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# Incorporation of Partial Least Squares-Discriminant Analysis with Ultra-High-Performance Liquid Chromatography Diode-Array Detector for Authentication of Skin Gelatine Sources

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**Abstract**

This research seeks to (1) authenticate sources of skin gelatine by combining putative 17 amino acids (AAs) analysis with chemometrics by Ultra-High-Performance Liquid Chromatography Diode-Array Detector (UHPLC-DAD) and (2) create AA profiles in skin gelatines. The classification capability of partial least square-discriminant analysis (PLS-DA) models was assessed to determine the most effective discriminant model. Principal component analysis (PCA) with quartimax rotation was utilised to accurately organise gelatine clusters and assign the significantly contributing AAs to each cluster. The PLS-DA model with 13 AAs (PLS-DAVIPAA) outperformed the PLS-DA model with 17 AAs (PLS-DAAA) because its $R^2Y$ (0.938), $R^2X$ (0.881), and $Q^2$ (0.929) values were greater. With 13 significant AAs, the PLS-DAVIPAA model obtained cluster classification accuracy of 100% on training and cross-validation datasets and 93.3% on testing and verification datasets. The chemical structure of gelatines may shed light on the interactions between AAs. Following six quartimax rotations, the gelatines were grouped correctly. The PCA showed the dominant presence of these AAs: L-Valine, L-Phenylalanine and L-Tyrosine in porcine gelatine; Glycine, L-Threonine, L-Arginine, L-Methionine, L-Histidine and L-Serine in fish gelatine; and L-Hydroxyproline, L-Leucine and L-Proline in bovine gelatine. The authority could use this technique to set a standard for authenticating skin gelatine samples.

## 1. Introduction

The gelatine industry was worth $1.34 billion in 2019. Approximately 80% of hydrolysed skin gelatine produces food gelatine products, such as marshmallows, gummies, and candies (Yap & Gam, 2019). Since most skin gelatines originate from porcine and bovine (Rather et al., 2022), some gelatine manufacturers commit food fraud via fraudulent adulteration, substitution and mislabeling of the source (Zia et al., 2020) due to the high demand and escalated cost to produce gelatine from various sources. This action was evident in previously reported food fraud on marshmallows, gumdrops, and Turkish delights containing declared halal gelatine (Garcia-Vaquero & Mirzapour-Kouhdasht, 2023). It could undermine the customers' right to consume gelatine-based products, especially for populations with certain religious beliefs. Our work addresses this action by establishing a method for authenticating skin gelatine sources, specifically porcine, bovine, and fish. This work used an Ultra-High-Performance Liquid Chromatography-Diode-Array Detector (UHPLC-DAD) and chemometrics to identify unique amino acids (AAs) as biomarkers in each type of skin gelatine.

In addition to UHPLC-DAD, Liquid Chromatography-Mass Spectrometer (LC/MS) and Liquid Chromatography Time-of-Flight Mass Spectrometer (LC-QTOF/MS) have been used to validate skin gelatine sources. Despite their high sensitivity, these mass spectrometer-based methods are costly (Rohman et al., 2020). Azilawati et al. (2015) used a High-Performance Liquid Chromatography-Fluorescence Detector (HPLC-FLD) in conjunction with principal component analysis (PCA) to measure amino acids (AAs) instead of LC/MS and LC-QTOF/MS. Yuswan et al. (2021) used HPLC-FLD to separate 17 AAs, validating and verifying the AA method and applying it to gelatine and collagen. Based on these works, we used this less expensive method in our study, except that we detected the separated AAs with a diode array detector, which has been shown to authenticate skin gelatine sources. This method was validated and verified on marshmallows, with the results published by Sani et al. (2021).

Previous studies validated and verified analytical methods for detecting AAs by establishing performance characteristics such as calibration curve, linearity range, the limit of detection and

quantification, sensitivity, and accuracy (Sani et al., 2020). Yuswan et al. (2021) performed a similar approach to comply with the requirement of ISO 17025: 2017 (Yuswan et al., 2020). However, Idris et al. (2021) proposed using putative biomarkers as an alternative and more expedient method for halal authentication, particularly in the case of ad-hoc analysis, due to the cost and time involved in performing these steps to achieve acceptable performance characteristics. In addition, a negligible number of reports mentioned this technique combined with chemometrics to authenticate skin gelatine sources. Consequently, our study utilised this method for the same objective.

Since it is the most prevalent chemometrics classification technique, discriminant analysis (DA) is utilised extensively in food authentication. Sani et al. (2021) also authenticated gelatine products using DA, whereas there are few reports of PLS-DA applications for authenticating gelatine sources. (Sani et al., 2020) noted that PLS-DA characteristics such as Fisher statistics and p-value could serve as discriminatory indicators for authentication purposes. In contrast, Sharin et al. (2021) stated that the importance of the variable in the projection (VIP) from the PLS-DA could identify the significantly contributing variables to the classification functions. Most previously reported chemometrics for food applications also conducted an authentication study at a significance level (α) of 0.05, allowing for a 5% error. Nevertheless, since the contamination of food with non-halal ingredients is a sensitive issue for Muslim consumers, our study authenticated the gelatine at α of 0.01 to increase the reliability of the results. The absence of employing Fisher statistics, p-value and α of 0.01 for halal authentication may impede the development of an authentication method for skin gelatine sources relative to other authentication methods, as these criteria have been shown to reduce the dimensionality of fatty acids methyl ester, thereby facilitating the authentication of oil-based products (Idris et al., 2021). Due to these considerations, our study explored the classification ability of PLS-DA by examining its features on a dataset of skin gelatine.

