotik sari buah terong belanda (Solanum betaceum Cav) dengan Lactobacillus sp. F213. Jurnal Ilmu Dan Teknologi Pangan (ITEPA), 9(2), 170–180.

GIFR. (2015). The Global Halal Industry: An overview. Global Institute of Forensic Research, 140–158. http://www.gifr.net/gifr2013/ch_13.pdf (Retrieved on February 6, 2016)

Griana, T. P., & Kinasih, L. S. (2020). Potensi Makanan Fermentasi Khas Indonesia Sebagai Imunomodulator. Jurusan Biologi, Fakultas Sains Dan Teknologi, UIN Alauddin Makassar, September, 401–412. http://journal.uin-alauddin.ac.id/index.php/psb/

Gulitz, A., Stadie, J., Wenning, M., Ehrmann, M. A., & Vogel, R. F. (2011). The microbial diversity of water kefir. International Journal of Food Microbiology, 151(3), 284–288. https://doi.org/10.1016/j.ijfoodmicro.2011.09.016

Halalmui.org. (2021). LPPOM MUI | Lembaga Pengkajian Pangan Obat-obatan dan Kosmetika Majelis Ulama Indonesia. In Halalmui.org. https://www.halalmui.org/mui14/main/detail/memahami-fatwa-mui-tentang-kadar-etanol-pada-produk-makanan-dan-minuman

Hasanah, U., Ratihwulan, H., & Nuraida, L. (2019). Sensory Profiles and Lactic Acid Bacteria Density of Tape Ketan and Tape Singkong in Bogor. AgriTECH, 38(3), 265. https://doi.org/10.22146/AGRITECH.30935

Ibrahim, Jalaluddin, Muhammad, R., Akhtar, R., & Muhammad, Z. (2022). Alcohol Content in the Fermentation Process of Cassava Tape utilising Garlic Extract as a Yeast Supplement. Research Journal of Biotechnology, 17(4), 20–23. https://doi.org/10.25303/1704rjbt20023

Jayanti, E. T. (2019). Kandungan Protein Biji dan Tempe Berbahan Dasar Kacang-Kacangan Lokal (Fabaceae) Non Kedelai (Seeds and *Tempeh* Protein Content From Non Soybean Fabaceae). Bioscientist: Jurnal Ilmiah Biologi, 7(1), 79–86. https://doi.org/10.33394/BJIB.V7I1.2454

Jiang, L. L., Gong, X., Ji, M. Y., Wang, C. C., Wang, J. H., & Li, M. H. (2020). Bioactive compounds from plant-based functional foods: A promising choice for the prevention and management of hyperuricemia. Foods, 9(8). https://doi.org/10.3390/foods9080973

Juniawati, Sri Usmiati, dan E. D. (2013). Pengembangan Keju Lemak Rendah sebagai Pangan Fungsional Development of

Low Fat Cheese as Functional Food. J. Litbang Pert, 34(1), 31–40.

Karahalil, E. (2020). Principles of halal-compliant fermentations: Microbial alternatives for the halal food industry. In Trends in Food Science and Technology (Vol. 98, pp. 1–9). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.01.031

Khoerunisa, T. K. (2020). Review: Pengembangan Produk Pangan Fungsional Di Indonesia Berbasis Bahan Pangan Lokal Unggulan. Indonesian Journal of Agricultural and Food Research, 2(1), 49–59.

Komisi Majelis Ulama Indonesia. (2010). Fatwa Majelis Ulama Indonesia Nomor 01 th 2010.

Krisnawati, A. (2017). Soybean as Source of Functional Food. Iptek Tanaman Pangan, 12(1), 57–65.

Kurniadi, M., & Frediansyah, A. (2017). Halal Perspective of Microbial Bioprocess Based-Food Products. Reaktor, 16(3), 147–160. https://doi.org/10.14710/REAKTOR.16.3.147-160

Kusuma, G. P. A. W., Nocianitri, K. A., & Pratiwi, I. D. P. K. (2020). Pengaruh Lama Fermentasi Terhadap Karakteristik Fermented Rice Drink Sebagai Minuman Probiotik Dengan Isolat Lactobacillus sp. F213. Jurnal Ilmu Dan Teknologi Pangan (ITEPA), 9(2), 181. https://doi.org/10.24843/itepa.2020.v09.i02.p08

Law, S. V, Bakar, A., Hashim, M., & Hamid, A. (2011). MiniReview Popular fermented foods and beverages in Southeast Asia. In International Food Research Journal (Vol. 18).

Lazo - Vélez, M. A., Serna - Saldívar, S. O., Rosales - Medina, M. F., Tinoco - Alvear, M., & Briones - García, M. (2018). Application of Saccharomyces cerevisiae var. boulardii in food processing: a review. In Journal of Applied Microbiology (Vol. 125, Issue 4). https://doi.org/10.1111/jam.14037

Liofilchem. (2017). Brain Heart Infusion Broth-Instructions for Use (Issue April). www.liofilchem.net

Lopes, M., Gomes, A. S., Silva, C. M., & Belo, I. (2018). Microbial lipids and added value metabolites production by Yarrowia lipolytica from pork lard. Journal of Biotechnology, 265, 76–85. https://doi.org/10.1016/J.JBIOTEC.2017.11.007

Maryam, S. (2015). Potensi Tempe Kacang Hijau (Vigna Radiata L) Hasil Fermentasi Menggunakan Inokulum Tradisional Sebagai Pangan Fungsional. JST (Jurnal Sains Dan Teknologi), 4(2), 635–641. https://doi.org/10.23887/jst-undiksha.v4i2.6055

Muhiddin, N. H., Ramlawati, R., Yanti, N. A., & Mun'im, A. (2019). Analisis Kuantitatif Mikroorganisme pada Ragi Tape Lokal dan Daya Terima Tape Jusinta yang dihasilkan. BioWallacea : Jurnal Penelitian Biologi (Journal of Biological Research), 6(2). https://doi.org/10.33772/biowallacea.v6i2.8950

Nuraida, L. (2015). A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. In Food Science and Human Wellness (Vol. 4, Issue 2, pp. 47–55). Elsevier. https://doi.org/10.1016/j.fshw.2015.06.001

Nurhidayanti, N. (2019). Pemanfaatan Darah Sisa Transfusi Dalam Pembuatan Media Bap Untuk Pertumbuhan Bakteri Streptococcus pyogenes. Indobiosains, 1(2), 63. https://doi.org/10.31851/indobiosains.v1i2.3189

Patel, A., & Shah, N. (2017). Fermented foods: An overview. Microorganisms in Sustainable Agriculture, Food, and the Environment, August, 3–65. https://doi.org/10.1201/9781315365824

Pinasti, L., Nugraheni, Z., & Wiboworini, B. (2020). Potensi tempe sebagai pangan fungsional dalam meningkatkan kadar hemoglobin remaja penderita anemia. AcTion: Aceh Nutrition Journal, 5(1), 19. https://doi.org/10.30867/action.v5i1.192

Purba, D. A., Gelgel, K. T. P., & Suarjana, I. G. K. (2022). Uji Kepekaan Streptococcus spp. yang Diisolasi dari Penyakit Saluran Pernapasan Kompleks Babi terhadap Kanamycin, Streptomycin dan Doxycycline. Buletin Veteriner Udayana, 158, 202. https://doi.org/10.24843/bulvet.2022.v14.i03.p02

Purukan, C., Siampa, J. P., & Tallei, T. E. (2020). Enkapsulasi Bakteri Asam Laktat Hasil Fermentasi Buah Salak (Salacca zalacca) Lokal Menggunakan Aginat dengan Pewarna Kembang Sepatu (Hibiscus rosa-sinensis L.). Jurnal Bios Logos, 10(2), 93. https://doi.org/10.35799/jbl.10.2.2020.29045

Qaradawi, Y. (1994). The Lawful and The Prohibited In-Islam. USA: American Trust Publication.

Rahayu, E. S., Nursiwi, A., N, B. S., & Supriyanto, S. (2018). Development of the Traditional Tape Ketan Into Probiotic Drink. Indonesian Food and Nutrition Progress, 15(1), 11. https://doi.org/10.22146/IFNP.33387

Rahmah, W. (2021). Karakterisasi Bakteri Asam Laktat (Bal) dari Fermentasi Tape Singkong. Jurnal Penelitian Farmasi Indonesia, 10(1), 1–5. https://doi.org/10.51887/jpfi.v10i1.1166

Ray, R. C., & Sivakumar, P. S. (2009). Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. International Journal of Food Science & Technology, 44(6), 1073–1087. https://doi.org/10.1111/J.1365-2621.2009.01933.X

Redi Aryanta, I. wayan. (2020). Manfaat Tempe Untuk Kesehatan. Widya Kesehatan, 2(1), 44–50. https://doi.org/10.32795/widyakesehatan.v2i1.609

Romulo, A., & Surya, R. (2021). Tempe: A traditional fermented food of Indonesia and its health benefits. International Journal of Gastronomy and Food Science, 26(December 2021), 100413. https://doi.org/10.1016/j.ijgfs.2021.100413

Shetty, Kalidas. (2007). Functional foods and biotechnology. CRC/Taylor & Francis.

Shurtleff, W., & Aoyagi, A. (2007). History of *Tempeh* A Special Report on The History of Traditional Fermented Soyfoods.

Siebenhandl, S., Lestario, L. N., Trimmel, D., & Berghofer, E. (2001). Studies on *Tape* ketan - An Indonesian fermented rice food. International Journal of Food Sciences and Nutrition, 52(4), 347–357. https://doi.org/10.1080/09637480120057585

Sine, Y., & Soetarto, E. S. (2018). Perubahan Kadar Vitamin Dan Mineral Pada Fermentasi Tempe Gude (Cajanus cajan L.). Jurnal Saintek Lahan Kering, 1(1), 1–3. https://doi.org/10.32938/slk.v1i1.414

Siregar, F. A., Yulika, F., Nofialdi, Harahap, I., Ridwan, B., & Syahputra, I. (2022). Merantau in the Ethnic Tradition of Minangkabau: Local Custom without Sharia Basis? Samarah, 6(1), 115–138. https://doi.org/10.22373/sjhk.v6i1.9954

Siti, H., Bintari, A., Dyah, P., Eka, V., & Citra, R. (2008). Efek Inokulasi Bakteri Micrococcus luteus Terhadap Pertumbuhan Jamur Benang dan Kandungan Isoflavon pada Proses Pengolahan Tempe (Effect Inoculation of Micrococcus luteus to Growth of Mold and Content Isoflavone at Tempe Processing). 1, 1–8.

Soenarno, M. S., Polii, B. N., Febriantosa, A., & Hanifah, R. (2013). Identifikasi Peptida Bioaktif dari Olahan Susu Fermentasi Tradisional Indonesia Sebagai Bahan Pangan Fungsional untuk Kesehatan. Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan, 01(3), 191–195.

Su, H. K., Tsai, M. H., Chao, H. R., Wu, M. L., & Lu, J. H. (2021). Data on effect of *Tempeh* Fermentation on patients with type II diabetes. Data in Brief, 38, 107310. https://doi.org/10.1016/j.dib.2021.107310

Sugitha, I. M., & Puspawati, N. N. (2018). *Dadih* susu sapi yang dibuat dalam bambu petung bali kering. Media Ilmiah Teknologi Pangan, 5(2).

Sujaya, I. N., Antara, N. S., Sone, T., Tamura, Y., Aryanta, W. R., Yokota, A., Asano, K., & Tomita, F. (2004). Identification and characterisation of yeasts in brem, a traditional Balinese rice wine. World Journal of Microbiology and Biotechnology, 20(2), 143–150. https://doi.org/10.1023/B:WIBI.0000021727.69508.19

Sukmarini, L., Mustopa, A. Z., Normawati, M., & Muzdhalifah, I. (2014). Identification of Antibiotic-Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. HAYATI Journal of Biosciences, 21(3), 144–150. https://doi.org/10.4308/hjb.21.3.144

Sulaiman, A., Othman, N., Baharuddin, A. S., Mokhtar, M. N., & Tabatabaei, M. (2014). Enhancing the Halal Food Industry by Utilising Food Wastes to Produce Value-added Bioproducts. Procedia - Social and Behavioral Sciences, 121, 35–43. https://doi.org/10.1016/J.SBSPRO.2014.01.1106

Sulistiani, S., & Hidayat, I. (2020). Identifikasi molekuler Bakteri Asam Laktat dari Tempe dan Tape Berdasarkan Sekuen Gen 16S rRNA. Majalah Ilmiah Biologi Biosfera: A Scientific Journal, 37(2), 69–77. https://doi.org/10.20884/1.MIB.2020.37.2.1149

Sunaryanto, R., & Marwoto, B. (2012). Isolasi, Identifikasi, dan Karakterisasi Bakteri Asam Laktat dari *Dadih* Susu Kerbau. Jurnal Sains Dan Teknologi Indonesia, 14(3), 228–233. http://blast.ncbi.nlm.nih.gov/Blast.cgi

Surono, I. S. (2016). Ethnic fermented foods and beverages of Indonesia. Ethnic Fermented Foods and Alcoholic Beverages of Asia, 341–382. https://doi.org/10.1007/978-81-322-2800-4_14/COVER

Surya, R., & Rahayu, W. P. (2012). Production and characteristics of canned tempe extract. Asian Journal of Food and Agro-Industry, 2012(04), 299–306. www.ajofai.info

Susanti, E., Tirta, S., Paramitha, A., Lutfiana, N., & Malang, U. N. (2019). Seleksi Bakteri Proteolitik dari Pangan Fermentasi Lokal Indonesia sebagai Sumber Protease untuk Produksi. MSOpen Book Chapter, 78–92.

Suter, K. (2013). Pangan Fungsional dan Prospek Pengembangannya. Tekonologi Pangan, 1–17.

Tratnik, L., Božanić, R., Herceg, Z., & Drgalić, I. (2006). The quality of plain and supplemented kefir from goat's and cow's milk. International Journal of Dairy Technology, 59(1), 40–46. https://doi.org/10.1111/j.1471-0307.2006.00236.x

Usmiati, S., & Risfaheri. (2013). Pengembangan *Dadih* Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat. J. Litbang Pert., 32(1), 20–29. https://doi.org/10.21082/jp3.v32n1.2013.p20-29

Usmiati, S., Risfaheri, D., Teknologi, B. P., Kepulauan, P., & Belitung, B. (2013). Pengembangan *Dadih* Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat; Improvement of *Dadih* as an Indigenous Probiotic Functional Food of West Sumatra. J. Litbang Pert, 32(1), 421797.

Utami, R., & Djaafar, T. F. (2014). Keberagaman umbi-umbian sebagai pangan fungsional. 22, 950–960.

Yap, C. K., & Al-Mutairi, K. A. (2023). Effective Microorganisms as Halal-Based Sources for Biofertilizer Production and Some Socio-Economic Insights: A Review. Foods, 12(8). https://doi.org/10.3390/FOODS12081702

Yarlina, V. P., & Astuti, D. I. (2021). Karakterisasi kandungan vitamin B12, folat dan isoflavon tempe kedelai dengan isolat murni Rhizopus oryzae, Rhizopus oligosporus, dan Rhizopus stolonifer sebagai bahan pangan fungsional. Teknologi Pangan : Media Informasi Dan Komunikasi Ilmiah Teknologi Pertanian, 12(1), 92–102. https://doi.org/10.35891/tp.v12i1.2219

Ye, J. H., Huang, L. Y., Terefe, N. S., & Augustin, M. A. (2019). Fermentation-based biotransformation of glucosinolates, phenolics and sugars in retorted broccoli puree by lactic acid bacteria. Food Chemistry, 286, 616–623. https://doi.org/10.1016/J.Foodchem.2019.02.030

Yongsmith, B., Kitpreechavanich, V., Tangjitjaroenkun, J., & Krusong, W. (2016). Functional Properties of Traditional Foods. Functional Properties of Traditional Foods, 17–37. https://doi.org/10.1007/978-1-4899-7662-8

Zainuddin, Sari, M., & Fajar, N. (2022). Sharia and Alcohol: Tape Ketan as Typical Fare of Muslims in Batusangkar. Journal of Legal, Ethical and Regulatory Issues, 25. https://heinonline.org/HOL/Page?handle=hein.journals/jnlolletl25&id=351&div=&collection=

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Bibliometric Analysis of Research on Cosmetic Products: Halal Cosmetics as an Emerging Research Area

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Abstract

The cosmetics industry is one of the most lucrative and high-growing industries that are driven by science and innovation. However, there is a lack of studies that analyze scholarly articles on cosmetic products. This study attempts to bridge this gap by reviewing the existing literature, with the specific purpose to explore the trends in the publications and themes related to research on cosmetic products. To achieve this purpose, a bibliometric analysis was conducted using the R application and R-based tools. The research retrieved 613 articles on the Scopus database that used the term 'cosmetic products' in their titles. The study reveals that the number of articles published on the topic of cosmetic products is increasing. Two main keywords, paraben, and high-performance liquid chromatography, are the most recurring themes. Halal cosmetics were identified as an emerging research area. In relation to this context, six major topics were identified, which are: regulations for halal certification of halal cosmetics, organization control, attributes of halal cosmetics, consumer purchase, and purchase intention. Overall, this bibliometric analysis provides essential information on the current trends and themes, and provides recommendations for future research on cosmetic products.