The next step in analysing the distribution of AAs in each source of skin gelatine is to use exploratory principal component analysis (PCA). Azilawati et al. (2015) and Ismail et al. (2021) performed the PCA. They assigned the contributing AAs by manually removing the skin gelatine samples from the dataset, which is undesirable because it presents a challenge to the testing laboratory, particularly in obtaining repeatability results due to an unstandardised procedure. To achieve correctly clustered samples, Ayerdi & Graña (2014) suggested using PCA with quartimax rotation, and our study used this strategy with varying values of varimax rotation until the skin gelatines were grouped into distinct clusters. This research could assign the contributing AAs to the various clusters and generate AA profiles for each. Because this approach has not been adopted and explained in previous studies, regulatory authorities are likely to be interested in issuing guidelines for the authentication of skin gelatine.

## 2. Methodology

### 2.1 Experimental design

The current study entailed skin gelatine preparation and AA analysis in skin gelatines and marshmallows via Ultra-High-Performance Liquid Chromatography Diode-Array Detector (UHPLC-DAD). The dataset was subjected to dataset pre-processing, PLS-DA and PCA with quartimax rotation. Figure 1 shows the experimental design of the current study.

### 2.2 Skin gelatine and marshmallow preparation

Cold-water fish (G7041), bovine (G9382) and porcine (G6144) skin gelatines were purchased from Sigma Aldrich, USA. Porcine, bovine, and fish gelatines were prepared to verify the AAs' presence in actual samples. A volume of 240 mL corn syrup (KHH Double Lion, Malaysia), 240 mL of deionised water and 300 g powdered sugar (MSM Malaysia Holdings Berhad, Malaysia) were mixed with 30 g porcine (Great Lakes Gelatin Co., USA). The mixture was homogenised at 200 rpm for 15 min under heating treatment at 80◦C to ensure the porcine gelatines bloom. Further works by Sani et al. (2021) on the preparation of the marshmallows pallets involving bovine (Halagel (M) Sdn. Bhd., Malaysia) and fish (Wani Erat Food Supplies Sdn Bhd., Malaysia) marshmallows before acid hydrolysis was followed.

Approximately 0.2 g gelatines and marshmallow pellets were acid-hydrolysed with 5 mL of 6 N HCL and incubated for 24 h at 110°C. The hydrolysate solution was mixed with 100 pmol/µL L-Aminobutyric acid (Aaba) in a 100 mL volumetric flask, marked up with distilled water and subjected to filtration with 0.45 µm cellulose acetate membrane to produce mixture A.

### 2.3 Amino acids analysis of skin gelatines and marshmallow

A volume of 10 µL mixture A was derivatised with 20 µL AccQ.Fluor reagent (Waters, Massachusetts, USA) and 70 µL AccQ.Fluor borate buffer (Waters, Massachusetts, USA) at pH maintained between 8.2 – 10.0 to produce mixture B. Then, mixture B was heated for 10 min at 55°C and spiked with 100 pmol/µL L-Aminobutyric acid (Aaba) solution (Waters, the USA) as an internal standard to produce mixture C. A volume of 1 µL mixture C was injected into UHPLC-DAD of Agilent, USA, and eluted by pre-filtered eluant A (AccQ.Tag™ concentrate (WAT052890)) and B (acetonitrile) for AA separation via a gradient elution set-up by Ismail et al. 2021. The AAs were separated at 36°C and 1 mL/min by a Waters AccQ.The tag column (3.9 mm x 150 mm) was subjected to detection by a diode array detector (DAD) at 260 nm.

The putatively detected AA peaks were confirmed by comparing their retention time with an injected mixture of 17 standard AAs and Aaba solutions, as shown in Figure 1. These AAs were selected based on the initial work by Sani et al. (2021). The performance characteristics of the 17 AAs, including method linearity and accuracy, had been validated and verified in our previous studies (Ismail et al., 2021a; Sani et al., 2021). The AA concentration in each skin gelatine was presented in percentage. The ratio of the peak area of AA over Aaba was computed for each gelatine and subjected to dataset pre-processing.

### 2.4 Dataset pre-processing

Four dataset types were prepared: training, cross-validation, testing and verification (marshmallow) datasets and these datasets were subjected to pre-processing, i.e. outlier treatment, dataset transformation, and dataset adequacy test at a significant level (α) of 0.01 using XLSTAT-Pro (2019) statistical tools (Addinsoft, Paris, France).

The training dataset of 40 porcine, 40 bovine and 40 fish skin gelatines was prepared for the chemometrics. Two types of cross-validation datasets of the PLS-DA model were also prepared: (1) 12 skin gelatines based on a 10-fold cross-

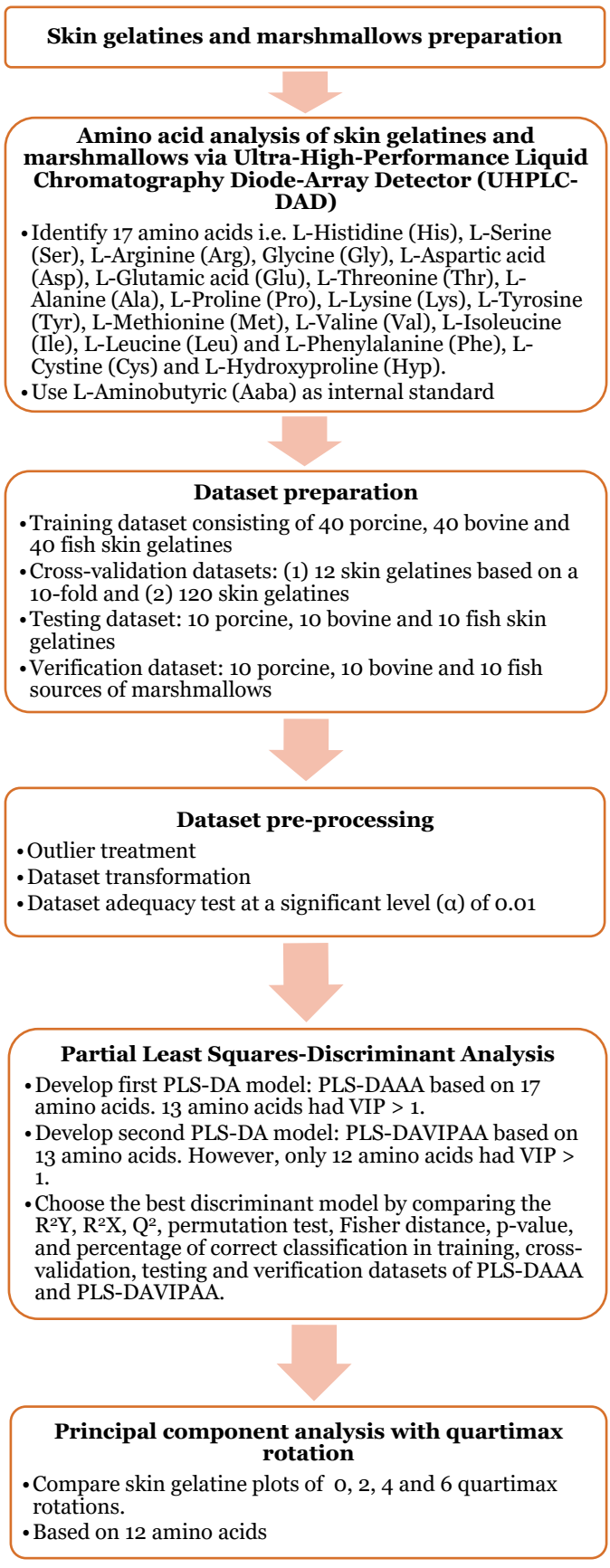


Figure 1: Experimental design of incorporation of partial least squares-discriminant analysis and quartimax rotation with ultra-high-performance liquid chromatography diode-array detector for authentication of skin gelatine sources.

validation dataset (120/10 = 12) (Sharin et al., 2021) and (2) 120 skin gelatines since the PLS-DA feature in XLSTAT-Pro (2019) allows the analysis of cross-validation against the training dataset.

A testing dataset consisting of 10 porcine, 10 bovine and 10 fish skin gelatines was prepared for PLS-DA based on the 80-to-20 minimum ratio of a training-to-testing dataset (Andrada et al., 2015). A verification dataset entailing 10 porcine, 10 bovine and 10 fish sources of marshmallows was also prepared to investigate the accuracy of the PLS-DA models in the actual sample.

The Grubbs and Dixons tests were carried out to identify outliers, replacing them with the mean value of individual AA. The dataset was transformed using the standardise (n-1) method to bring the dataset near normal distribution (Sani et al., 2021).

The dataset adequacy test was carried out using the Kaiser-Meyer-Olkin (KMO) test. The average KMO value was evaluated according to the criteria in the work of Ismail et al. (2021b). The KMO > 0.5 was acceptable for chemometrics.

Post dataset pre-processing, the dataset was subjected to PLS-DA and quartimax rotation of PCA.

### 2.5 Partial least square-discriminant analysis

The PLS-DA established a discriminating model (DM) for the skin gelatine sources. A new column labelled 'cluster' was added to the training, cross-validation, testing and verification datasets and each gelatine was assigned as 'porcine', 'bovine' and 'fish' clusters. The PLS-DA was carried out twice; the first PLS-DA was carried out involving 17 AAs (PLS-DAAA), while the second PLS-DA (PLS-DAVIPAA) was carried out involving significant AAs with variable importance in the projection () score > 1 (Idris et al., 2022). The significant AAs for PLS-DAVIPAA were selected from the PLS-DAAA's result.

The PLS-DA was carried out at α of 0.01 on the training dataset to establish a DM for the skin gelatines via Equation 1:

$$F(y_i, a_k) = b_0 + \sum_{j=1}^{p} b_i x_{ij} \text{ (Equation 1)}$$

Where $F$ is the function, $k$ is the number of skin gelatine classes for $y$ dependant variable, $a$ is the skin gelatine cluster, $b_0$ is the DM's intercept, $p$ is the number of AAs, $b_i$ Is DM's coefficient, and $x$ is the observation. The DM discriminated skin gelatines into $a$ class via Equation 2:

$$x = argmax_k F(y_i, a_k) \text{ (Equation 2)}$$

The DM returns the argument (arg) of the $F$ to the maximum (max) class, i.e., k = 3 in this study. The goodness of fit for the DM was evaluated via the R²Y cumulated index, while the quality of the AAs as contributors to the DM was evaluated via the R²X cumulated index. The ability of the DM to classify the skin gelatine clusters was measured via cross-validation of the PLS-DA and represented as a Q² cumulated index.

The classification robustness and accuracy of the PLS-DA models were evaluated using permutation tests with 100 random permutations ($p < 0.01$) via MetaboAnalyst 5.0 (McGill University, Canada). The permutation test calculated the p-value against the hypothesis null ($H_0$), i.e., no difference among the skin gelatine clusters. The PLS-DA model rejected the $H_0$

when the p-value was less than 0.01, indicating 99% accuracy in classifying the skin gelatine clusters.

The predictive ability of the DMs was further evaluated on the percentage of correct classification of the porcine, bovine and fish skin gelatines. Cluster dissimilarity was also assessed via the Fisher distance and their distance p-values (Sani et al., 2023). To validate the predictive ability of the DM, the established DM was validated and tested on cross-validation and testing datasets, respectively, and their percentage of correct classifications was evaluated. Based on the $R^2Y$, $R^2X$, $Q^2$, permutation test, Fisher distance, p-value, and percentage of correct classification in training, cross-validation, testing and verification datasets, the classification ability of PLS-DAAA and PLS-DAVIPAA was compared, and the best DM was selected.