1. Introduction

Cosmetic products can be considered a necessity as their various daily uses are ubiquitous among many people. The term 'cosmetic products' refers to products used externally for cleaning or washing the face or body parts to make the user more comfortable; specific items include cleanser, shampoo, soap, and perfume. The technical definition is that cosmetic products include any substance or preparation used for cleansing, perfuming, enhancing the body's appearance, or correcting body odour. The term also refers to products applied to the body's outer layer, such as the face and lips, to keep them moisturized (Standard Malaysia, 2019). Cosmetic products such as lipstick, eyeliner, foundation, and eye concealer are used to beautify the human face. Likewise, products such as body wash, shampoo, and perfume are used to clean or perfume human body parts. The worldwide cosmetics market was projected to be valued at over USD 503 billion in 2021, making it one of the most lucrative industries in the world (Statista Research Department, 2022). It is predicted that by 2024, the cosmetics industry in Malaysia will generate a revenue, estimated at USD451 million (Statista, 2023).

Some people might associate the word 'cosmetics' with women, as most makeup and skincare cosmetics users are female. However, the term does not imply that a cosmetic product is aimed solely at female consumers as such products are used by everyone, regardless of gender. Deodorant and soap, which are used by everyone, are examples of products that fall into the category of cosmetics. Despite growing demand for cosmetic products in the market, too few studies have reviewed the literature specifically related to cosmetic products. The study attempted to bridge this gap by using R software to conduct a bibliometric analysis of the literature on cosmetic products. The specific purpose of the study was to explore the trends in the publications and themes related to research on cosmetic products.

2. Dataset and methods

To achieve the purpose of the study, the Scopus database was searched for articles relevant to cosmetic products. This database is recognized as a central search system that enables researchers to find articles related to a chosen research topic (Gusenbauer & Haddaway, 2020). The Scopus database is a comprehensive collection of scholarly literature from various sources, such as journals, conference proceedings, and books. It contains peer-reviewed literature and publications worldwide from various academic disciplines, including sciences, social sciences, engineering, medicine, and others, making it suitable as a source for conducting bibliometric analysis (Elsevier, 2023). The Scopus database is also available in BibTeX format, making it a helpful resource for analysing collaboration patterns and other analyses in bibliometric

evaluation (Elsevier, 2023). In this study, a bibliometric study was conducted to review the most recent publications of cosmetic products on the Scopus database using the R and R-based tools. R is a programming language used for statistical computing and graphics. It offers a wide range of statistical and graphical techniques and is highly customizable. For this bibliometric analysis, the specific R-based tools that were utilised include RStudio, Bibliometrix R Package, and Biblioshiny. RStudio serves as an integrated development environment (IDE) for R and acts as a tool that runs on R, while Bibliometrix R package software is the specific open-source software used to conduct bibliometric research. In addition, this study used Biblioshiny, an application that offers a user-friendly web interface for the Bibliometrix R package. It is capable of producing high-quality graphical statistics on article publications derived from the Scopus database (Ahmi, 2022; Aria & Cuccurullo, 2017; R Core Team, 2020).

Figure 1 shows an overview of the step-by-step Scopus search for publications on cosmetic products conducted in this study, adapted from Zakaria *et al.* (2020). The study utilized only the Scopus database, and not other databases due to authors' accessibility to the Scopus database and the availability of the BibTeX format dataset in Scopus. The BibTeX format in Scopus is different from that of other databases such as WOS. Currently, the software package utilized for the study (R, RStudio, Bibliometrix R package and Biblioshiny) is only able to analyse a dataset of a single format. Hence, the study utilised only the Scopus database.

The search focused on cosmetic products and utilised variations of the words in the article title search, including 'cosmetic product,' 'cosmetic products,' and 'cosmetics products'. Using these keywords adhered to the objective of obtaining appropriate articles whose main focus was cosmetic products. Using the advanced search function in Scopus, the scope of the search was narrowed to include only articles published in journals, and those written in English and include years from 1955 to 2021. The dataset was derived in September 2022; hence, the researchers excluded publications from the years 2022 to 2023. This search generated 613 scientific articles, which have cosmetic products in the title. The information on the 613 articles was then exported to BibTeX format. The raw data (in BibTeX format) were then imported into the Biblioshiny application to conduct bibliometric analysis (Aria & Cuccurullo, 2017).

3. Analysis and results

3.1 Descriptive analysis

3.1.1 Main information

Table 1 shows the main information regarding the dataset obtained from Scopus. In total, the documents examined are 613. Sources in this study are exclusively from journals. As shown in the table, within the 1955 to 2021 timeline, the publication growth rate was 6.2% annually.

3.1.2 Annual publication trends

Figure 2 shows the trend in the annual publications on cosmetic products from 1955 to 2021. Overall, the Scopus database search produced 613 scientific articles. The highest productivity was in 2021, with a total of 53 documents, while the lowest was between 1955 and 1977 when only one publication was produced each year. More recently, between 2018 and 2020, the average number of annual articles increased, with 43 to 45 publications each year. The number dropped slightly in 2017 when 29 articles were published. Overall, the number of articles published is increasing.

Table 1: Main information of the documents

Description	Results
Main Information About Data	
Timespan	1955:2021
Sources (Journals)	293
Documents	613
Annual Growth Rate %	6.2
Document Average Age	13.1
Average citations per doc	17.99
References	15284
Document Contents	
Keywords Plus (ID)	4311
Author Keywords (DE)	1484
Authors	
Number of Authors	2081
Authors of single-authored docs	44
Authors Collaboration	
Single-authored docs	55
Co-Authors per Doc	4.15
International co-authorships %	12.72
Document Types	
Articles in Journals	**613**

3.1.3 Most productive authors

Table 2 shows the most productive authors of research on cosmetic products. They all published at least eight articles involving research on cosmetic products. Two authors, Gagliardi L. and Tonelli D., each published 19 articles. Referring to Table 2, the h-index values for both authors (11) were the joint-highest of all the authors. The two authors mentioned can be considered the most prominent in terms of researching cosmetic products. Chisvert A. and Salvador A. published 14 articles on topics related to cosmetic products, while Cavazzutti G. published 13 such articles. As can be seen, researchers from the Istituto Superiore Di Sanita dominated the field of cosmetic product research.

3.1.4 Most cited papers

Table 3 shows the ten most frequently cited papers on topics related to cosmetic products. Global citations refer to the number of citations of a paper, counted from all papers published in the Scopus database. Table 3 shows that the most-cited paper was published in the journal Regulatory Toxicology and Pharmacology, which is not included in the ten most

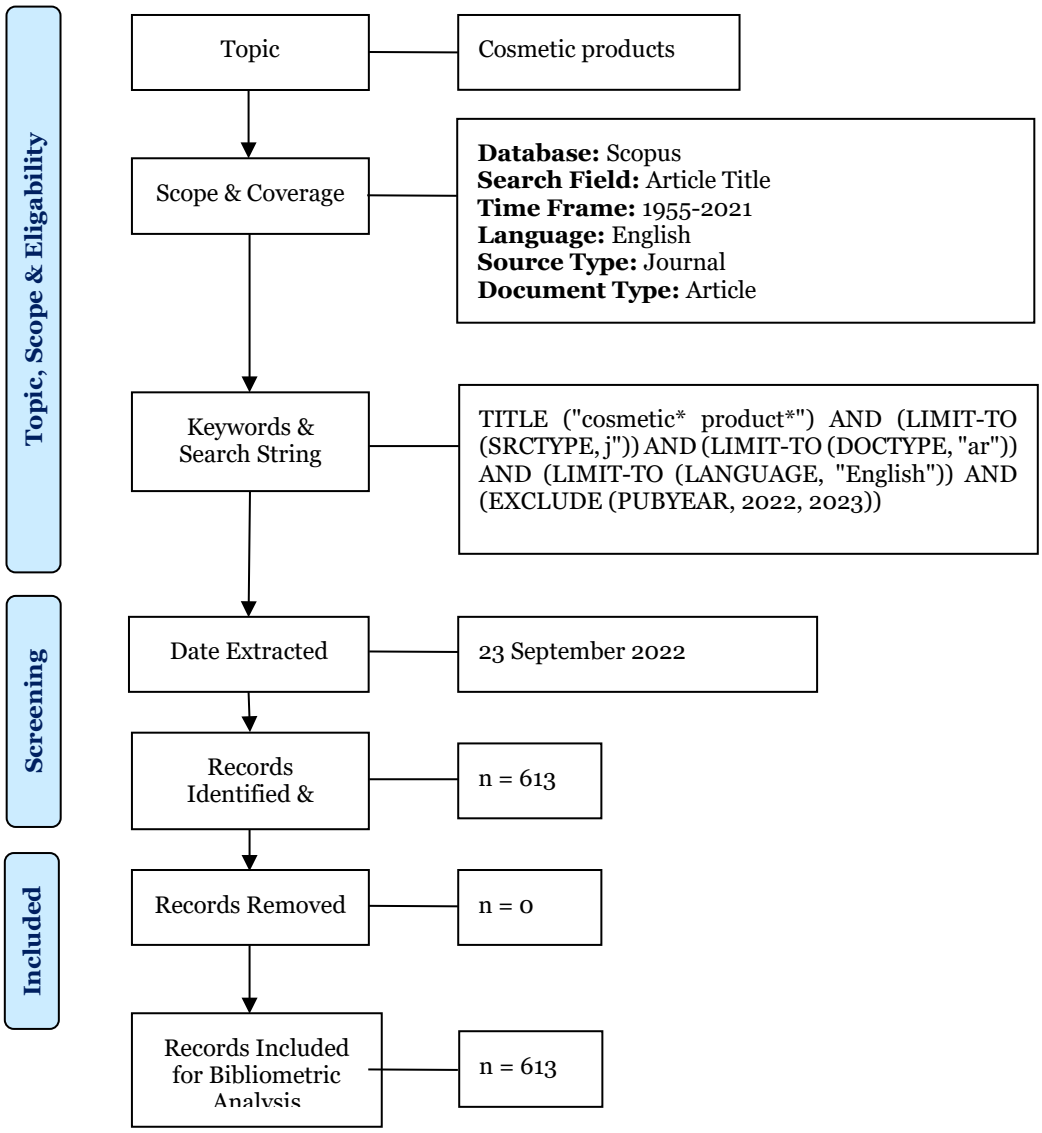

Figure 1: Step-by-step search of articles in Scopus for bibliometric analysis. Adapted from Zakaria *et al.* (2020).

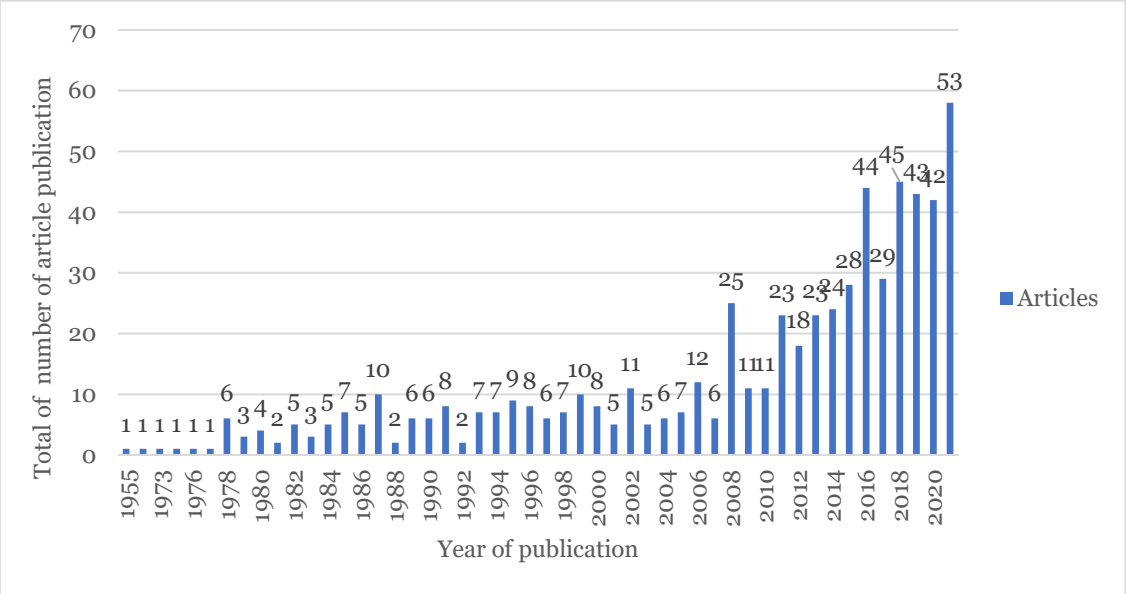

Figure 2: Trend in the annual publications on cosmetic products.

Table 2: Most productive authors of research on cosmetic products

Author	Number of Publications	Affiliation	Country	*h*-index	g-index	m-index	Total Citations	Publication Year*start
Gagliardi L.	19	Istituto Superiore Di Sanita	Italy	11	18	0.282	326	1984
Tonelli D.	19	Alma Mater Studiorum Università di Bologna	Italy	11	17	0.282	304	1984
Chisvert A.	14	Universitat de València	Spain	9	13	0.6	171	1984
Salvador A.	14	Universitat de València	Spain	9	13	0.6	171	2008
Cavazzutti G.	13	Istituto Superiore Di Sanita	Italy	8	13	0.205	204	2008
Ficheux A. S.	10	Université de Brest	France	7	10	0.778	172	1984
Roudot A. C.	10	Université de Brest	France	7	10	0.778	172	1995
Amato A.	9	Istituto Superiore Di Sanita	Italy	7	9	0.179	146	2014
De Orsi D.	9	Università degli Studi di Siena	Italy	7	9	0.25	149	2014
Rastogi S. C.	8	Nationalt center for miljø og energi	Denmark	7	8	0.2	357	1988

Table 3: List of top 10 cited cosmetic products articles in the Scopus database

No.	Author/Year	Journal	Title	Global Citations	Total Citation Per Year
1.	Safford *et al.* (2015)	Regulatory Toxicology and Pharmacology	Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic product	961	120.13
2.	Buschmann & Schollmeyer (2002)	Journal of Cosmetic Science	Applications of cyclodextrins in cosmetic product: A review	243	11.57
3.	Lopez-Galindo *et al.* (2007)	Applied Clay Science	Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic product	236	14.75
4.	Handjani-Vila *et al.* (1979)	International Journal of Cosmetic Science	Dispersions of lamellar phases of non-ionic lipids in cosmetic product	209	4.75
5.	Rastogi *et al.* (1995)	Contact Dermatitis	Contents of methyl-, ethyl-, propyl-, butyl- and benzylparaben in cosmetic product	174	6.21
6.	Shen *et al.* (2007)	Journal of Separation Science	Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods	145	9.06
7.	Wang *et al.* (2009)	Environment International	Low molecular weight cyclic volatile methylsiloxanes in cosmetic product sold in Canada: Implication for dermal exposure	144	10.29
8.	Loretz *et al.* (2005)	Food and Chemical Toxicology	Exposure data for cosmetic products: Lipstick, body lotion, and face cream	138	7.67
9.	Abd Rahman *et al.* (2015)	Journal of Islamic Marketing	Consumers and Halal cosmetic product: knowledge, religiosity, attitude and intention.	131	16.38
10.	Lei *et al.* (2017)	Regulatory Toxicology and Pharmacology	Microplastics releasing from personal care and cosmetic products in China.	120	20

productive journals. In the paper with the most citations (961), Safford *et al.* (2015) used a probabilistic model to evaluate the aggregate exposure of consumers to fragrance ingredients in all forms of personal care products. They discovered the Creme FIRM model risk assessment to be superior to the conservative method. In the paper with the second highest number of citations, 243, Buschmann and Schollmeyer (2002) gave an overview of the application of cyclodextrins in cosmetic products. They emphasized the advantages of using cyclodextrins in these products, such as the derivatives of cyclodextrins not being nutrient mediums for bacteria. Cyclodextrins can also reduce the number of preservatives used in a final product and they are an inert material. While the article by Safford *et al.* (2015) had the most citations per year, 120.13, Abd Rahman *et al.* (2015) produced the work with the second-highest number of annual citations, 16.38, despite the article itself being cited 131 times. This shows that the article gained the attention of other researchers.

3.1.5 Most productive countries

Figure 3 shows the most productive countries in terms of publishing articles on topics related to cosmetic products. The the most productive country was Italy, which produced 20% (123) articles. The articles from Italy were contributed by five of the ten most productive authors, such as Gagliardi L., Tonelli D., Cavazzutti G., Roudot A. C., and Amato A., all of whom belonged to the same institution. The second-most productive country was the USA, which produced 18% (112) articles about cosmetic products. This aligned with the fact that the USA was listed among the three leading cosmetic product manufacturers worldwide in 2020 (GlobalData, 2020). Third, 17% (102) of cosmetic product articles were published by authors in France. Other than that, authors in China published 15% (89) articles on cosmetic products, while authors in Malaysia published 8% (52) such articles.

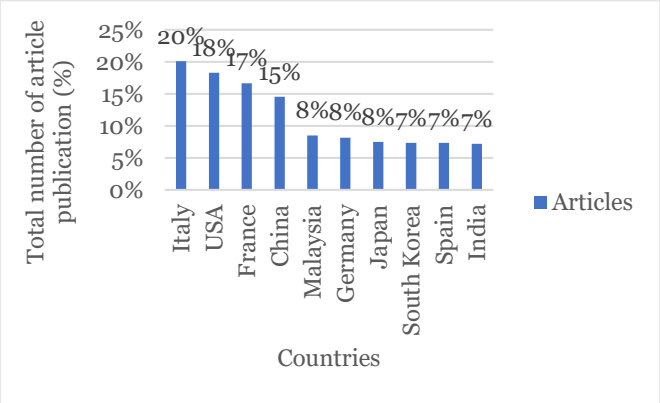

Figure 3: Most productive countries that publish articles on cosmetic products.

3.1.6 Most productive affiliations

Figure 4 shows the most productive institutions in terms of publishing articles on cosmetic products-related topics. Research on cosmetic products was usually conducted by institutions or research divisions commissioned by cosmetic manufacturers. The Istituto Superiore di Sanita and the Food and Drugs Administration each published 22 articles. In terms of their affiliation, the most productive authors, such as Gagliardi L., Cavazzutti G., and Amato A., were affiliated with the Istituto Superiore di Sanita (the Italian National Institute of Health). Alma Mater Studiorum Università di Bologna published 19 articles. The University of Valencia published 14 articles, while companies also published articles, with L'oreal S.A., for instance, publishing ten. The Faculté des Sciences et Techniques of the Université de Brest (UBO) published nine articles. Three institutions and companies published eight articles each, including the University of Brest (UBO), the University of Ferrara, Procter and Gamble, and Unilever.

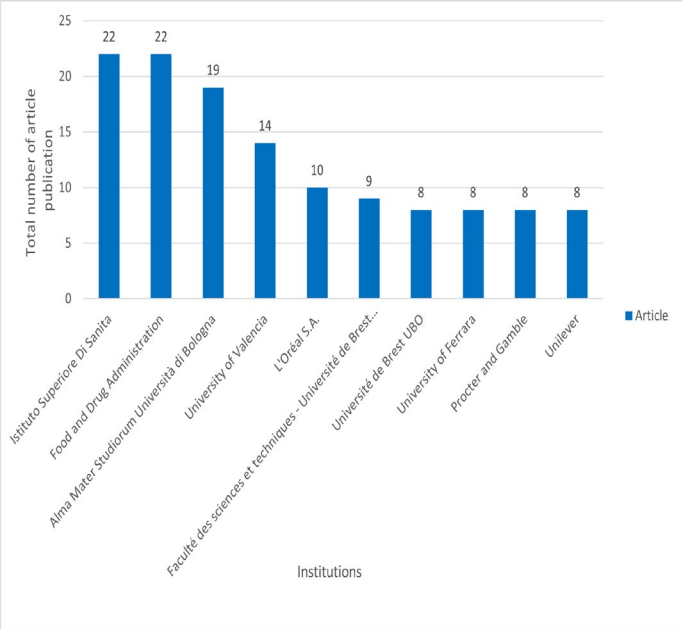

Figure 4: Most productive institutions that publish articles on cosmetic products topics.

3.1.7 Most frequent journals

Figure 5 shows the journals that published articles on cosmetic products-related topics most frequently. It shows that 37 articles were published in the International Journal of Cosmetic Science, while the Journal of Chromatography A had 35 articles. Contact Dermatitis published 26 articles, and Food and Chemical Toxicology published 18. Although the International Journal of Cosmetics Science published the most articles, the journal's cite score was 4.5, lower than that of Talanta, which was 10.6.

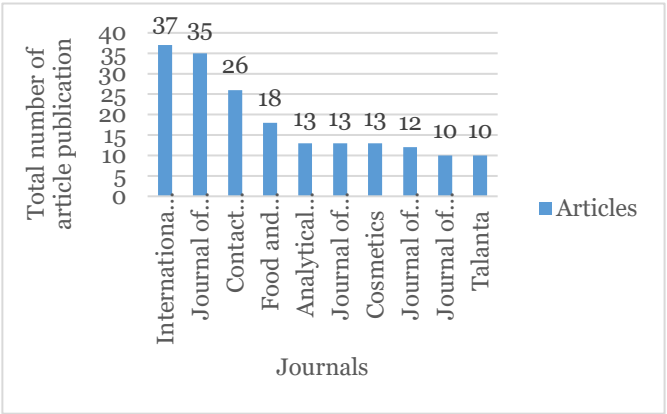

Figure 5: Most frequent journals of cosmetic products topics.

3.1.8 Most frequent keywords

Figure 6, a word cloud based on the authors' keywords, shows the most frequently used keywords in the articles on cosmetic products. There are two types of keywords, which are author

keywords and Keyword Plus. Author Keywords are listed by authors when their papers are published, and generally, they are more specific than Keyword Plus. Word cloud analysis shows that the most frequently used author keywords were 'cosmetics, preservatives, paraben, allergic contact dermatitis, HPLC (high-performance liquid chromatography), and liquid chromatography,' which are considered natural science terms. As the word cloud illustrates, some of the keywords commonly used in the social science articles included 'purchase intention, halal cosmetics, attitude, and consumption.'

Figure 6**:** Most frequent author keywords in cosmetic products articles.

3.2 Network analysis

3.2.1 Collaboration analysis

Figure 7 displays the patterns of inter-country collaboration on cosmetic products-related topics. This analysis of collaboration between countries is initiated based on author affiliation. The countries appeared in four main clusters, as the figure shows. The collaboration links between France and the USA, Italy, and Germany were quite strong, as can be seen through the publication of more articles from these countries than from other countries. In the dataset, an example of the collaboration of authors from different countries is the article written by Ribet, Nobile, and Rossi (2019). The level of thickness of the collaboration link for Germany, Portugal, and Sweden is fairly substantial compared to other collaboration links.

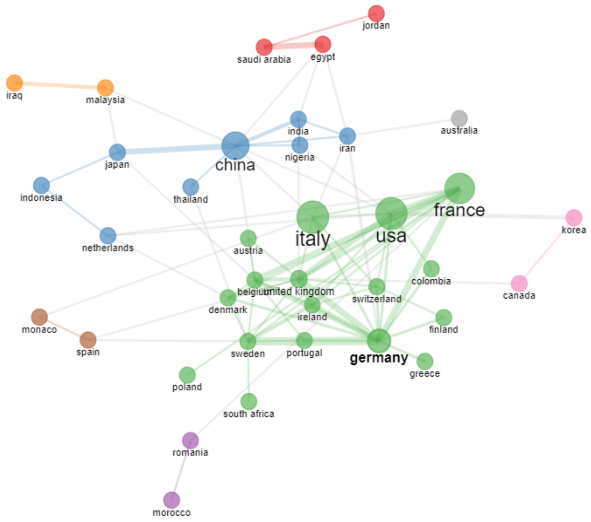

Figure 7: The collaboration analysis between countries.

3.2.2 Co-word analysis

Figure 8 shows the co-word analysis of the author keywords. A co-word analysis is a structure in which words clump to each other once they are understood to refer to the most critical and up-to-date issues; this is described as a research frontier. A co-word analysis of author keywords might help to identify topics that will potentially evolve in the future. For this study, the co-word analysis involved words that occurred together in the articles on cosmetic products (Aria & Cuccurullo, 2017; Callon *et al.*, 1991). The different colours represent different categories of keywords that were strongly linked to each other. For example, 'cosmetics' was mentioned with several keywords, including 'paraben, preservative, risk assessment, personal care products, and sunscreen agents.' The link between cosmetics and paraben is quite thick, so the frequency with which the keywords were mentioned together was quite high, compared to links between other keywords. The size of the circle of each keyword (for example, safety testing), as shown in Figure 8, indicates the frequency the keyword is used by authors.

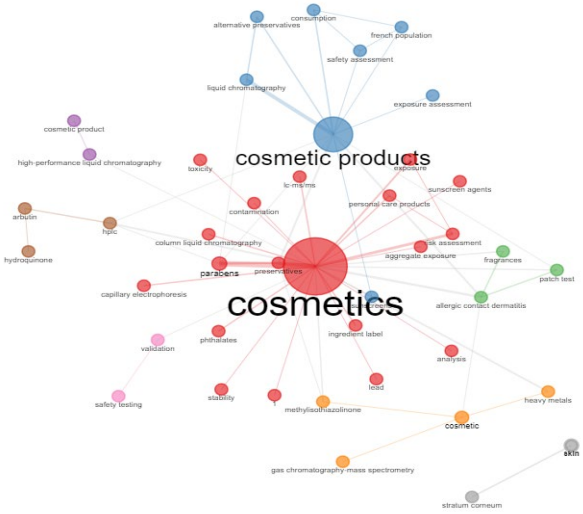

Figure 8: The co-word analysis of author keywords.

Figure 9 shows the trend in cosmetic products-related topics, based on the author's keywords. The topics leading the research trends included 'health risks, heavy metals, stability, cadmium, halal cosmetics, liquid chromatography, toxicity, and attitude.' These topics became popular among researchers in 2020. Topics such as hydroquinone were not prominent after 2018, while topics such as cosmetic and purchase intention were not prominent after 2019. The topics that became popular among researchers can be divided based on discussions from different perspectives: natural science and social science. The topics of halal cosmetics and attitude were examined from a social science perspective, while other topics came under natural science. Meanwhile, several topics were discussed between the 1980s and 2020. In the 1980s, stratum corneum and skin became cosmetic products-related trends in terms of author discussions, which lasted until 2013. In the 1990s, topics such as cosmetic products, high-performance liquid chromatography, and column liquid chromatography became widespread in discussions among researchers. In the 2000s, studies on parabens, preservatives, eye irritation, patch test, allergic contact dermatitis, and HPLC were some of the central topics of discussion among researchers. In the 2010s, popular topics included 'risk assessments, sunscreen, cosmetics, toxicity, halal

cosmetics, exposure, hydroquinone, liquid chromatography, purchase intention, attitude, personal care products, stability, health risk, heavy metals, and cadmium.' These observations show that especially in the social sciences, cosmetic products-related research on topics such as halal cosmetics, purchase intention, and attitude increased between 2014 and 2021. In the natural sciences, studies on cosmetic products have evolved from the 1980s until now, and they have become a reliable resource for future researchers.

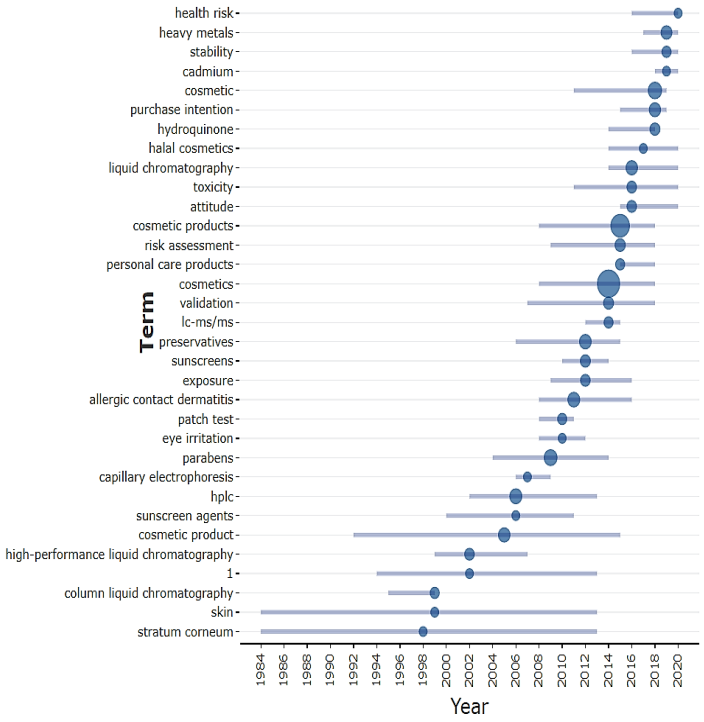

Figure 9: The trend topics of author keywords.

Figure 10 displays a thematic map of the articles on cosmetic products, based on the author's keywords. Thematic maps have four quadrants, with two different indicators that determine the character of each quadrant (Callon *et al.*, 1991; Della Corte *et al.*, 2019). In Figure 10, the coloured boxes contain the author's keywords; the red arrow shows the degree of relevance, and the blue arrow shows the degree of development. First, the motor theme can be considered to have high degrees of development and relevance, indicating that the keywords are significant and represent excellent development in research on cosmetic product topics. An example of a pink box is halal cosmetics, inside the upper-right quadrant of the thematic map. The term halal cosmetics is one of the most frequently mentioned by authors, but not the most frequent keyword. This relationship indicates that the degree of development of the halal cosmetics topic was lower than that of other terms (such as *HPLC*). The degree of development refers to a topic's level of maturity, while the degree of relevance refers to its level of significance to the body of knowledge. There is a high potential for halal cosmetics to be developed further as a body of knowledge in the future because the current development of halal cosmetics literature was found to be low.

Secondly, basic themes with a high degree of relevance are significant, indicating that the keywords are essential in research concerning cosmetic products. These keywords included 'paraben and high-performance liquid chromatography.' However, the development of these keywords for cosmetic products had not fully matured. Meanwhile, niche themes have a high degree of relevance and a low degree of development, suggesting that these keywords were relevant to cosmetic products but low on centrality, indicating that on the whole, keywords such as 'antioxidant activity, green purchase behaviour, and contamination' are not important in the cosmetic products field. Moreover, those keywords did not appear in the list of the most frequently used keywords. Lastly, emerging or declining themes, which have low degrees of relevance and development, refer to keywords that were not well developed in articles on cosmetic products or used to be relevant to the topic but might be starting to decline because authors began to focus on other keywords. One example, 'purchase intention,' is mentioned in the thematic map. Research on purchase intention may be declining because authors are focusing more on consumers' actual behaviour.

3.2.3 Three-field plots

A three-field plot shows relationships between countries, journals, and author keywords. Figure 11 shows the three-field plot between the authors, countries, and author keywords for this study. Authors from the most productive countries, including the USA, Italy, China, South Korea, France, Germany, the United Kingdom, Spain, Iran, and Japan, published articles in the International Journal of Cosmetics Science. This also indicates that the authors from these countries used keywords such as cosmetics or cosmetic products in the articles published in this journal. The link between France and the International Journal of Cosmetic Science is fairly thick, possibly indicating that more articles from France were published in this journal. The sizes of the boxes placed after the countries may indicate the number of article publications. Compared to India, the size of the box for Malaysia is quite small, possibly because the country published fewer articles in the journals shown in Figure 11. The single link to the Cosmetics journal indicated that Malaysia published an article only in that journal but in none of the others mentioned in Figure 11.

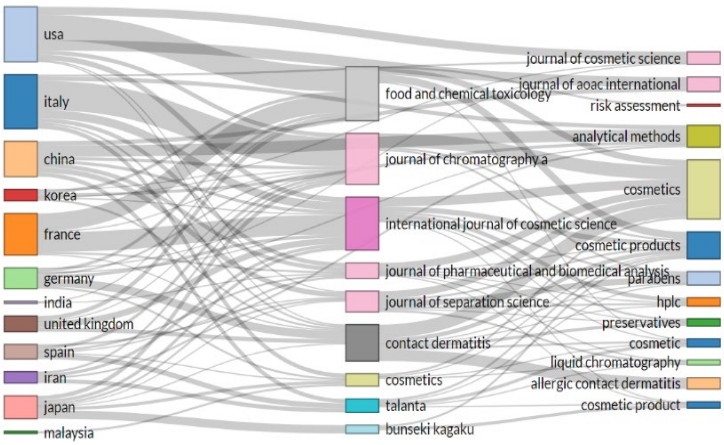

Figure 11: The three-field plots between countries, journals, and author keywords.