### 2.6 Principal component analysis with quartimax rotation

A Pearson correlation of principal component analysis (PCA) at α of 0.01 was employed to group skin gelatines into porcine, bovine and fish clusters and identify the AA distribution. Twelve significant AAs ($p < 0.01$) identified by PLS-DAVIPAA were transformed and underwent PCA to generate 12 principal components (PCs) known as independent variables. Component score $C$ for $y$ PC number and $n$ sample number as expressed in Equation 3:

$$C_{yn} = f_{y1}a_{n1} + f_{y2}a_{n2} + \cdots + f_{yi}a_{ni} \quad \text{(Equation 3)}$$

Where $f$ is the factor loading (FL), $a$ is the AA concentration, and $i$ is the total number of AA.

Cumulative variability (CV) of two-dimensional PCs entailing PC1 and PC2 were computed for the AA profile exploratory. To group the skin gelatines into porcine, bovine and fish clusters, quartimax rotation at zero (no rotation), two, four and six rotations were carried out. The quartimax rotation was stopped at six rotations as the skin gelatine sources had been clustered with the highest CV. The comparison among the numbers of quartimax rotation was depicted as skin gelatine plots.

The strong, moderate and weak contributing AAs to the clusters were evaluated based on factor loading (FL) criteria: $FL \geq |0.750|$ for strong, $|0.500| < FL < |0.749|$ for moderate and $FL \leq |0.499|$ for weak contributing AAs. The AA profile in each cluster was assessed via a biplot of skin gelatines and amino acids with six quartimax rotations.

## 3. Results and discussion

### 3.1 Amino acids content in porcine, bovine and fish skin gelatines

This study investigated the distribution of AA content in porcine, bovine and fish skin gelatines. Table 1 shows the amino acid content in each skin gelatine. The presence of 17 AAs in the gelatine was confirmed with a retention time of SS. The chromatograms and validation and verification of the 17 AAs' performance characteristics, including method linearity and accuracy, were retrievable in our previous studies (Ismail et al., 2021a; Sani et al., 2021). Glycine was dominant, while His was undetected in the porcine skin gelatine. The ranking of AA concentration in the porcine skin gelatine was as follows: Gly (33.66%) > Pro (12.16%) > Hyp (10.63%) > Ala (9.77%) > Glu (6.54%) > Arg (6.30%) > Lys (3.92%) > Asp (3.48%) > Ser (3.08%) > Leu (2.46%) > Val (2.37%) > Thr (1.79%) > Phe (1.56%) > Ile (1.05%) > Met (0.77%) > Tyr (0.45%) > His (0.00%). In comparison with Hafidz & Yaakob (2011), Azilawati et al. (2015) and Widyaninggar et al. (2012), our study had a similar AA distribution: Gly > Pro, Asp > Ser, and Ile > Met > Tyr. The AA distribution in porcine skin gelatine analysed by a validated and verified method by Sani et al. (2021) showed a similar distribution. Although the porcine bone could also be used to produce gelatine, to the authors' knowledge, no report was found on the AA distribution from the porcine bone gelatine.

Based on the ranking of bovine skin gelatine, i.e. Gly (33.83%) > Pro (11.90%) > Hyp (10.89%) > Ala (9.95%) > Glu (6.72%) > Arg (5.95%) > Lys (3.84%) > Asp (3.65%) > Ser (3.11%) > Leu (2.46%) > Val (2.23%) > Thr (1.79%) > Phe (1.47%) > Ile (1.26%) > Met (0.65%) > Tyr (0.28%) > His (0.00%) (Table 1), the AA distribution was similar to the AA distribution of porcine skin gelatine probably due to both of porcine and bovine are mammals. This similarity may render difficulty in differentiating the porcine and bovine skin gelatines. This AA distribution contradicted the findings of Azilawati et al. (2015) and Hafidz & Yaakob (2011) except Gly > Pro, Asp > Ser and Ile > Met > Tyr > His distributions. Valipour et al. (2008) identified the AA distribution of bovine bone gelatine as follows: Gly (17.24%) > Glu (15.56%) > Asp (11.47%) > Pro (9.4%) > Ala (6.67%) > Lys (3.78%) > Thr (3.15%) > Phe (3.15%) > Ser (2.94%) > Arg (2.38%) > Leu (2.27%) > Val (2.09%) > Ile (1.15%) > Met (0.78%) > His (0.67%) > Tyr (0.66%). From this distribution, both bovine skin and bone gelatines had Gly as the dominant AA, similar to the Ile > Met distribution. Conversely, Valipour et al. (2008) identified 0.67% His in the bovine bone gelatine, while our result found no His in the bovine skin gelatine.