3.2.4 Historiography

Figure 12 shows the historiography of the articles on cosmetic products. A histography shows the direct historical citations of a paper. Seven coloured clusters of direct historical citations are illustrated, based on biblioshiny. Starting with the blue cluster, Wang *et al.* (2006) were cited by Miralles P. *et al.* (2018) and Miralles P. *et al.* (2019). Both cited this paper because Wang *et al.* (2006) used the liquid chromatography

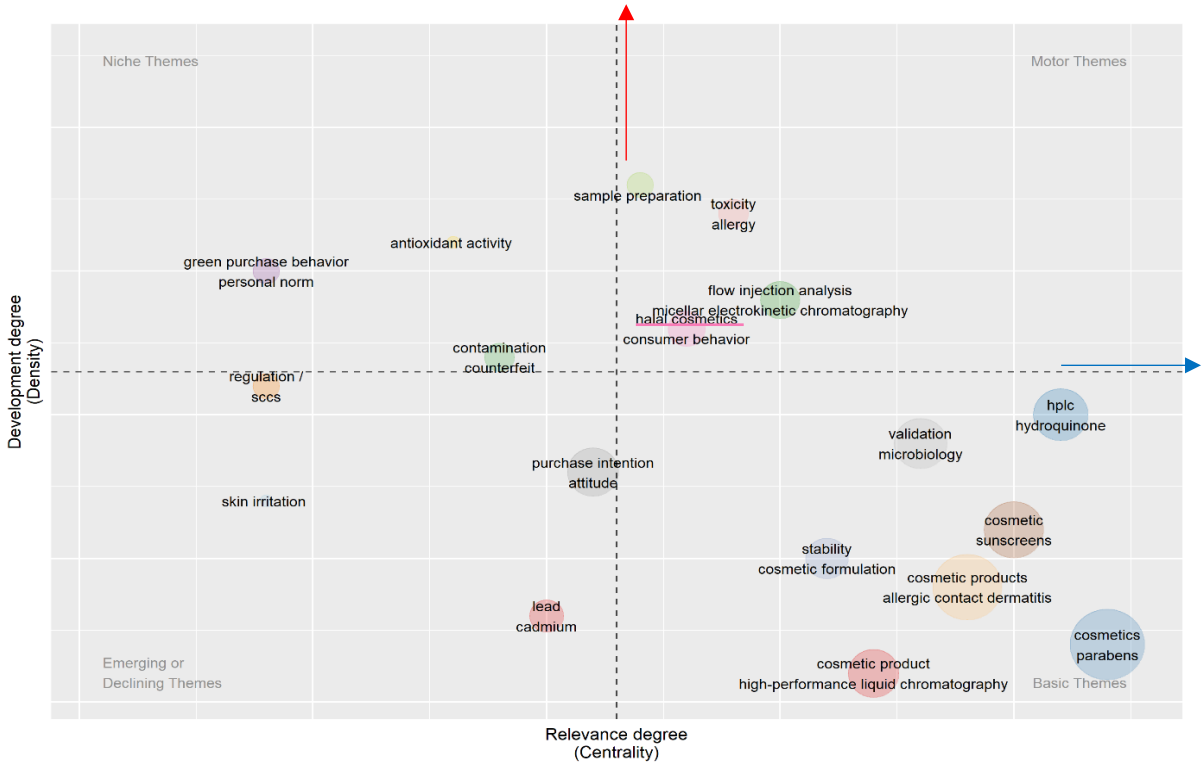

Figure 10: Thematic maps of cosmetic product articles.

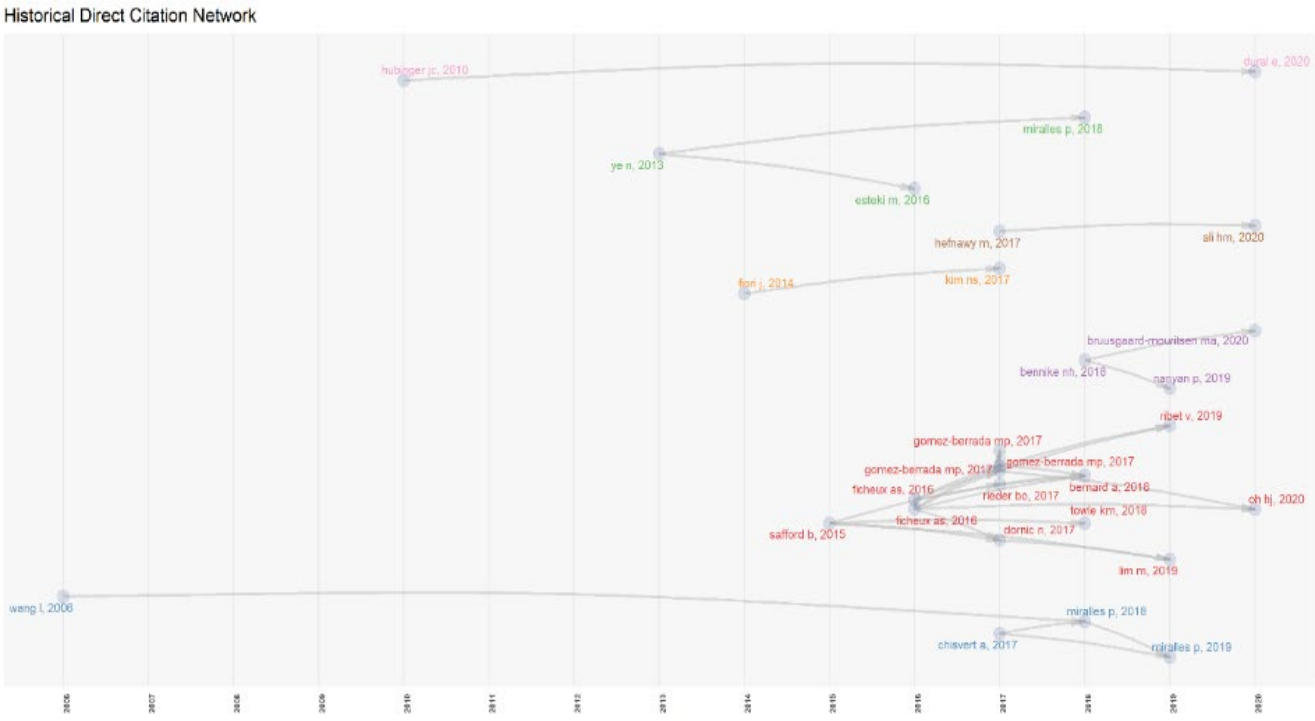

Figure 12: The historiography of cosmetic products articles.

the technique when examining cosmetic products. For the red cluster, Safford *et al.* (2015) are cited in an article that used the former work as a main reference when examining consumer exposure to cosmetic products. For the green cluster, Ye *et al.* (2013) developed a method to extract paraben from cosmetic products. For the brown cluster, Hefnawy *et al.* (2017) developed a liquid chromatography technique to extract preservatives such as salicylic acid and parabens. For the pink cluster, Hubinger (2010) surveyed cosmetic products with five phthalate esters. For the purple cluster, Bennike *et al.* (2018) investigated the fragrance contact allergen in 5,588 cosmetic products, identifying the allergen using a novel smartphone application. Bruusgard *et al.* (2020) and Nanyan (2019) cited the paper as a reference for fragrance contact allergens. For the orange cluster, Fiori and Andrisano (2014) investigated the simultaneous determination of glucosteroids in counterfeit cosmetic products and pharmaceutical formulations using the LC-MS method. Kim *et al.* (2017) cited the paper to record how consumers were unaware of the side effects because the latter were unlabeled on cosmetic product packaging.

4. Emergence of halal cosmetics as research focus

Halal cosmetics is a major theme that emerged from the study's findings. Halal cosmetics refers to products made in conformity with Islamic law, for instance, should its ingredients are sourced from animal origins, the animals must be slaughtered according to Islamic law (Azmi, Noor & Elgharbawy, 2021). The significance of halal cosmetics as an emerging trend can be seen from two aspects: first, halal cosmetics is an emergent topic in the thematic maps and second, it is one of the trend topics that emerged from 2014 until 2021. The finding that emerged from thematic maps indicates that the term halal cosmetics is located in the quadrant of motor themes, which means the halal cosmetics keyword is significant to the research related to cosmetic products. The position of halal cosmetics in the low side of the quadrant indicates the term halal cosmetics is included as one of the most frequent keywords used by authors, although not the most frequent keyword used by authors (see Figure 6). This finding can be interpreted that the current development of halal cosmetics in the cosmetic products field is low, but it has a high potential to develop further. Secondly, the trend topics show that research on cosmetic products related to halal cosmetics increased consistently from 2014 to 2021. It shows there is a high potential for halal cosmetics to be developed further as a body of knowledge in the future.

Within the analysed dataset of 613 articles, 13 articles included 'halal cosmetics' in the title. Of these 13 articles, 11 articles are from the social sciences, with eight from the marketing perspective and three from the management perspective respectively, and two articles are on natural sciences. The eight articles on marketing focused on the topic of consumer behaviour, with attributes of halal cosmetics, consumer purchase, and purchase intention as the major themes. On the theme of attributes, Daud *et al.* (2012) proposed specific attributes regarding halal cosmetics, which include religious compliance, safety, purity, and quality. On the theme of consumer purchase, the research conducted by Shahid *et al.* (2018) suggested that trust, halal awareness, and attitude towards the products are the key factors that determine the behaviour of consumers when purchasing halal cosmetic products. Six articles focused on consumer purchase intention, highlighting several factors that influence the purchase intention of halal cosmetic products purchase: religiosity (Abd Rahman *et al.*, 2015), attitude (Briliana & Mursito; Haque *et al.*, 2018), awareness and trust of halal cosmetic products (Handriana *et al.*, 2020), the halal logo of the product and ingredients (Khan *et al.*, 2021). Arbak *et al.* (2019) claimed that sexism in halal cosmetics advertising leads to ethical violations and negatively impacts consumer purchase intention.

From the management perspective, the authors of the relevant three articles discussed the organization's context concerning halal cosmetics. Widjaja and Sijabat (2021) studied the halal certification of halal cosmetic products that complied with the law and regulations set by the government of Indonesia. The other two articles studied the statistical process control implemented in halal cosmetics companies (Husain, 2015; Husain *et al.*, 2019). According to these two articles, management's commitment has an impact on the implementation of statistical control processes, which in turn will benefit the company.

Two studies on halal cosmetics from the natural science perspective include articles by Kim *et al.* (2018) and Salae *et al.* (2018). These studies focused on testing methods to examine the ingredients used in the production of halal cosmetic products.

Figure 13 shows the nine journals that published halal cosmetics articles. Most authors publish their articles in the Journal of Islamic Marketing with four articles. It indicates that most authors publish their halal cosmetics articles in a journal with specialized Islamic focus. Malaysian Journal of Consumer and Family Economics published two articles, whereas other journals, namely Asia Pacific Management Review, Management Science Letters, Humanities and Social Sciences Reviews, Journal of Applied Sciences Research, International Journal of Applied Business and Economic Research, and Journal of Legal, Ethical and Regulatory Issues and Applied Biological Chemistry respectively publish one article each that related to halal cosmetics.

Overall, existing literature regarding halal cosmetics focused mostly on consumer purchase intention, which is from the marketing perspective. Given the current development of halal cosmetic products literature from a natural science perspective is low, there is a potential for more exploration of natural science perspective related to testing methods of halal cosmetics, such as the method to determine the halal (or haram) ingredients in finished cosmetic products. Such testing methods, which can be used for verifying the halal-ness of ingredients, help manufacturers address potential issues of fraudulent practices among their suppliers.

5. Conclusion and implications

The study was conducted to explore the trends in the publications and themes related to research on cosmetic products. Halal cosmetics have been identified as an emerging area for future research as there is increasing academic interest in the development of the body of knowledge. Six major topics related to halal cosmetics were attributes of halal cosmetics, consumer purchase, purchase intention, regulations for halal certification of halal cosmetics, organization control and testing method. Most articles related to halal cosmetics were published in the Journal of Islamic Marketing, which is a specialized Islamic-theme journal. The published articles related to halal cosmetics are mostly within realm of the social science, while articles from the natural sciences are lacking. On reflection, there is a need and considerable potential to further explore halal cosmetics from the natural science perspective such as articles by Kim *et al.* (2018) and Salae *et al.* (2018).

Most research concerning cosmetic products has been conducted in European countries, but many Muslim consumers, particularly in Asia, use readily-made cosmetics produced by both Muslim and non-Muslim manufacturers. The

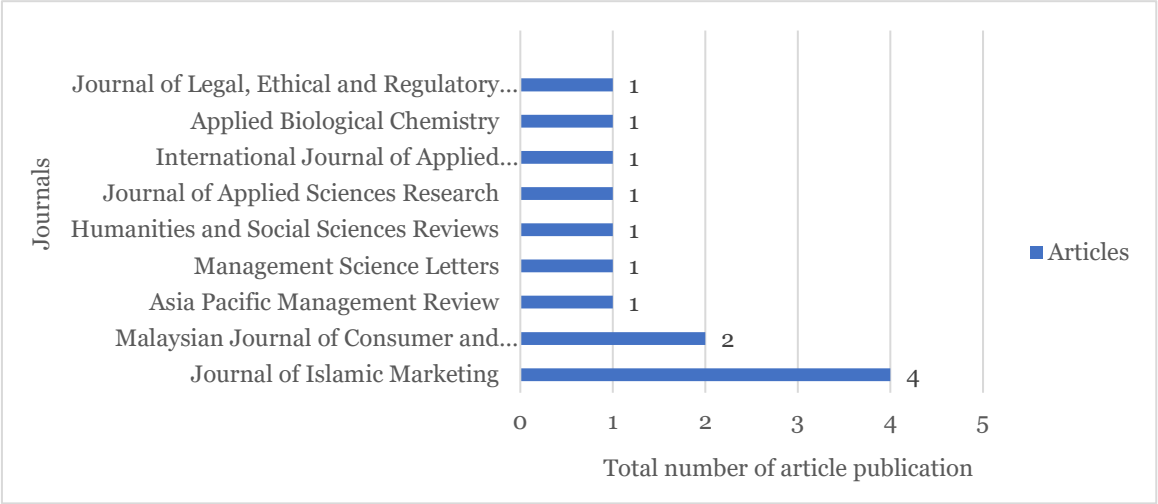

Figure 13: Halal cosmetics articles published in journals (from 613 articles on cosmetic products).

size of the halal cosmetics market increases exponentially year on year. Researchers, particularly Muslim researchers need to conduct more research on halal cosmetics and publish in reputable journals so that the knowledge about halal cosmetics can be rapidly disseminated globally. Such knowledge serves as the foundation for the effective development of halal industry practices that serves the needs and concerns of Muslims. This knowledge is essential given that Muslims' needs and requirements regarding cosmetics generally differ from those of non-Muslims, for instance, in terms of the requirements of the ingredients that can be used, processing methods, packaging, transportation, and delivery to consumers, all which must follow the Islamic law.

The findings of this study contribute toward enriching the body of knowledge on cosmetic products by reporting the publication trends of published articles and highlighting the main themes in cosmetic products research over time. This understanding provides avenues through which to identify specific research gaps that could guide future research. To date, most cosmetic product research has come from a natural science perspective, focusing on sample preparation, toxicity of raw materials, and flow injection analysis. Based on the findings of this bibliometric analysis, research on cosmetic products from the social science perspective can be said as less developed. Collaboration between natural science and social science researchers is highly recommended as their research and perspectives could complement each other. In particular, future researchers should focus on providing new knowledge to mitigate issues with existing cosmetic products, among others, products that could be dangerous or harmful for human consumption. For this purpose, halal cosmetics, which concept addresses issues of prohibition of harmful and non-clean ingredients (as per halal requirements), as well as hygiene and processing aspects, would be an excellent area for future research and exploration.

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References

Ahmi, A. (2022). Bibliometric Analysis using R for non-coders: A practical handbook in conducting bibliometric analysis studies using Biblioshiny for Bibliometrix R package: (n.p.).

Abd Rahman, A., Asrarhaghighi, E., & Ab Rahman, S. (2015). Consumers and halal cosmetic products: knowledge, religiosity, attitude and intention. Journal of Islamic Marketing, 6(1), 148-163.

Arbak, S., Islam, R., & Al Rasyid, H. (2019a). Influence of Islamic advertising: ethic violation on customer purchase intention of halal cosmetic products in Malaysia. Humanities and Social Sciences Reviews, 7(3), 671-682.

Aria, M., & Cuccurullo, C. (2017). Bibliometrix: An R-tool for comprehensive science mapping analysis. Journal of Informetrics, 11(4), 959-975.

Azmi, N. A. N., Noor, N. A. M., & Elgharbawy, A. A. (2021). A Bibliometric analysis on Halal cosmetics over twenty years. Halalpshere, 1(1), 53-71.

Bennike, N. H., Oturai, N. B., Müller, S., Kirkeby, C. S., Jørgensen, C., Christensen, A. B., Zachariae, C., & Johansen, J. D. (2018). Fragrance contact allergens in 5588 cosmetic products identified through a novel smartphone application. Journal of the European Academy of Dermatology and Venereology, 32(1), 79-85.

Briliana, V., & Mursito, N. (2017). Exploring antecedents and consequences of Indonesian Muslim youths' attitude towards halal cosmetic products: a case study in Jakarta. Asia Pacific Management Review, 22(4), 176-184.

Bruusgaard-Mouritsen, M. A., Johansen, J. D., Zachariae, C., Kirkeby, C. S., & Garvey, L. H. (2020). Natural ingredients in cosmetic products - a suggestion for a screening series for skin allergy. Contact Dermatitis, 1-20.

Buschmann, H. J., & Schollmeyer, E. (2002). Applications of cyclodextrins in cosmetic product: a review. Journal of Cosmetic Science, 53(3), 185-192.

Callon, M., Courtial, J. P., & Laville, F. (1991). Co-word analysis as a tool for describing the network of interactions between

basic and technological research: The case of polymer chemistry. Scientometrics, 22(1), 155-205.

Daud, N. M., Aziz, H. A., Baharudin, N. H., & Shamsudin, S. F. (2012). Identifying the determinant attributes of halal cosmetics products that influence its positioning strategy in the Malaysian market. Journal of Applied Sciences Research, 8(1), 301-313.

Della Corte, V., Del Gaudio, G., Sepe, F., & Sciarelli, F. (2019). Sustainable tourism in the open innovation realm: A bibliometric analysis. Sustainability, 11(21), 6114.

Elsevier (2023). What is Scopus about? https://service.elsevier.com/app/answers/detail/a_id/15100/supporthub/scopus/

Fiori, J., & Andrisano, V. (2014). LC–MS method for the simultaneous determination of six glucocorticoids in pharmaceutical formulations and counterfeit cosmetic products. Journal of Pharmaceutical and Biomedical Analysis, 91, 185-192.

GlobalData (2020, October 29). Malaysia's Cosmetics & Toiletries Industry to Grow at a CAGR of 2.8% Through 2019-24. GlobalData. https://www.globaldata.com/media/consumer/malaysias-cosmetics-toiletries-industry-grow-cagr-2-8-2019-24-says-globaldata/

Gusenbauer, M., & Haddaway, N. R. (2020). Which academic search systems are suitable for systematic reviews or meta-analyses? Evaluating retrieval qualities of google scholar, PubMed, and 26 other resources. Research Synthesis Methods, 11(2), 181-217.

Haque, A., Anwar, N., Tarofder, A., Ahmad, N., & Sharif, S. (2018). Muslim consumers' purchase behaviour towards halal cosmetic products in Malaysia. Management Science Letters, 8(12), 1305-1318.

Handriana, T., Yulianti, P., Kurniawati, M., Arina, N. A., Aisyah, R. A., Aryani, M. G. A., & Wandira, R. K. (2020). Purchase behaviour of millennial female generation on halal cosmetic products. Journal of Islamic Marketing, 12(7), 1295-1315.

Handjani-Vila, R. M., Ribier, A., Rondot, B., & Vanlerberghie, G. (1979). Dispersions of lamellar phases of non-ionic lipids in cosmetic product. International Journal of Cosmetic Science, 1(5), 303-314.

Hefnawy, M., Al-Majed, A., Mohammed, M., Al-Ghusn, A., Al-Musallam, A., Al-Sowidan, N., Al-Hamid, M. & Al-Homoud, A. (2017). Fast and sensitive liquid chromatography method for simultaneous determination of methylisothiazolinone, salicylic acid and parabens in cosmetic products. Current Analytical Chemistry, 13(5), 430-438.

Husain, R., Shariff, S. R., & Zahari, S. M. (2019). Modelling the effects of statistical process control implementation on halal cosmetics products. Malaysian Journal of Consumer and Family Economics, 23, 1-22.

Husain, R. (2015). Implementation of statistical process control by management in cosmetic production organization: case of halal cosmetics companies in Selangor, International Journal of Applied Business and Economic Research, 13(7), 5899-5919.

Hubinger, J. C. (2010). A survey of phthalate esters in consumer cosmetic products. Journal of Cosmetic Science, 61(6), 457-465.

Khan, N., Sarwar, A., & Tan, B. C. (2021). Determinants of purchase intention of halal cosmetic products among generation y consumers. Journal of Journal of Islamic Marketing, 12(8), 1461-1476.

Kim, Y. S., Yu, H. K., Lee, B. Z., & Hong, K. W. (2018). Effect of DNA extraction methods on the detection of porcine ingredients in halal cosmetics using real-time PCR. Applied Biological Chemistry, 61(5), 549-555.

Kim, N. S., Yoo, G. J., Lee, J. H., Park, H.-J., Cho, S., Shin, D. W., Kim, Y. & Baek, S. Y. (2017). Determination of 43 prohibited glucocorticoids in cosmetic products using a simultaneous LC-MS/MS method. Analytical Methods, 9(13), 2104–2115.

Lei, K., Qiao, F., Liu, Q., Wei, Z., Qi, H., Cui, S., Yue, X., Deng, Y., & An, L. (2017). Microplastics releasing from personal care and cosmetic products in China. Marine Pollution Bulletin, 123(1-2), 122-126.

Loretz, L., Api, A., Barraj, L., Burdick, J., Dressler, W., Gettings, S., Han Hsu, H., Pan, Y., Re, T., Renskers, K., Rothenstein, A., Scrafford, C., & Sewall, C. (2005). Exposure data for cosmetic products: Lipstick, body lotion, and face cream. Food and Chemical Toxicology, 43(2), 279-291.

Lopez-Galindo, A., Viseras, C., & Cerezo, P. (2007). Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic product. Applied Clay Science, 36(1-3), 51-63.

Nanyan, P. (2019). Fragrance allergens in hair removal cosmetic products. Dermatitis, 30(4), 268–271.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for statistical computing. https://R-project.org

Rastogi, S. C., Schouten, A., De Kruijf, N., & Weijland, J. W. (1995). Contents of methyl-, ethyl-, propyl-, butyl-and benzylparaben in cosmetic products. Contact Dermatitis, 32(1), 28-30.

Ribet, V., Nobile, V., & Rossi, A. B. (2019). In situ antioxidant activity of a dermo-cosmetic product: a randomized controlled clinical study. Experimental Dermatology, 28(11), 1219-1226.

Salae, K., Sirikwanpong, S., Ngamukotel, S., Katelakha, K. Nopponpunth, V. & Dahlan, W. (2018). Classification of plant- and animal-based glycerin by using ATR-FTIR: A rapid screening tool applicable for halal cosmetics. Malaysian Journal of Consumer & Family Economics, 21(2), 1-7.

Safford, B., Api, A., Barratt, C., Comiskey, D., Daly, E., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., & Tozer, S. (2015). Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regulatory Toxicology and Pharmacology, 72(3), 673-682.

Shahid, S., Ahmed, F., & Hasan, U. (2018). A Qualitative investigation into consumption of halal cosmetic products: the evidence from India. Journal of Islamic Marketing, 9(3), 484-503.

Shen, H. Y., Jiang, H. L., Mao, H. L., Pan, G., Zhou, L., & Cao, Y. F. (2007). Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. Journal of Separation Science, 30(1), 48-54.

Standard Malaysia. (2019). Malaysian standard ms2634 halal cosmetics - general requirements (first revision). Selangor, Malaysia: Department of Standards Malaysia.

Statista (2023). Beauty & personal care - Malaysia. Statista. https://www.statista.com/outlook/cmo/beauty-personal-care/cosmetics/malaysia

Statista Research Department (2022, March 8). Revenue of the beauty & personal care market worldwide from 2013 to 2026. Statista. https://www.statista.com/forecasts/1244578/beauty-and-personal-care-global-market-value

Wang, L. H., Hsia, H. C., & Wang, C. C. (2006). Simultaneous determination of five volatile and non-volatile N-nitrosamines in biological fluids and cosmetic products by liquid chromatography with photodiode array detection. Journal of Liquid Chromatography & Related Technologies, 29(12), 1737-1751.

Wang, R., Moody, R. P., Koniecki, D., & Zhu, J. (2009). Low molecular weight cyclic volatile methylsiloxanes in cosmetic products sold in Canada: implication for dermal exposure. Environment International, 35(6), 900-904.

Widjaja, G., & Sijabat, H. H. (2021). Technical and regulatory aspects of alcohol use in halal certification for cosmetics products. Journal of Legal, Ethical and Regulatory Issues, 24(1), 1-7.

Ye, N., Shi, P., Li, J., & Wang, Q. (2013). Application of graphene as solid phase extraction absorbent for the determination of parabens in cosmetic products by capillary electrophoresis. Analytical Letters, 46(13), 1991-2000.

Zakaria, R., Ahmi, A., Ahmad, A. H., & Othman, Z. (2020) Worldwide melatonin research: a bibliometric analysis of the published literature between 2015 and 2019, Chronobiology International, 38(1), 2

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Halal Concerns on Lard in Food Products and its Detection Methods

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Abstract

Lard refers to a creamy, white, soft substance made from pig fat and used in cooking and baking. Lard not only has the risk of biological complications and health problems associated with regular consumption, but also has other issues such as the restriction on use in the food industry from the perspective of the Muslim religion. There are few literature studies that show several issues related to the adulteration of food products, especially halal meat. This issue must be resolved to avoid confusion for Muslims. Therefore, laboratory analysis on meat species authentication is highly necessary. There are different available methods to detect different species in food samples, including Polymerase Chain Reaction (PCR), Electronic Nose (e-nose), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimeter (DSC), and Rapid PCR. This article aims to review the application of lard in food, cases related to halal and methods to detect the presence of lard.

1. Introduction

Food is one of the fundamental and essential needs for human survival; hence, natural and raw foods will be subjected to multiple industrial processes to retain its nutrient and functionality while maintaining it to fit for human consumption. However, its complex matrix and issues such as health and religion bound to it have made it a subject of interest worldwide among researchers and scholars. Recently, food has been processed based on modern science and technology advancements, and the ingredients used can be permitted or prohibited (Fadzlillah *et al.,* 2011). As technology improves, a few problems occur, and one of the most frequent problems is adulteration in food. Adulteration is the addition of undeclared substances to boost the bulk product or weight and make the product more valuable than the actual product. In the case of meat products, adulteration refers to substituting ingredients and unsuitable information regarding the origin of raw materials. The choice of food typically represents aspects of lifestyle, culture, faith, nutrition and health issues. From the perspective of Muslims, choosing one food over another is solely based on its halal status, as Muslims observe strict dietary rules expressed in the *Qur'an*.

Historically, meat was not commonly associated with adulteration for Muslim consumption, and this may be due to the fact that it was sold fresh at joints that were easily identifiable. Today, the food supply chain is too long, cross geographical origin, and people's lifestyles have changed greatly (Nakyinsige *et al.,* 2012). The issue with meat products is that the meat is adulterated with lard. This is because lard is one of the cheapest edible oils subsequently added to food products to reduce production costs (Mohamad *et al.,* 2015). Lard or industrially modified lard could be effectively blended with other vegetable oils to produce shortenings, margarine, and other food oils. In addition to the risk of biological complications and health problems associated with regular consumption, there is a restriction on the use of this animal product in the food industry from the perspective of the Muslim religion (Azir *et al.,* 2017).

The most frequent food fraud cases, found in 95% of reports, are replacing the real ingredient with an ingredient that is akin but has a cheaper cost, hard to notice by the consumer and hard to identify via usual analytical methods. The species authentication is vital to the user as there is an economic loss since the food frauds happen, and health issues are also affected, such as food allergies and religious reasons (Abbas *et al.,* 2018).

Halal foods are foods free from any ingredients or components that Muslims are forbidden from eating. Both good and clean foods are halal, which has been stated in the *Qur'an*. In comparison, haram means anything that is forbidden in Islam. Consequently, excluding those explicitly forbidden by the al-*Qur'an* and *Sunnah*, almost all plant and animal-origin foods are considered halal (Ariff *et al.,* 2018). Therefore, any food products that are contaminated or have contact with haram food are considered haram food and cannot be consumed by Muslims. Few literature studies show several issues related to the adulteration of food products, especially in halal meat (Salahudin *et al.,* 2018). From Mahpar *et al.* (2016), few restaurants in Malaysia displayed signs of 'no pork'; however, it is not guaranteed that the vendors do not use other pig derivatives. For example, the author mentions a woman working at a restaurant that displays the sign 'no pork' and says that sometimes the vendors use the same utensil to cook pork and other meat or food. This will cause an issue as Muslims do not know the vendor uses the same utensil to cook pork and other food, which is unacceptable for Muslims. This issue must be resolved to avoid confusion for Muslims.

Therefore, laboratory analysis on meat species authentication is highly necessary. Among the available method to detect different species in food samples are Polymerase Chain Reaction (PCR), Electronic Nose (e-nose), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimeter (DSC) and Rapid PCR. Enzyme-linked Immunosorbent Assay (ELISA) can also be concluded in meat species authentication due to the specificity, simplicity and sensitivity (Kök & Atalay, 2018).

The studies on the detection of lard were mostly conducted using either infrared spectroscopy, high-performance liquid chromatography, gas chromatography or differential scanning calorimetry (Rohman & Man, 2008). Research by Saeed *et al.* (1986) determines the presence of lard in beef and mutton mixtures by gas chromatography. Most detection methods used ELISA were applied to samples like milk (Chughtai *et al.,* 2017), meat, pasta, flour and egg (Febo *et al.,* 2018). Therefore, the present paper will review the application of lard in food, cases related to halal, and methods to detect lard's presence. The study will be a significant endeavour for the food industry to the consumer as it protects the interest of consumers and public health, scientist and technologies are continuously established and advanced to check the validity of various food products (Abbas *et al.,* 2018).

2. Lard

From the Oxford Dictionary, lard is defined as the abdomen fat of a swine that is rendered and refined to be used in cooking. It can also be characterised as a soft, unctuous white substance with a bland taste and a distinctive odour (Bergfeld *et al.,* 2017). According to Cambridge Dictionary, lard is a creamy, white, soft substance made from pig fat and used in cooking and baking. It leads to a flakier finished product because lard has especially large fat crystals, which is one reason why people choose to use lard instead of other fat or oil. That is also why traditional empanadas are so delicious, based on the same crust logic. Many savoury uses abound. Typically lard for homemade sausage, *paté*, Mexican homemade tamales or carnitas. It also has a high smoke point so that it can be used without problems at elevated temperatures, and it is a perfect fat for deep and shallow frying (Reisner, 2020).

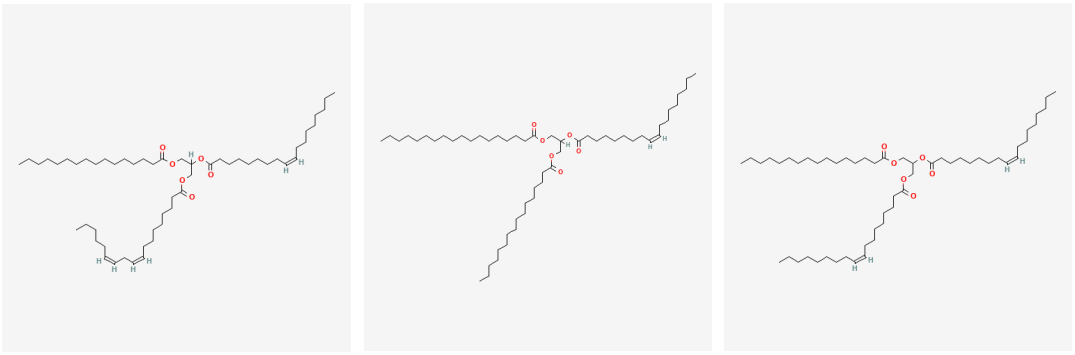
Lard contains about 200 fatty acids, and stearic, palmitic, and oleic acids are the main constituents (Bergfeld *et al.,* 2017). Lard is made of 100 % fat from pork. Via a method called rendering, it is extracted from the fatty parts of a pig. Portions such as pork belly, butt of pork, or shoulder of pork would make the most lard. At room temperature, the separated fat is solid and opaque and, depending on its purity, transforms into a transparent liquid at about 95 to 113 degrees Fahrenheit (Ferguson, 2019). Lard is obtained shortly after slaughter by a dry or wet rendering of fresh fatty porcine tissues from cuttings and trimmings. Lard produced by processes of wet-rendering is known as prime steam lard. Rendered lard can be bleached or deodorised and bleached (Bergfeld *et al.,* 2017). There are several advantages to cooking with lard; more chefs prefer lard over conventional cooking oils or shortenings. It does not contain trans fats, which makes it a healthier choice than hydrogenated fats, has fewer saturated fat and cholesterol than butter, like olive oil, contains healthy monounsaturated fats, and has a high smoke point, making it perfect for frying food (Ferguson, 2019). Lard contains less polyunsaturated fatty acids, which implies that when exposed to heat, it is more stable, less likely to turn bad and cause free radicals. Thus, to make it last longer, there is no need to add any chemical components (Pisuthipan, 2015).

In addition, it is cheaper than other consumable oil; therefore, it is purposely added to food products to lower the production cost (Mohamad *et al.,* 2015). Some religions, such as Islam and Judaism, believe that the existence of lard in any food products is prohibited. Besides that, Henry (2013) stated that although lard is healthier than butter, it still contains saturated fat. Therefore, it cannot be consumed in a huge amounts as saturated fat increases low-density lipoproteins, also known as LDLs, which are poor cholesterol and decreases healthy cholesterol, which is high-density lipoproteins (HDLs). It is associated with heart disease, hypertension, diabetes, and obesity, but metabolism and cell function are also important (Henry, 2013). Consequently, some analytical procedures, either physical or chemical-based methods, were developed to determine lard in food products (Rohman *et al.,* 2012).