Table 1 also presents the AA distribution of fish skin gelatine, which followed this ranking: Gly (35.44%) > Ala (9.73%) > Pro (9.43%) > Arg (6.77%) > Glu (6.25%) > Hyp (6.22%) > Ser (6.02%) > Lys (3.81%) > Asp (3.79%) > Thr (2.76%) > Val (2.02%) > Leu (2.02%) > Met (1.81%) > Phe (1.43%) > Ile (1.20%) > His (0.96%) > Tyr (0.33%). The AA distribution of yellowfin tuna (*Thunnus albacares*) skin gelatine was in line with our finding at the Pro > Ar > Glu and Ile > His > Tyr ranking (Nurilmala et al., 2019). Nevertheless, Nawaz et al. (2020) stated that cold-water fish skin gelatine had lower Hyp than the skin gelatine of warm-water fish. This finding was supported by a higher Hyp in tilapia (*Oreochromis mossambicus*), yellowfin tuna (*Thunnus albacares*) and blackcarp (*Mylopharyngodon piceus*) than cod (*Gadus morhua*), hake (*Merluccius capensis*) and alaska pollock (*Gadus chalcogrammus*). Of the 17 AAs, our fish skin gelatines had similar AA distribution of Met > Phe > Ile > His > Hyl > Tyr in cod; Gly > Ala > Pro, Glu > Hyp, Thr > Val, and Met > Phe in hake; and: Gly > Ala > Pro and Met > Phe > Ile > His > Hyl > Tyr (Derkach et al., 2020). The bone gelatine of *Ephinephelus sp*. has Gly > Pro > Glu > Ala > Arg > Asp > Leu > Ser > Lys > Thr >Val > Phe > Ile > His > Tyr distribution where only Thr > Val and Phe > Ile > His distribution were similar to our study (Suprayitno, 2019). These findings indicated that cold-water fish's skin and bone gelatine had their individual AA distribution, although some similarities are recorded. For this reason, the skin gelatine of porcine, bovine, and fish was differentiated via statistical analysis.

The ANOVA test in Table 1 shows a significant difference in the mean value of AA among the skin gelatines of porcine, bovine, and fish, where skin gelatines with different superscript alphabets were significantly different ($p < 0.01$). The Arg, Pro,

Tyr, Met, Val and Ile significantly differed among the three skin gelatines. Specifically, the gelatine of porcine skin had the highest Pro, Tyr and Val content and the lowest Ile content. The gelatine of bovine skin had the highest Ile percentage and the lowest Arg, Tyr and Met. The fish skin gelatine had the highest Arg and Met content and the lowest Pro and Val content.

The Arg, Pro, Tyr, Met, Val and Ile could be utilised to differentiate porcine and bovine skin gelatines, although the AA distribution between these skin gelatines was similar. However, the content differences between porcine and fish skin gelatines were significant in all AAs except Ala and Lys. Likewise, our study observed a significant difference in all AAs except Asp, Ala, Lys, and Phe in bovine and fish skin gelatines. Nevertheless, the application of the ANOVA test was insufficient to discriminate the three gelatines since more than one AA characterised the gelatines; hence, Sani et al. (2021) and Ismail et al. (2021a) proposed the chemometric application to discriminate the skin gelatines.

### 3.2 Outlier treatment and dataset adequacy

Prior to the chemometrics, the skin gelatine datasets underwent pre-processing to ensure the dataset fulfilled the chemometrics prerequisite, including outlier treatment, dataset transformation, and dataset adequacy test (Ismail et al., 2021b). The training dataset had 29, 12 and 21 outliers in the porcine, bovine and fish skin gelatines (Table 1), respectively, where our method replaced the outliers with the mean value of each AA (Sani et al., 2021). Then, this training dataset was transformed via the standardised (n-1) method. Although only negligible reports carried out dataset transformation in their works, our study performed the transformation to fulfil the prerequisite of chemometrics (Azilawati et al., 2015). Additionally, various dataset transformations are available for chemometrics, e.g., standardise (n), standard deviation$^{-1}$ (n-1), standard deviation$^{-1}$ (n), centre, 0 to 1 rescaling, 0 to 100 rescaling, Pareto and log methods (Ismail et al., 2021b); however, our study adopted standardised (n-1) as proposed by Sani et al. (2021) for gelatine matrix since high AA numbers achieved normality post this transformation.

Table 1 shows the individual KMO value for each AA, where Met (0.9274) and Ile (0.6075) had the highest and lowest KMO values, respectively. A comparison of the average KMO value (0.7874) with the guideline from Williams and Brown's (2012) study indicated that the dataset adequacy fell on the good ranking (0.7 < KMO < 0.8). Yuswan et al. (2021) and Azilawati et al. (2015) employed chemometrics without declaring the fulfilment of the dataset adequacy; hence, comparing the results may not be possible. Nevertheless, other gelatine studies found that KMO > 0.7 signified that the dataset was adequate for chemometrics (Sani et al., 2021). Our KMO value (0.7874) was higher than the gelatine study by Ismail et al. (2021) (KMO = 0.7542). Based on these comparisons, our KMO value indicated that the dataset was adequate for chemometrics.

### 3.3 Development of a model of partial least square discriminant analysis for skin gelatine sources

In this study, the partial least square-discriminating analysis (PLS-DA) model generated two components to explain the classification ability of the sources of skin gelatine. Table 2 shows two PLS-DA models, i.e., the PLS-DA model for 17 AAs (PLS-DAAA) and the PLS-DA model for AA with variable importance in the projection (VIP) score > 1 (PLS-DAVIPAA). Sharin et al. (2021) recommended a VIP score > 1 since a high VIP score could explain most of the variance among the porcine, bovine and fish gelatines. The best PLS-DA model was selected by evaluating the performance of the PLS-DAAA and PLS-DAVIPAA models.

The quality of both PLS-DA models was evaluated via $R^2Y$, $R^2X$ and $Q^2$ cumulated indices on each component. The $R^2Y$ (0.938) and $R^2X$ (0.881) of PLS-DAVIPAA were higher than the PLS-DAAA ($R^2Y$ = 0.894 and $R^2X$ = 0.771), indicating that the PLS-DAVIPAA was better in explaining the gelatine clusters and AA contribution to the gelatine clusters, respectively. These findings were associated with the definition of $R^2Y$, which is a sum of determination coefficients between the gelatine clusters and two components. The $R^2Y$ also indicated the variance proportion of the gelatine clusters explained by the PLS-DA model, showing the model's goodness of fit. The $R^2Y \sim 1$ represented a perfect goodness of fit. The $R^2X$ was computed as the sum of determination coefficients between the actual and predicted AAs within the two components. The $R^2X$ value indicated the variability of the AAs where the $R^2X \sim 1$ showed the highest quality of the AAs; hence measured the AAs quality as contributors to the PLS-DA model.

The $Q^2$ of PLS-DAVIPAA (0.929) was also higher than the $Q^2$ of PLS-DAAA (0.873), signifying that the two components generated by the PLS-DAVIPAA model had a significant contribution to predictive quality for skin gelatine sources. Since $Q^2$ was a predictive ability evaluation from the cross-validation dataset, it was also an indicator that the predictive ability of PLS-DAVIPAA for the new dataset was better than PLS-DAAA, where $Q^2 \sim 1$ was the perfect prediction.

Table 2 shows that the AAs with VIP scores > 1 significantly contributed to the predictive quality of skin gelatine sources. The PLS-DAAA identified 13 significant AAs with descending VIP scores, i.e., Hyp (1.417) > Met (1.406) > Thr (1.384) > Tyr (1.356) > Ser (1.351) > His (1.343) > Phe (1.278) > Pro (1.228) > Arg (1.207) > Gly (1.182) > Val (1.177) > Leu (1.171) > Ile (1.023) where the Hyp and Ile were the most and least significant AA, respectively. Based on the VIP score, these AAs explain most of the variance among the porcine, bovine and fish skin gelatines. Hence, these AAs could be used to differentiate the skin gelatine sources. Nevertheless, all 13 AAs of the PLS-DAVIPAA except Ile (0.984) yielded VIP scores > 1, confirming the AA significance in discriminating against the gelatine sources (Table 2).

The calculated permutation tests for PLS-DAAA and PLS-DAVIPAA were similar ($p < 0.01$), indicating they could classify the clusters with 99% accuracy, which could be further explained in the correct classification results. Table 2 also exhibits the correct classification of PLS-DAAA on the porcine, bovine and fish gelatines via 17 AAs. The training and 12 gelatines in cross-validation datasets showed 100% total classification of the porcine, bovine and fish skin gelatines (Table 2), indicating the PLS-DAAA was able to discriminate the gelatines at a 99% confidence level using these datasets. The correct classification of the PLS-DAAA model reduced to 98.8%, 93.3% and 93.3% for 120 skin gelatines in cross-validation, testing and verification datasets, respectively, indicating that the classification ability was negatively affected in the actual sample. On the contrary, the PLS-DAVIPAA had improved the correct classification (100%) using the training and 12 and 120 skin gelatines in cross-validation datasets using 13 significant AAs.

Table 1: Amino acid percentage, number of outliers, and sampling adequacy result of porcine, bovine and fish skin gelatines

| Amino acid | Retention time, min[1] | Amino acid concentration in testing dataset, %[1,2] | | | Number of outliers in testing dataset[1,3,4] | | | Sampling adequacy value[1,5] |
|---|---|---|---|---|---|---|---|---|
| | | Porcine skin gelatine | Bovine skin gelatine | Fish skin gelatine | Porcine skin gelatine | Bovine skin gelatine | Fish skin gelatine | |
| L-Hydroxyproline (Hyp) | 1.774 | 10.63 ± 0.45[a] | 10.89 ± 0.67[a] | 6.22 ± 0.19[b] | 4 (P3, P18, P19 and P40) | 1 (B24) | 1 (F37) | 0.9048 |
| L-Histidine (His) | 1.887 | 0.00 ± 0.01[b] | 0.00 ± 0.00[b] | 0.96 ± 0.05[a] | 3 (P18, P19, P21, P28 and P40) | 1 (B29) | 0 | 0.8519 |
| L-Serine (Ser) | 2.543 | 3.08 ± 0.15[b] | 3.12 ± 0.15[b] | 6.02 ± 0.16[a] | 1 (P40) | 1 (B24) | 1 (F9) | 0.7196 |
| L-Arginine (Arg) | 2.610 | 6.30 ± 0.29[b] | 5.95 ± 0.25[c] | 6.77 ± 0.20[a] | 1 (P9) | 1 (B24) | 1 (F37) | 0.6317 |
| Glycine (Gly) | 2.776 | 33.66 ± 1.05[b] | 33.83 ± 0.93[b] | 35.44 ± 0.88[a] | 1 (P9) | 1 (B24) | 1 (F9 and F39) | 0.7586 |
| L-Aspartic acid (Asp) | 2.987 | 3.48 ± 0.36[b] | 3.65 ± 0.34[ab] | 3.79 ± 0.28[a] | 2 (P4, P15) | 0 | 1 (F13 and F30) | 0.8428 |
| L-Glutamic acid (Glu) | 3.365 | 6.54 ± 0.48[a] | 6.72 ± 0.47[a] | 6.25 ± 0.33[b] | 1 (P15) | 0 | 1 (F13 and F30) | 0.8320 |
| L-Threonine (Thr) | 3.720 | 1.79 ± 0.08[b] | 1.79 ± 0.07[b] | 2.76 ± 0.08[a] | 1 (P9) | 1 (B24) | 1 (F9) | 0.7721 |
| L-Alanine (Ala) | 4.077 | 9.77 ± 0.51[a] | 9.95 ± 0.57[a] | 9.73 ± 0.33[a] | 1 (P15) | 0 | 2 (F13 and F30) | 0.7139 |
| L-Proline (Pro) | 4.668 | 12.16 ± 0.32[a] | 11.90 ± 0.36[b] | 9.43 ± 0.18[c] | 2 (P9, P15) | 1 (B13) | 2 (F13 and F39) | 0.8938 |
| L-Lysine (Lys) | 6.009 | 3.92 ± 0.31[a] | 3.84 ± 0.40[a] | 3.81 ± 0.20[a] | 1 (P15) | 0 | 1 (F13 and F30) | 0.7901 |
| L-Tyrosine (Tyr) | 6.107 | 0.45 ± 0.03[a] | 0.28 ± 0.03[c] | 0.33 ± 0.03[b] | 2 (P6 and P40) | 0 | 1 (F38) | 0.8049 |
| L-Methionine (Met) | 6.351 | 0.77 ± 0.04[b] | 0.65 ± 0.18[c] | 1.81 ± 0.08[a] | 1 (P8) | 0 | 3 (F9, F37 and F38) | 0.9274 |
| L-Valine (Val) | 6.624 | 2.37 ± 0.04[a] | 2.23 ± 0.06[b] | 2.02 ± 0.04[c] | 2 (P9 and P15) | 1 (B13) | 1 (F13) | 0.7890 |
| L-Isoleucine (Ile) | 7.940 | 1.05 ± 0.03[c] | 1.26 ± 0.03[a] | 1.20 ± 0.04[b] | 2 (P8 and P15) | 1 (B5) | 0 | 0.6075 |
| L-Leucine (Leu) | 8.052 | 2.46 ± 0.04[a] | 2.46 ± 0.07[a] | 2.02 ± 0.06[b] | 2 (P9 and P15) | 1 (B13) | 1 (F13) | 0.7797 |
| L-Phenylalanine (Phe) | 8.184 | 1.56 ± 0.05[a] | 1.47 ± 0.10[b] | 1.43 ± 0.05[b] | 2 (P10 and P11) | 2 (B12 and B24) | 3 (F8, F26 and F37) | 0.6588 |
| Total outliers | nr | nr | nr | nr | 29 | 12 | 21 | nr |
| Average KMO value | nr | nr | nr | nr | nr | nr | nr | 0.7874 |