2.1 Lard composition

Most fats and oils have different fatty acid compositions. In determining the source of lipids, the structure and configuration of fatty acids in fats and oils can act as an indicator (Fadzlillah *et al.,* 2016). The most important element of edible fats and oils is fatty acids (FAs). The fatty acids are commonly discovered in the ester form with a glycerol backbone called triglycerides. Lard consists of four main fatty acids, which are palmitic, stearic, oleic and linoleic (Rohman *et al.,* 2012). The facts on fatty acid composition are crucial for health consciousness and religious commitment. Nevertheless, the fatty acids compositions, particularly from animal fats, are difficult to determine because of the complicated length of the chain, branching, degree of unsaturation, geometry and position of the double bonds (Fadzlillah *et al.,* 2016). The fatty acids identified in lard are myristic, palmitic, palmitoleic, margaric, stearic, oleic and linoleic (Fadzlillah *et al.,* 2016), linolenic, arachidic and gadoleic acids (Rohman *et al.,* 2012). The fatty acids detected in lard are 7.2% palmitic, 39.7% elaidic, 16.5% oleic, 17.6% linoleic, and 4.28% linolenic acid (Sairin *et al.,* 2019).

According to Rohman *et al.* (2012), most fats and oils were mostly composed of triacylglycerols (TAG), diacylglycerols (DAGs), free fatty acids and other minor components like phospholipids, sterols, tocopherols, carotenoids, and



(A) (B) (C)

Figure 1: (A)-linoleoyloleoylpalmitoylglycerol (POL), (B)-palmitooleostearin (POS), (C)-dioleoypalmitoylglycerol (POO)
Source: National Centre for Biotechnology Information (NCBI)

fat-soluble vitamins. The major TAGs that make up lard are palmitooleoolein (POO), palmitooleostearin (POS), and palmitooleopalmitin (POP) (Rohman *et al.,* 2012). Azir *et al.* (2017) reported that lard contain 20.21% of linoleoyloleoylpalmitoylglycerol (POL), 18.58% of palmitooleostearin (POS), and 17.25% of dioleoypalmitoylglycerol (POO) as can be seen in figure 1.

2.1.2 Pig and lard in Islam

Muslims are taught to avoid eating pork flesh and its derivatives. These prohibitions have been mentioned strictly in the *Qur'an* and have been a Muslim guideline. As stated in the *Qur'an surah al-Maidah, Allah* says:

> *"Forbidden to you is anything that dies by itself, and blood and pork, as well as whatever has been consecrated to something besides Allah, and whatever has been strangled, beaten to death, trapped in a pit, gorged, and what some beast of prey has begun to eat unless you give it the final blow; and what has been slaughtered before some idol, or what you divide up in a raffle; (all) that is immoral!". (Al-Ma'dah, 5:3)*

Therefore, as referred to *Qur'an* and *Sunnah*, consensus Muslim jurist *(Ijma')* has forbidden pork and anything related. Among other verses that highlight the ban on pigs can be seen in *surah al-Baqarah, Allah* says:

> *"Indeed, Allah only forbids you to eat carrion, and blood, and pork, and animals that were slaughtered not for the sake of Allah, so anyone is forced (to eat it due to emergency) while he does not desire it and does not exceed the limit (in the amount of things eaten), then it is not a sin. Indeed, Allah is Forgiving, Most Merciful". (Al-Baqarah, 2:173)*

As stated earlier, lard is a pork fat extracted from pork belly, butt and shoulder and subsequently subjected to the rendering process to churn out the final product. In the food industry, its characteristic of large fat crystal, stable fat structure and high smoke point makes it the perfect choice to imply in food than conventional fat and oils (Ferguson, 2019). Also, lard is less likely to cause free radicals when heated due to less polyunsaturated fatty acid structure. Nevertheless, despite all the advantages demonstrated by the lard, Muslims must abide by the rules prescribed in the *Qur'an*. Prohibiting pork is only meant to protect Muslims from all possible harm, and only *Allah* knows the wisdom behind it. The advent of material like lard has raised much concern among Muslims and other parties like Judaism. Therefore, transparency in the food industry is vital to halt the concern about halal authenticity.

2.2 Application of lard in food

Many applications of lard in food make it a popular substance for food products. Among the justification for using lard in food is 1) the glycerolized lard, which is glycerolysed into diacylglyceride and monoacylglycerol, can increase the emulsifying activity and emulsion stability in meat products, 2) lard-based diacylglycerol can increase water holding capacity of meat product, 3) lard can reduce the hardness of cookie dough and 4) lard aids in controlling pests such as mite in dry-cured ham. This section is not intended to highlight the superiority of lard, however, it is meant to set the benefits of lard that are favoured by food manufacturers worldwide. Thus, justifies the need to have substantial techniques to detect its presence in food.

2.2.1 The glycerolized lard and purified glycerolized lard emulsions increase emulsifying activity and emulsion stability in meat products

Emulsion-type meat products such as sausages, frankfurters, bolognas, and wieners are very common in the meat industry and are produced in the presence of water and lipids by pounding the raw meat and creating an aqueous protein stage in which fat droplets are dispersed. Myofibrillar proteins have stronger emulsion properties in meat products to keep water and oil together. In contrast, immobilised fat droplets in the protein matrix play a crucial role in reducing purge and cooking loss, increasing water holding capacity and offering outstanding mouthfeel and juiciness.

Scientists proposed the use of lard to create the emulsion complex. Lard is inexpensive and very significant for meat product processing, and lard glycerolysis can be used to prepare DAGs. Diacylglycerols (DAGs) are glycerol esters in which fatty acids esterify two hydroxyl groups. Since DAGs have hydrophilic polar groups in their molecular structure, it shows higher surface activity as well as interfacial properties, DAGs can be emulsified more easily than TAGs. Therefore, DAGs in emulsions have beneficial effects and are ideal for use as emulsifiers. Furthermore, because of their higher melting points compared with TAGs, DAGs can enhance food texture (Diao *et al.,* 2016). In lard, glycerolysis was done to obtain the DAGs part used as an emulsion stabiliser that the authors did in their research (Diao *et al.,* 2016).

In general, emulsifying activity indices are determined by protein-lipid and protein-protein interactions and continuous and scattered phases are correlated with emulsion stability indices. The probability of creating a stable emulsion depends on the protein molecules remaining to stabilise oil droplets at the interface after adsorption. The emulsifying activity index and emulsion stability index of emulsion with lard, glycerolized lard, and purified glycerolized lard are shown in Figure 2.

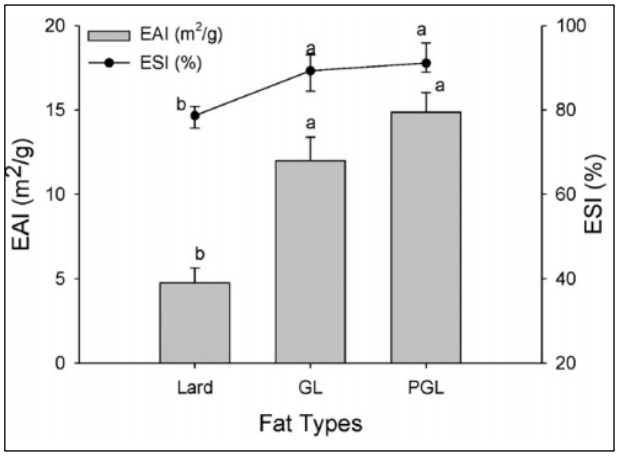


Figure 2: Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions prepared with myofibrillar proteins and lard, glycerolized lard (GL), or purified glycerolized lard (PGL).

Source: (Diao *et al.*, 2016).

The emulsion emulsifying activity index for lard was 4.76 (m^2/g), which was slightly lower than for glycerolized lard, which is 12.0 (m^2/g) and purified glycerolized lard is 14.9 (m^2/g), respectively. The findings showed that in emulsions, glycerolized lard and purified glycerolized lard could disperse more effectively than lard, which can be due to the hydrophilic groups in DAGs that are correlated more strongly with water droplets scattered in the emulsions. Glycerolized lard and purified glycerolized lard's acylglycerol structures are more versatile than lard and can interact more strongly with proteins at the water-oil interface. To conclude, the study conducted by Diao *et al.* (2016) reported that DAGs can greatly increase emulsion properties. Compared with the emulsion prepared with lard, the emulsion prepared with myofibrillar proteins and purified glycerolized lard had higher emulsifying behaviour and emulsion stability. Thus, in emulsion-type meat products, such as frankfurters, bolognas, and wieners, lard DAGs have very good application prospects (Diao *et al.,* 2016).

2.2.2 Lard-based diacylglycerol can increase water holding capacity of meat product

The rheological and physicochemical properties of pork myofibrillar protein gels at different pH levels are influenced by lard-based diacylglycerol. Myofibrillar protein is an essential functional protein primarily responsible for meat products' physicochemical and thermally mediated gelation properties. In the characteristics of processed meat products, the shaped myofibrillar protein gels play a crucial role, such as microstructure, texture, and sensory properties. Adding fat to meat products affects the physicochemical properties of meat products. In the three-dimensional protein gel matrix, emulsified fats as fillers occupy voids, reducing the porosity of processed meat products (Zhou *et al.,* 2020).

According to Zhou *et al.* (2020), the addition of impurified diacylglycerol or purified diacylglycerol of lard to the gel of pork myofibrillar protein has a higher water-holding capacity than the control gel of pork myofibrillar protein. The enhanced water holding capacity can be due to the increased number of hydrogen bonding sites between protein and water with increased pH (Zhou *et al.,* 2020). The increased water-holding capacity of the gel of pork myofibrillar protein was correlated with myofibril's swelling resulting from increased pH since swelled myofibrils promote retaining moisture and fat during heat treatment. The space-filling effect of the fat globules and the interaction between the membrane proteins of the fat globules and the proteins in the emulsions can result in increasing the water-holding capacity of the gel of pork myofibrillar protein treated with fats. In addition, due to the free hydroxyl group in their structures, the improved water-holding capacity of the gel of pork myofibrillar protein can be obtained by the addition of unpurified diacylglycerol and purified diacylglycerol (Zhou *et al.,* 2020).

Furthermore, the addition of lard-based diacylglycerol and the shift in pH had a major effect on the physicochemical properties of the thermally induced gel of pork myofibrillar protein. As the pH increases or there is an addition of impurified diacylglycerol or purified diacylglycerol, the water-holding capacity of the myofibrillar protein gels increases (Zhou *et al.,* 2020). As the authors used composite gel in their research, they stated that the results obtained give a theoretical basis for using lard-based diacylglycerol in emulsified meat products (Zhou *et al.,* 2020).

2.2.3 Lard reduces hardness in cookie dough

Cookies are among the most popular snacks people of all ages, races and cultures enjoy. They are products made from wheat flour, sugar and fat that are baked. Most cookies are formulated from either plant or animal fat, with around 10 to 30% of the shortening made (Manaf *et al.,* 2019). Shortening plays an important role in dough production as it interacts efficiently with the matrix of protein and starch to avoid excessive gluten development throughout mixing. Shortening is also believed to act as a lubricating agent during dough making, allowing aeration to capture and hold air. Shortening could also impart desirable quality attributes to the texture and flavour of baked products.

Fat plays a significant role in the mechanical properties and fracturing behaviour of cookies. It is widely accepted that various types of fats, such as butter, lard, palm olein, palm stearin, and mid-fraction palm, have different effects on the textural properties of cookie dough. For instance, solid fat softens the dough and reduces viscosity (Manaf *et al.,* 2019). Hardness plays an important role in baked goods, as it can lead to the freshness of products (Noor Raihana *et al.,* 2017). They further claimed that this led to an increase in the length and a decrease in cookies' weight and thickness (Manaf *et al.,* 2019).

In a previous study, Yanty *et al.* (2014) noted that cookie lipids formulated with palm-based fats and lard showed different thermal behaviours due to their compositional variations. Manaf *et al.* (2019) have attempted to create binary, ternary, and quaternary plant-fat mixtures as alternative plant-fat mixtures since halal and kosher food regulations commonly forbid the use of lard in cookie formulations. The maximum force has been reported to determine the hardness of the

dough. Based on the authors ' research results, cookie dough made from binary shortening was the hardest, which is 231.56 g. This is because it requires more force to compress. This may be attributed to the components of palm stearin and Mee (Madhucalongifolia) fat, which are difficult due to the dominance of di-saturated molecules, 40.97% and 1.38% tri-saturated molecules. The softest was dough made from lard which is 218.33 g because its compression needed the least force. The presence of low concentrations of di-saturated and tri-saturated molecules, which are 26.6% and 2.50%, respectively, may be attributed to this soft existence.

Cookie dough made of lard and quaternary shortenings should have been more cohesive and viscous, contributing to a smoother texture (Manaf *et al.,* 2019). The set times for Mee (Madhucalongifolia) fat, palm stearin (99:1), and lard-prepared cookies were 7 mins, while those for other shortening forms were 6 mins. However, no major variations in width and thickness between the cookies of all fat mixtures have been found. This may be attributed to the standardised expansion of cookies made from formulated plant-based shortenings and lard shortening during baking (Manaf *et al.,* 2019).

2.2.4 Lard aids in controlling pests in dry cured ham

In the ageing process, dry-cured hams can become infested with ham mites, red-legged beetles, cheese skippers, and larger beetles. Methyl bromide is the only fumigant available that is effective in controlling ham mites in dry-cured ham plants in the United States, although other methods can be used for beetles and cheese skippers (Zhou *et al.,* 2016). Nonetheless, methyl bromide will be phased out of all industries by roughly 2015. In the European Union, methyl bromide cannot be used to regulate mites. In the European Union, mites are considered a quality issue regulated by relative humidity, the use of lard on the surface and other good production practices (Zhou *et al.,* 2016).

In the United States, industry and university scientists are exploring possible solutions to methyl bromide as it is being phased out of use in the United States by the industry. These potential alternatives may include certain methods, such as using hot lard and relative humidity control, currently used in Europe to manage mite populations (Zhou *et al.,* 2016). A popular practice in Spain is coating hams with vegetable oils or hot lard to control mite infestations in dry-cured ham. Some vegetable oils protect cured fish from pests and give fish a more appealing look, including coconut, sunflower, groundnut palm, maise and sesame oil. As dipping on dry-cured ham cubes, a list of animal and vegetable oils, including soybean oil, canola oil, corn oil, olive oil, mineral oil and lard, sorbic salts like sodium, potassium and calcium propionate, iodide salts such as sodium, potassium and calcium iodide, sodium, potassium, calcium citrate which is an example of citrate salts, short-chain alcohols (1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol), organic acids like maleic acid, citric acid, 3,3-thiodipropionic acid and butylated phenol preservatives including BHT and BHA have been analysed. According to Zhou *et al.* (2016), approximately 2.5 cm^3 cubes of dry-cured ham were immersed in a test compound of a given concentration for 1 min for food dips, then put in a ventilated glass jar and inoculated with 20 adults consisting mainly of female mites. As a result, ham cubes dipped in water from 20 parents created around 600 mites per container. 100% lard, 50 % propylene glycol (1, 2-propanediol) and 10 % butylated hydroxytoluene (BHT) were successful in regulating mite reproduction under laboratory conditions among all substances tested (Zhou *et al.,* 2016).

2.2.5 Adulteration issues related to lard

The global halal market accounts for more than 20% of the entire food industry, and by 2050, demand for halal products is estimated to continue to rise by up to 70% (Ruslan *et al.,* 2018). *Halal* food products are generally manufactured in Malaysia by certified Multinational companies and Small and Medium Enterprises (SMEs). As per the Syariah Legislation, the halal food industry involves manufacturing, preparation, preservatives, distribution, food service, and beverages. The industry embodies a dynamic and multinational array of diverse businesses that supply Muslim consumers worldwide with most halal food products (Ruslan *et al.,* 2018). Recently, the regular reports in the mass media of food fraud controversies in the food supply chain have raised serious concerns among Muslim consumers regarding halal food products. Several examples of food fraud are the contamination of undeclared porcine species meat products in sausages and burger patties, the detection of porcine DNA in commercial candy products such as marshmallows, gummies, hard candies and complex candies and issues in halal slaughterhouses where they do not follow the halal practices in the slaughtering process (Ruslan *et al.,* 2018).

Adulteration is a legal concept for a food product that fails to meet certain requirements. According to the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA), adulteration commonly applies to non-compliance with health or safety requirements. Food adulteration is purposely lowering the quality of the food offered for sale by combining or replacing inferior substances or withdrawing some important ingredients. Fats and oils are important nutrients for humans. Industrially, manufacturing has played an important role in developing the various fields of chemicals, pharmaceuticals, cosmetics and foodstuffs. Using animal fats in foods was very popular in the first 50 years of the twentieth century (Fadzlillah *et al.,* 2011). For example, lard or pig fat was the most commonly used commodity in the mass production of breads and cakes for domestic frying and raw material. Lard remains an essential ingredient for the food industry in formulating some food products, mainly embedded products. Usually, the label of the ingredients does not list the origin of the ingredients. A serious problem for Muslim buyers is secret ingredients from different sources (Fadzlillah *et al.,* 2011). However, lard can be replaced by other fat such as butter, shortening, margarine and coconut oil (Jaron, 2020).