Note: [1]nr = not related.

[2]Different superscript alphabets indicated a significant difference in average relative error mean.

[3]Number of detected outliers by Grubbs and Dixon tests.

[4]Skin gelatine in parenthesis indicates the outlier presence.

[5]Sampling adequacy test by Kaiser-Meyer-Olkin (KMO) test.

Table 2: Classification matrix of training, validation and testing datasets of partial least square – discriminant analysis

| Discriminating model (DM) | Discriminating model quality | | Dataset | Correct classification, % | Number of gelatines, Fisher distance value and p-value of Fisher distance in skin gelatine cluster[3,4] | | | Total |
|---|---|---|---|---|---|---|---|---|
| | $R^2Y$, $R^2X$ and $Q^2$ cumulated indices and permutation test ($p < 0.01$) | Ranking of significant amino acid ($p < 0.01$)[1,2] | | | Porcine skin gelatine | Bovine skin gelatine | Fish skin gelatine | |
| Partial least square–discriminant analysis for 17 amino acids (PLS-DAAA) | $R^2Y$ : 0.894; $R^2X$ : 0.771; $Q^2$ : 0.873; and $p < 0.01$ | Hyp (1.417) > Met (1.406) > Thr (1.384) > Tyr (1.356) > Ser (1.351) > His (1.343) > Phe (1.278) > Pro (1.228) > Arg (1.207) > Gly (1.182) > Val (1.177) > Leu (1.171) > Ile (1.023) | Training dataset (120 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 40 (0, 1) | 0 (294, < 0.0001) | 0 (2764, < 0.0001) | 40 |
| | | | Bovine gelatine | 100 | 0 (294, < 0.0001) | 40 (0, 1) | 0 (2762, < 0.0001) | 40 |
| | | | Fish gelatine | 100 | 0 (2764, < 0.0001) | 0 (2762, < 0.0001) | 40 (0, 1) | 40 |
| | | | Total | 100 | 40 | 40 | 40 | 120 |
| | | | Cross-validation dataset (10-fold – 12 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 4 (0, 1) | 0 (294, < 0.0001) | 0 (2764, < 0.0001) | 4 |
| | | | Bovine gelatine | 100 | 0 (294, < 0.0001) | 4 (0, 1) | 0 (2762, < 0.0001) | 4 |
| | | | Fish gelatine | 100 | 0 (2764, < 0.0001) | 0 (2762, < 0.0001) | 4 (0, 1) | 4 |
| | | | Total | 100 | 4 | 4 | 4 | 12 |
| | | | Cross-validation dataset (120 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 40 (0, 1) | 0 (294, < 0.0001) | 0 (2764, < 0.0001) | 40 |
| | | | Bovine gelatine | 96.5 | 2 (294, < 0.0001) | 38 (0, 1) | 0 (2762, < 0.0001) | 40 |
| | | | Fish gelatine | 100 | 0 (2764, < 0.0001) | 0 (2762, < 0.0001) | 40 (0, 1) | 40 |
| | | | Total | 98.8 | 42 | 38 | 40 | 120 |
| | | | Testing dataset (30 skin gelatines) | | | | | |
| | | | Porcine gelatine | 90 | 9 (0, 1) | 1 (294, < 0.0001) | 0 (2764, < 0.0001) | 10 |
| | | | Bovine gelatine | 90 | 1 (294, < 0.0001) | 9 (0, 1) | 0 (2762, < 0.0001) | 10 |
| | | | Fish gelatine | 100 | 0 (2764, < 0.0001) | 0 (2762, < 0.0001) | 10 (0, 1) | 10 |
| | | | Total | 93.3 | 10 | 10 | 10 | 30 |
| | | | Verification dataset (30 marshmallows) | | | | | |
| | | | Porcine gelatine | 90 | 9 (0, 1) | 1 (294, < 0.0001) | 0 (2764, < 0.0001) | 10 |
| | | | Bovine gelatine | 90 | 1 (294, < 0.0001) | 9 (0, 1) | 0 (2762, < 0.0001) | 10 |
| | | | Fish gelatine | 100 | 0 (2764, < 0.0001) | 0 (2762, < 0.0001) | 10 (0, 1) | 10 |
| | | | Total | 93.3 | 10 | 10 | 10 | 30 |
| PLS-DA for AA with variable importance in the projection (VIP) > 1 (PLS-DAVIPAA) | $R^2Y$ : 0.938; $R^2X$ : 0.881; $Q^2$ : 0.929; and $p < 0.01$ | His (1.282) > Tyr (1.275) > Met (1.257) > Ser (1.249) > Phe (1.198) > Thr (1.183) > Hyp (1.160) > Pro (1.121) > Arg (1.110) > | Training dataset (120 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 40 (0, 1) | 0 (227, < 0.0001) | 0 (2901, < 0.0001) | 40 |
| | | | Bovine gelatine | 100 | 0 (227, < 0.0001) | 40 (0, 1) | 0 (3316, < 0.0001) | 40 |
| | | | Fish gelatine | 100 | 0 (2901, < 0.0001) | 0 (3316, < 0.0001) | 40 (0, 1) | 40 |
| | | | Total | 100 | 40 | 40 | 40 | 120 |
| | | | Cross-validation dataset (10-fold – 12 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 4 (0, 1) | 0 (227, < 0.0001) | 0 (2901, < 0.0001) | 11 |
| | | | Bovine gelatine | 100 | 0 (227, < 0.0001) | 4 (0, 1) | 0 (3316, < 0.0001) | 15 |
| | | | Fish gelatine | 100 | 0 (2901, < 0.0001) | 0 (3316, < 0.0001) | 4 (0, 1) | 14 |