Halal requires numerous procedures, such as slaughter, storage, display, preparation, hygiene, and sanitation. Thus, Muslim consumers are worried since most halal food items are imported from non-Muslim countries. Generally, non-Muslim countries such as Brazil, Australia, India, France, China, the Netherlands, and Spain are responsible for global halal meat supplies (Ruslan *et al.,* 2018). Some cases have reported a high risk of cross-contamination between halal and haram products. Cases such as when halal meats and non-halal meats are stored together. As reported, there has been a case of the Malaysian Quarantine and Inspection Service (MAQIS) Department seizing cargo shipments of halal and non-halal frozen meat stored together at Tanjung Pelepas Port (Said, 2017). Due to complex food supply chains, halal meat adulteration can occur in many forms. In the case of meat adulteration, in addition to the slaughter of animals in a manner that does not comply with the Syariah law as being addressed in the halal food industry, it includes not only substituting the ingredients but mislabelling these items from the country of origin (Ruslan *et al.,* 2018). Zakaria (2008) argued that it is hard to check the halal status of food items when the product is pre-packaged or processed.

Thus, the production of methods for studying food ingredients has been improved by the high demand for transparency in the food industry (Fadzlillah *et al.,* 2011).

3. Detection method of lard in food

The wide application of lard in food has been a concern for certain parties like Muslims and vegans. Undeclared substances in food products nowadays have catalysed the need and importance of detection methods for food adulteration. Many methods have been developed to distinguish the presence of lard or pork in meat or food products. For instance, Polymerase Chain Reaction (PCR), Gas Chromatography (GC), Enzyme-linked Immunosorbent Assay (ELISA), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Rapid PCR, Electronic Nose technology (E-nose) and Fourier Transform Infrared (FTIR) are the methods that have been employed to detect lard in foods and food products where the summarised information can be seen in table 1. Mohamad *et al.* (2015) summarised that few techniques can be used to detect the existence of lard in food products, and it involves labelling and non-labelling-based techniques. A labelling-based technique characterises Polymerase Chain Reaction (PCR), and the non-labelling technique are Electronic Nose (E-Nose), Fourier Transform Infrared (FTIR) Spectroscopy and Gas Chromatography (GC).

3.1 Polymerase chain reaction (PCR)

Several procedures supported by PCR are planned as advantageous means for recognising species of origin in foods due to their high specificity and sensitivity from qualitative PCR over restriction fragment length polymorphism (RFLP) to quantitative PCR, referred to as real-time PCR (Rohman *et al.,* 2016). Consistent with Perestam *et al.* (2017), the recognition of species within treated meat products uses the procedures of Polymerase Chain Reaction (PCR), a DNA-based method. The PCR assay was an alternative method for species recognition in treated meat products when a small recognition limit is needed. Furthermore, Ulca *et al.* (2013) stated that the polymerase chain reaction could distinguish less than 0.1% porcine DNA from meat mixture and recognise the right meat species. Lubis *et al.* (2016) noted that PCR-based DNA analysis has several drawbacks, such as the expense and time required for DNA extraction. DNA extraction can be labelled as slow due to the longer extraction time required. The extraction process might take up to 6 hours or more for certain samples due to different compositions. Other than that, DNA is prone to deterioration in treated food, which promotes difficulty in detecting the targeted DNA and possibly gives rise to false negatives.

Moreover, Rosman *et al.* (2016) investigated the inhibitory effect exerted by each of the chocolate components, four basic chocolate components, sugar, milk powder, cocoa butter, and cocoa powder, whereby they found that cocoa powder, as the only component that prevents DNA extraction of lard in chocolate. No substantial polymerase chain reaction inhibition was detected, and thus confirms the cocoa powder's inhibition on DNA extraction of lard from lard-adulterated chocolate.

3.2 Rapid PCR

Previous studies have reported that meat adulteration is ruining users' attraction towards food. Wu *et al.* (2020) suggested that it is crucial to establish a fast, uncomplicated, cost-effective and sensitive methodology to recognise meat species. In addition, according to Wu *et al.* (2020), in the experiment that was conducted, as little as 0.01% pork contents in binary mixes can be identified, and the entire identification method manages to be completed in 20 mins from the sampling steps to the step where the outcome is obtained. The developed methodology has great potential for fast identifying pork meat and recognising meat species (Wu *et al.,* 2020). To create a quick, easy and cost-effective method for extracting meat nucleic acids, a new meat extraction method was developed and carried out. The method would be completed in 5 mins, where the changing time between water baths is also considered (Wu *et al.,* 2020). Previous studies have reported that nucleic acids can be magnified in mins. This can be done by rotating the plastic capillary between the two water baths (Zhuo *et al.,* 2018). The author uses the glass capillary as the reaction vessel to transfer heat efficiently between water baths and the reaction solution (Wu *et al.,* 2020). Moreover, the molarity of polymerase and primers were correspondingly raised 10 times and 2 times to equal the kinetics of primer annealing and polymerase extension under faster temperature cycling. To verify the period consumed in each water bath, a thermocouple was utilised to measure the temperature change of the reaction solution (Wu *et al.,* 2020).

Next, rapid PCR linked with the portable device was utilised to distinguish pork meat in binary mixes. The strong green fluorescence with 520 nm wavelength was formed to identify raw beef meat or cooked beef meatball containing 100%, 10%, 1%, 0.1%, and 0.01% pork meat. It was found that the colour of negative samples continued to be black. The fluorescence signal was comparatively low in recognising 0.01% of pork meats in beef meat, however, the identification of outcomes could still be distinguished by the naked eye if compared with the negative samples (Wu *et al.,* 2020). The process of visual recognition could be completed in 30 seconds, and the risk of carryover contamination could be prevented as there is no uncapping step (Wu *et al.,* 2020). Rapid PCR is used to amplify pork meat DNA, which can be achieved using two water baths in 5 mins. A SYTO 9-based visual detection system is created to make it simpler and more convenient to classify the amplification effects (Wu *et al.,* 2020).

3.3 Enzyme-linked immunosorbent assay (ELISA)

Other methods to determine lard's existence in food products are Enzyme-linked Immunosorbent Assay, also known as ELISA. The simplicity of sample preparation, the absence of complex equipment, trained staff and the high productivity of serial testing define immune techniques. Indeed, electrochemical immunosensors are an alternative detection method for food authentication and are highly viable for on-site use (Mandli *et al.,* 2018). Perestam *et al.* (2017) research shows that in terms of sensitivity, ELISA could persistently identify pork in the binary mixture at levels down to 10.0% w/w. Even though the pork was identified at levels as low as 5.00% w/w in another sample prepared with different concentrations of pork spiked in beef, this outcome was only detected with one of the duplicate samples. The ELISA test showed better sensitivity than the pork-specific test, with the lowest recognition at 0.50% w/w and the lowest uniform recognition level at 1.00% w/w for beef within a binary mixture (Perestam *et al.,* 2017). Mandli *et al.* (2018) used the anti-pig IgG (whole molecule), a peroxidase antibody produced in rabbits, and pure IgG from porcine serum used as a standard to detect pork in meat. Compared to the competitive ELISA, the developed direct ELISA detects low adulteration levels of 0.01% in 14 hours and 15 mins compared to the competitive ELISA, which allows the determination of 0.1% as the adulteration level in 45 mins. However, with the developed immunosensor, 0.1% of pork adulteration can be detected in 2 hours, and a low level of pork

Table 1: Comparison method for detection of lard in food

Method	Limit of Detection	Reliability	Drawbacks	Types of samples
Polymerase Chain Reaction (PCR)	• Able to distinguish <0.1% porcine DNA (Ulca *et al.,* 2013).	1. Portray high sensitivity method. 2. Required only small number of samples to analyse.	1. Slow method. 2. Required longer time for DNA extraction depending on composition of food matrices. 3. Susceptible to DNA deterioration in process food.	• Preferably only on treated meat products. Eg; nugget and sausage.
Rapid PCR	• Study reported as low as 0.01% pork content needed for recognition of adulteration (Wu *et al.,* 2020).	1. Highly sensitive method available. 2. Cost effective. 3. Fast method. Time required to analyse is only 20 minutes (Wu *et al.,* 2020).	• Susceptible to DNA deterioration in process food. Eg; chocolate-based product (Rosman *et al.,* 2016).	• Suits for treated meat products. Eg; patties
Enzyme-linked Immunosorbent Assay (ELISA)	• Capable of detection 0.01% pork adulteration (Mandli *et al.,* 2018).	1. High sensitivity. 2. Uncomplicated procedures. 3. Easily operated. No qualified personnel and equipment needed	1. Expensive due to antibody involved. 2. Antibody instability due to improper care of storage procedure. 3. Possibility of false positive and negative results (Sakamoto *et al.,* 2018).	• Applicable for treated meat products. Eg, minced meat
Gas Chromatography	• Previously reported only 0.5% of or lard adulteration could be detected (Hussain 2022).	• Capable of providing fine details of fatty acid composition and quality.	1. Laborious 2. Complex procedures 3. Dependent. Method like PCA analysis required to distinguish types of animals presents.	• High fat content food. Eg, biscuits
Duplex PCR Enzyme-linked Oligonucleotide Assay (ELONA)	• Pork adulteration range from 0.5% - 1% w/w (Skouridou *et al,* 2019).	1. Sensitive and cost-effective 2. Small number of samples required	1. Multiple procedures involved 2. Trained personnel required	• Raw meat and processed meat products.

adulteration can be detected with the competitive immunosensor (0.01%) in only 20 mins. The developed immunosensor was effective in detecting IgG pork in processed foods and can detect pork in a studied range of 1 to 100% in boiling beef meatballs and was successfully applied to species screening tests. Due to its sensitivity, specificity, simplicity and low cost, the assay is suitable for food authentication (Mandli *et al.,* 2018).

3.4 Gas chromatography

Research by Hussain (2022) reported that the long-wave NIR (LW-NIR) spectroscopy system at 1350 – 2450 nm region in combination with chemometrics analysis was used for detecting and quantifying lard adulteration in palm oil (PO). The result has shown that the samples with a minimum level of adulteration as low as 0.5% could still be easily detected with an overall correct classification rate using linear discriminant analysis (LDA) in the Open-source R software.

Meanwhile, Marikkar *et al.* (2021) differentiate fatty acid and triacylglycerol compositions of native lard (NL), beef tallow (BT), mutton tallow (MT), and chicken fat (CF) by using gas-liquid chromatography (GLC). GLC analysis showed that comparing the overall fatty acid data might not be suitable for discriminating different animal fats, but using the principal component analysis and the % palmitic acid enrichment factor [PAEF (%)] calculations were useful.

The study by Azizan *et al.* (2021) was conducted to detect lard adulterated wheat biscuits using chemometrics and machine learning–assisted GCMS. Oil was extracted from the laboratory-prepared wheat biscuits using the Soxhlet extraction method, converted to fatty acid methyl ester and analysed using GCMS. The result shows that principal component analysis (PCA) and hierarchical cluster analysis (HCA) could categorise lard, wheat biscuits and lard-adulterated samples based on their fatty acid distribution. Random forest outperformed partial least squares-discriminant analysis (PLS-DA) in sample classification. Feature selection using random forest identified two fatty acids as potential biomarkers. The researcher proposed C18:3n6 as the potential biomarker to differentiate pure wheat and lard-adulterated biscuits.

3.5 Duplex PCR enzyme-linked oligonucleotide assay (ELONA)

Skouridou *et al.* (2019) have pursued an alternative and flexible platform for the identification of meat adulteration. It was based on PCR amplification and direct post-PCR detection with a colourimetric enzyme-linked oligonucleotide assay (ELONA) using specially developed species-specific tailed primers. The authors have informed that PCR–Enzyme Linked Oligonucleotide Assay (ELONA) is a susceptible and decent procedure for determining the presence of pork in beef and chicken products (Skouridou *et al.,* 2019). The design of the primers was based on incorporating the tails of ssDNA and a PCR stopper to receive amplicons at each end with separate ssDNA tails. This design aimed to facilitate the detection of ssDNA tails by hybridisation with capture and detection probes. Control genomic DNA was used from each species in PCR-ELONA assays and was collected from the various animal tissues using the High Pure PCR Template Preparation Kit following the manufacturer's instructions (Skouridou *et al.,* 2019). Meat from cows, chicken and pigs was obtained from local commercial sources to test the sensitivity of the developed assay and used after grinding for the preparation of binary mixtures containing 0.1, 1 and 10% pork in beef or chicken (w/w) (Skouridou *et al.,* 2019). Using the Gene JET Genomic DNA Purification Kit according to the manufacturer's protocol, DNA was extracted from approximately 20 mg of each mixture and analysed by agarose gel electrophoresis (Skouridou *et al.,* 2019). To detect pig DNA in mixtures with cow or chicken DNA, duplex PCR amplification using species-specific tailed primer pairs was optimised, followed by Enzyme-Linked Oligonucleotide Assay (ELONA) (Skouridou *et al.,* 2019). For duplex amplification, the primers utilised were the species-specific tailed primers. To allow for simultaneous verification of the origin of the principal species comprising the food product, duplex amplification was sought, bypassing the additional use of universal eukaryotic primers (Skouridou *et al.,* 2019). Therefore, the approach's potential flexibility and consumer-friendly features are highlighted at a lower cost relative to commercially available kits, without needing costly reagents or sacrificing detection sensitivity. It was authenticated using DNA add mixtures and DNA extracted from raw meat mixtures, and 0.5 – 1% *w/w* pork could be easily distinguished if mixed with beef or chicken. Skouridou *et al.* (2019) demonstrated that in preparing PCR-ELONA assays, each species' control genomic DNA was implemented, and the animal's animal tissue was extracted using the High Pure PCR Template Preparation kit. The two duplex PCR-ELONA assays were evaluated by adding mixtures of pig DNA with cow or chicken DNA. Mixtures containing 0.1 – 25% pig genomic DNA in cow or chicken DNA solutions were prepared using each species' control genomic DNA (Skouridou *et al.,* 2019). A PCR reaction followed by duplex amplification and ELONA detection of the pig amplicon was then applied to each DNA pool. The detection of pig DNA combined with cow DNA was more sensitive than that of the chicken/pig duplex. According to Skouridou *et al.* (2019), the circumstances that provide optimal performance of the two duplex PCR–ELONA assays were then employed for the building of calibration curves for all the targets via both synthetic ssDNA and genomic DNA (Skouridou *et al.,* 2019). The sensitivity achieved was high, detecting pig genomic DNA as low as 71 pg, while the presence of 0.5 - 1% pig DNA in mixtures with cow or chicken DNA could easily be visually identified compared to pure DNA-containing samples from only one animal. It also successfully identified the inclusion of 1% pork in beef or chicken raw meat mixtures. The output of the strategy established demonstrated its suitability for food product screening and the identification of adulterant levels of tissue extracted from pork (Skouridou *et al.*, 2019).

3.6 Electronic nose technology (E-nose)

The electronic nose acts like a biological nose in detecting the form of vapour that reaches its 'receptors'. An electronic nose's sensors can be selected to recognise an interesting odour. The electronic nose is typically a combination of an odour delivery system, a sensor (array), a data acquisition and a data processing unit (Latief *et al.,* 2017). In environmental and medical investigations, electronic noses have different applications. They also play a significant role in the food sector. The identification of various flavours of milk, meat, tea, spoiled beef and spoiled fish are some applications of electronic noses (Latief *et al.,* 2017).

According to Che Man *et al.* (2005), the electronic nose was shown to be able to differentiate between different types of vegetable oils and control the storage stability of RBD palm olein and the authors decided to use an electronic nose to detect lard. The gas sensor used is specified to be sensitive to volatile organic compounds (Latief *et al.,* 2017). There are many

chemical compositions of animal fats, which mainly contain triglycerides. A triglyceride is a chemical compound formed from one glycerol molecule and three fatty acids. The principal component of animal fat, like pig fat, is triglycerides. Moreover, lard primarily comprises saturated fatty acids (Latief *et al.,* 2017). Lard is reported to contain up to 10% linoleic acid and small amounts of arachidonic acid, both of which are in the unsaturated fatty acid group. For this analysis, the gas sensor selected can quickly detect volatile organic compounds (Latief *et al.,* 2017).

Metal oxide gas sensors are widely used in electronic noses, including pork adulteration, quality control, and a formaldehyde sensor. Tin dioxide [SnO_2] and tungsten trioxide [WO_3] are sensing materials used in metal oxide sensors, as both materials are said to be extremely sensitive to different forms of volatile compounds (Latief *et al.,* 2017). When exposed to the sensor, it was revealed that decanal, an aldehyde, gives a major signal shift (Latief *et al.,* 2017). As such, predicted to be significantly more sensitive to lard than other fats. A SnO_2 layer is coated with the sensor used in the author's report (Latief *et al.,* 2017).

On the other hand, an electronic nose was used to authenticate lard in refined, bleached, deodorised palm olein (Che Man *et al.,* 2005). The samples were prepared in proportions ranging from 1% to 10% of animal fat, in 1% increments (w/w) and from 10% to 20% of animal fat, in 5% increments (w/w), refined, bleached, deodorised palm olein and lard were blended. The electronic nose result is displayed as a chromatogram, the derivative of the frequency shift versus time shown graphically. The peak area was correlated to the concentration of the compound and was expressed in the count. RBD palm olein contained compounds that were less reactive than lard. This was due to the different nature of the processing methodology between the two oils. The palm olein used was processed, bleached and deodorised, but the lard was crude oil with a strong and distinctive swine odour. The findings show that with the increase of lard in the samples, the peak area of these components substantially increased. There was a substantial difference between the detector counts of 18.00 and 21.00 for 0 % lard and 1 % lard. Although the fine details of fatty acid composition and consistency parameters of adulterated RBD palm olein could be given by gas chromatography and chemical tests, they are of very little use for the qualitative identification and quantitative determination of adulterants such as lard in RBD palm olein. In contrast, using the e-Nose in RBD palm olein provided a more sensitive tool for detecting lard.

3.7 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy was used in halal authentication for the analysis of lard in a binary mixture with other animal fats by means of multivariate calibration in conjunction with discriminant analysis and for the analysis of lard in cake and chocolate formulations by means of partial least square calibration (Kurniawati *et al.,* 2014). Fourier transform infrared has also been used to distinguish the presence of lard in animal fat mixtures, other vegetable fats in cocoa butter, the characterisation of edible oils and lard, and the Spanish fatty acid shortening composition (Syahariza *et al.,* 2005). Syahariza *et al.* (2005) used Fourier transform infrared to identify lard in the cake using Perkin–Elmer spectrum RXI Fourier transform infrared spectrometer equipped with a LITA (Lithium tantalate) detector. The results show a range of spectra. The entire range of spectra looks almost the same, but when it was observed closely at the frequency range of 3009-2800 cm $^{-1}$which is due to CH stretching absorption, the carbonyl absorption of the triacylglycerol ester relation at 1744-1739 cm $^{-1}$, the bands associated with the fingerprint area (1500-1000 cm $^{-1}$), the trans double bond C=CAH bending vibration at 990-950 cm $^{-1}$ and the overlap of the methylene rocking vibration and the out-of-plane bending vibration of cis-disubstituted olefins at 723 cm $^{-1}$ (Syahariza *et al.,* 2005). This Fourier transform infrared analytical approach is likely to be adaptable to detect and quantify the adulterated lard level in cake formulation, particularly if the same type of shortening was used in the formulation. The authors said that using 1117 - 1097 and 990 - 950 cm-1 regions can verify the presence of lard when combined with this shortening in cake formulation. The authors have shown here that it is possible to extract meaningful information from mid-infrared spectroscopy (MIR) spectra by combining attenuated total reflectance (ATR) with partial least square (PLS) regression (Syahariza *et al.,* 2005).

In another study, lard in 'rambak' cracker was detected using Fourier transform infrared (FTIR) spectroscopy combined with partial least square and principal component analysis chemometrics. FTIR spectroscopy at wavenumber regions of 1200 – 1000 cm−1 was successfully used to quantify and classify lard in 'rambak' crackers (Erwanto *et al.,* 2016).

4. Conclusion

In conclusion, adulterating pig sources in food products is banned in Islam. From the Islamic point of view, this restriction involves all parts of pigs, such as meat, skin, and even their derivatives like lard, enzyme, and others. Products containing lard must also be indicated in the labelling of foods. Several detection approaches have been developed, for instance, FTIR, ELISA, ELONA, e-nose, DSC and PCR. The review shows few lard applications in food, especially when lard is in the diacylglyceride form. The diacylglyceride form of lard can increase the emulsion activity and stability and increase the water-holding capacity of meat products. At the same time, the unsaturated part of the lard plays a role in increasing the stability of lipid and protein oxidation in minced pork. Lard also has a role in making cookies. It will help reduce the hardness of cookie dough so that the cookie produced has the properties of a good cookie, as there is an ever-growing demand from consumers worldwide for knowledge and trust relating to the origin and content of food purchased. Food producers have no choice but to include and validate the genuineness of the sources of their food ingredients in this regard. It is no longer feasible to detect adulteration using physical properties such as the refractive index, viscosity, melting point, saponification, and iodine value, in view of the availability of more modern, sophisticated procedures and approaches. However, at a known stage, each oil and fat have a particular component, and their existence and quality should be considered as a method for detection. Therefore, it is important to consider advanced, sophisticated, highly sensitive methods to detect and measure adulteration. Many methods can be used to detect the adulteration of lard, and from the review, a few methods are PCR, rapid PCR, ELISA, ELONA, e-nose, FTIR and DSC.

As the Muslim population have increased worldwide, the authorised person should take the initiative to produce and commercialise kits for halal analysis. There is clearly a great demand for steps to support Muslims with the religious duty to ensure that halal is their food. As technology has improved from time to time, it will be easier to determine any contamination in food, particularly the adulteration of lard in the food product.

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References

Abbas, O., Zadravec, M., Baeten, V., Mikuš, T., Lešić, T., Vulić, A., Prpiće, J., Jemeršić, L., & Pleadin, J. (2018). Analytical methods used for the authentication of food of animal origin. Food Chemistry, 246, 6–17.

Ariff, M. I., Alias, M. N., Mat Zin, D. M., Mohamed Said, N. L., Zakaria, Z., Salleh, A. D., & Jamsari, E. A. (2018). Istihalah and its effects on food: an Islamic perspective. International Journal of Civil Engineering and Technology (IJCIET), 9(1), 755-762.

Azir, M., Abbasiliasi, S., Ibrahim, T. T., Manaf, Y., Sazili, A., & Mustafa, S. (2017). Detection of Lard in Cocoa Butter—Its Fatty Acid Composition, Triacylglycerol Profiles, and Thermal Characteristics. Foods, 6(11), 98.

Azizan, N. I., Mokhtar, N. F. K., Arshad, S., Sharin, S. N., Mohamad, N., Mustafa, S., & Hashim, A. M. (2021). Detection of Lard Adulteration in Wheat Biscuits Using Chemometrics-Assisted GCMS and Random Forest. Food Analytical Methods, 14(11), 2276-2287.

Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Liebler, D. C., Marks, J. G., . . . Synder, P. W. (2017). Amended Safety Assessment of Lard and Lard-Derived Ingredients as Used in Cosmetics. Cosmetic Ingredient Review.

Che Man, Y. B., Tan, C. P., Nazimah, S., Noraini, I., & Gan, H. L. (2005). Detection of lard adulteration in RBD palm olein using an electronic nose. Food Chemistry, 90(4), 829-835.

Chughtai, M. I., Maqbool, U., Iqbal, M., Shah, M. S., & Fodey, T. (2017). Development of in-house ELISA for detection of chloramphenicol in bovine milk with subsequent confirmatory analysis by LC-MS/MS. Journal of Environmental Science and Health, Part B, 52(12), 871-879.

Diao, X., Guan, H., Zhao, X., Chen, Q., & Kong, B. (2016). Properties and oxidative stability of emulsions prepared with myofibrillar protein and lard diacylglycerols. Meat Science, 115, 16-23.

Erwanto, Y., Muttaqien, A. T., Sugiyono, Sismindari, & Rohman, A. (2016). Use of Fourier Transform Infrared (FTIR) Spectroscopy and Chemometrics for Analysis of Lard Adulteration in "Rambak" Crackers. International Journal of Food Properties, 2718-2724.

Fadzlillah, N. A., Che Man, Y. B., Jamaludin, M. A., Rahman, S. A., & Al Kahtani, H. A. (2011). Halal Food Issues from Islamic and ModernScience Perspectives. 2nd International Conference on Humanities, Historical and Social Sciences, 17, 159–163.

Fadzlillah, N. A., Rohman, A., Rosman, A. S., Yusof, F. M., Ismail, A., Mustaffa, S., Minhat, A. E., & Khatib, A. (2016). Differentiation Of Fatty Acid Composition Of Butter Adulterated With Lard Using Gas Chromatography Mass Spectrometry Combined With Principal Component Analysis. Jurnal Teknologi, 78(2).

Febo, T. D., Schirone, M., Visciano, P., Portanti, O., Armillotta, G., Persiani, T., Giannatale, E. D., Tittarelli, M., & Luciani, M. (2018). Development of a Capture ELISA for Rapid Detection of Salmonella enterica in Food Samples. Food Analytical Methods, 12(2), 322-330.

Ferguson, M. (2019, November 12). What is Lard? Retrieved December 26, 2020, from https://www.webstaurantstore.com/blog/3068/what-is-lard.html

Henry, M. (2013, May 14). Why lard's healthier than you think. Retrieved January 22, 2021, from https://www.thestar.com/life/health_wellness/nutrition/2013/05/14/why_lards_healthier_than_you_think.html#:~:text=Cons%3A%20Not%20squeal%2Dworthy,lowers%20HDLs%2C%20the%20good%20cholesterol.

Hussain, M.N., Basri, K.N., Arshad, S., Mustafa, S., Khir, M.F.A. & Bakar, J. (2022). Analysis of Lard in Palm Oil Using Long-Wave Near-Infrared (LW-NIR) Spectroscopy and Gas Chromatography-Mass Spectroscopy (GC–MS). Food Analytical Methods

Jaron. (2020, June 10). The Best Substitutes For Shortening – What You Should Use. Retrieved January 21, 2021, from https://foodsguy.com/best-substitutes-for-shortening/

Kök, S., & Atalay, S. (2018). Determination of the Fraud of Processed Meat Products by ELISA. Research Article, 58(2), 95-98.

Kurniawati, E., Rohman, A., & Triyana, K. (2014). Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics. Meat Science, 96(1), 94-98.

Latief, M., Khorsidtalab, A., Saputra, I., Akmeliawati, R., Nurahikin, A., Jaswir, I., & Witjaksono, G. (2017). Rapid lard identification with portable electronic nose. IOP Conference Series: Materials Science and Engineering, 6, 1-8.

LARD: Meaning in the Cambridge English Dictionary. (n.d.). Retrieved November 26, 2022, from https://dictionary.cambridge.org/dictionary/english/lard

Lubis, H. N., Mohd-Naim, N. F., Alizul, N. N., & Ahmed, M. U. (2016). From market to food plate: Current trusted technology and innovations in halal food analysis. Trends in Food Science & Technology, 58, 55–68.

Mahpar, N., Parzi, M. N., & Satar, N. M. (2016). The restaurant boasts a 'no pig' sign raising the question. BH Online.

Manaf, Y. N., Marikkar, J. M., Mustafa, S., Van Bockstaele, F., & Nusantoro, B. P. (2019). Effect of three plant-based shortenings and lard on cookie dough properties and cookies quality. International Food Research Journal, 26(6), 1795-1802.

Mandli, J., Fatimi, I. E., Seddaoui, N., & Amine, A. (2018). Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat. Food Chemistry, 255, 380–389.

Marikkar, N., Alinovi, M., & Chiavaro, E. (2021). Analytical approaches for discriminating native lard from other animal fats. Italian Journal of Food Science, 33(1), 106-115.

Mohamad, F., Sairin, M. A., Nizar, N. N. A., Aziz, S. A., Hashim, D. M., Misbahulmunir, S., & Rokhani, F. Z. (2015). Investigation on interdigitated electrode design for impedance spectroscopy technique targeting lard detection application. 2015 9th International Conference on Sensing Technology (ICST), 739–744.

Nakyinsige, K., Man, Y. B., & Sazili, A. Q. (2012). Halal authenticity issues in meat and meat products. Meat Science, 91(3), 207-214.

Noor Raihana, A. R., Marikkar, J. M., Jaswir, I., Nurrulhidayah, A. F., & Miskandar, M. S. (2017). Effect of pink guava oil-palm stearin blends and lard on dough properties and cookies quality. International Food Research Journal, 24, 355-362.

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 9544086, 1-Palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol. Retrieved May 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol.

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 11366450, 1-Palmitoyl-2-oleoyl-3-stearoylglycerol. Retrieved May 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Palmitoyl-2-oleoyl-3-stearoylglycerol.

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 25240174, 1,2-Dioleoyl-3-palmitoylglycerol. Retrieved May 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/1_2-Dioleoyl-3-palmitoylglycerol.

Pisuthipan, A. (2015, December 8). Lard vs vegetable oil. Retrieved January 21, 2021, from: https://www.bangkokpost.com/life/social-and-lifestyle/788169/lard-vs-vegetable-oil

Perestam, A. T., Fujisaki, K. K., Nava, O., & Hellberg, R. S. (2017). Comparison of real-time PCR and ELISA-based methods for the detection of beef and pork in processed meat products. Food Control, 71, 346–352.

Reisner, A. (2020, March 16). What Is Lard and Is It Bad for You? Retrieved January 21, 2021, from https://www.eatthis.com/lard/

Rohman, A., & Man, Y. C. (2008). Physico-Chemical Methods for Determination of Lard In Food Products For Halal Authentication Study A. Agritech, 28(4).

Rohman, A., Himawati, A., Triyana, K., Sismindari, & Fatimah, S. (2016). Identification of pork in beef meatballs using Fourier transform infrared spectrophotometry and real-time polymerase chain reaction. International Journal of Food Properties, 20(3), 654–661.

Rohman, A., Triyana, K., Sismindari, & Erwanto, Y. (2012). Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis. International Food Research Journal, 16(2), 475-479.

Rosman, N. N., Mokhtar, N. F. K., Ali, M. E., & Mustafa, S. (2016). Inhibitory Effect of Chocolate Components Toward Lard Detection in Chocolate Using Real Time PCR. International Journal of Food Properties, 19(11), 2587–2595.

Ruslan, A. A., Kamarulzaman, N. H., & Sanny, M. (2018). Muslim consumers' awareness and perception of Halal food fraud. International Food Research Journal, 25(1), 87-89.

Saeed, T., Abu-Dagga, F., & Rahman, H. A. (1986). Detection of Pork and Lard as Adulterants in Beef and Mutton Mixtures. Journal of AOAC INTERNATIONAL, 69(6), 999-1002.

Said, H. 2017. Maqis seized containers of unseparated halal, non-halal meat at Tanjung Pelepas. The New Straits Times. Retrieved from website: https://www.nst.com.my/news/crime-courts/2017/07/258158/maqis-seizes-containers-unseparated-halal-nonhalal-meat-tanjung

Sairin, A., Aziz, A., Tan, C. P., Mustafa, S., Abd Ghani, S. S., & Rokhani, F. Z. (2019). Lard classification from other animal fats using Dielectric Spectroscopy technique. International Food Research Journal, 26(3), 773-782.

Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H., & Morimoto, S. (2018). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. Journal of natural medicines, 72(1), 32–42.

Salahudin, A., Ramli, M. A., Zulkepli, M. I. S., & Razak, M. I. A. (2018). Issues in Halal Meat Product and Authentication Technology from Islamic Perspectives. International Journal of Academic Research in Business and Social Sciences, 7(12).

Skouridou, V., Tomaso, H., Rau, J., Bashammakh, A. S., El-Shahawi, M. S., Alyoubi, A. O., & Osullivan, C. K. (2019). Duplex PCR-ELONA for the detection of pork adulteration in meat products. Food Chemistry, 287, 354–362.

Syahariza, Z., Man, Y., Selamat, J., & Bakar, J. (2005). Detection of lard adulteration in cake formulation by Fourier transform infrared (FTIR) spectroscopy. Food Chemistry, 92(2), 365-371.

Ulca, P., Balta, H., Çağın, I., & Senyuva, H. Z. (2013). Meat species identification and Halal authentication using PCR analysis of raw and cooked traditional Turkish foods. Meat Science, 94(3), 280–284.

Wu, H., Qian, C., Wang, R., Wu, C., Wang, Z., Wang, L., Zhang, M., Ye, Z., Zhang, F., He, J., & Wu, J. (2020). Identification of pork in raw meat or cooked meatballs within 20 min using rapid PCR and visual detection. Food Control, 109, 106905.

Yanty, N. A. M., Marikkar, J. M. N. and Abdulkarim, S. M. 2014. Determination of the type of fat ingredient in some commercial biscuit formulations. International Food Research Journal 21(1): 277–282.

Zakaria, Z. 2008. Tapping into the world halal market: Some Malaysian laws and standards discussions. Shariah Journal 16 (Special Edition): 603-616.

Zhou, Y., Abbar, S., Amoah, B., Phillips, T. W., & Schilling, M. W. (2016). Controlling pests in dry-cured ham: A review. Meat Science, 111, 183 191.

Zhuo, Z., Wang, J., Chen, W., Su, X., Chen, M., Fang, M., He, S., Zhang, S., Ge, S., Zhang, J., & Xia, N. (2018). A Rapid On-Site Assay for the Detection of Influenza A by Capillary Convective PCR. Molecular Diagnosis & Therapy, 22(2), 225–234.

Zhou, X., Han, G., Sun, Q., Liu, H., Liu, Q., & Kong, B. (2020). Influence of lard-based diacylglycerol on the rheological and physicochemical properties of thermally induced pork myofibrillar protein gels at different pH levels. LWT - Food Science and Technology, 117, 1-8.