| Discriminating model (DM) | Discriminating model quality | | Dataset | Correct classification, % | Number of gelatines, Fisher distance value and p-value of Fisher distance in skin gelatine cluster[3,4] | | | Total |
|---|---|---|---|---|---|---|---|---|
| | $R^2Y$, $R^2X$ and $Q^2$ cumulated indices and permutation test ($p < 0.01$) | Ranking of significant amino acid ($p < 0.01$)[1,2] | | | Porcine skin gelatine | Bovine skin gelatine | Fish skin gelatine | |
| | | Gly (1.102) > Val (1.041) > Leu (1.015) > Ile (0.984) | Total | 100 | 4 | 4 | 4 | 40 |
| | | | Cross-validation dataset (120 skin gelatines) | | | | | |
| | | | Porcine gelatine | 100 | 40 (0, 1) | 0 (227, < 0.0001) | 0 (2901, < 0.0001) | 40 |
| | | | Bovine gelatine | 100 | 0 (227, < 0.0001) | 40 (0, 1) | 0 (3316, < 0.0001) | 40 |
| | | | Fish gelatine | 100 | 0 (2901, < 0.0001) | 0 (3316, < 0.0001) | 40 (0, 1) | 40 |
| | | | Total | 100 | 40 | 40 | 40 | 120 |
| | | | Testing dataset (30 skin gelatines) | | | | | |
| | | | Porcine gelatine | 90 | 9 (0, 1) | 1 (227, < 0.0001) | 0 (2901, < 0.0001) | 10 |
| | | | Bovine gelatine | 90 | 1 (227, < 0.0001) | 9 (0, 1) | 0 (3316, < 0.0001) | 10 |
| | | | Fish gelatine | 100 | 0 (2901, < 0.0001) | 0 (3316, < 0.0001) | 10 (0, 1) | 10 |
| | | | Total | 93.3 | 10 | 10 | 10 | 30 |
| | | | Verification dataset (30 marshmallows) | | | | | |
| | | | Porcine gelatine | 90 | 9 (0, 1) | 1 (227, < 0.0001) | 0 (2901, < 0.0001) | 10 |
| | | | Bovine gelatine | 90 | 1 (227, < 0.0001) | 9 (0, 1) | 0 (3316, < 0.0001) | 10 |
| | | | Fish gelatine | 100 | 0 (2901, < 0.0001) | 0 (3316, < 0.0001) | 10 (0, 1) | 10 |
| | | | Total | 93.3 | 10 | 10 | 10 | 30 |

Note: [1]Value in parenthesis was an F-statistic value of significant amino acid with variable importance in the projection (VIP) > 1.

[2]Hyp = L-Hydroxyproline, His = L-Histidine, Ser = L-Serine, Arg = L-Arginine, Gly = Glycine, Asp = L-Aspartic acid, Glu = L-Glutamic acid, Thr = L-Threonine, Ala = L-Alanine, Pro = L-Proline, Lys = L-Lysine, Tyr = L-Tyrosine, Met = L-Methionine, Val = L-Valine, Ile = L-Isoleucine, Leu = L-Leucine and Phe = L-Phenylalanine.

[3]Values in parenthesis were the Fisher distance value and p-value of Fisher distance, respectively.

[4]Calculated p-value of Fisher distance < 0.01 indicated that the three clusters were significantly different.

The correct classification was also reduced to 93.3% for testing and verification datasets, where the verification dataset was the actual sample (marshmallow). The PLS-DAVIPAA also had a higher and significant Fisher distance value ($p < 0.0001$), i.e., porcine vs bovine (227), porcine vs fish (2901) and bovine vs fish (3316) compared to PLS-DAAA. These results showed that the clusters produced by PLS-DAVIPAA were significantly separated, thus allowing higher authentication accuracy for the skin gelatine sources than the PLS-DAAA. Based on these results, PLS-DAVIPAA with 13 significant AAs was the best DM model to authenticate the skin gelatine sources.

Figure 2 (b) also supported the finding for higher accuracy of PLS-DAVIPAA than PLS-DAAA in Figure 2 (a). The PLS-DAVIPAA showed that each porcine, bovine and fish skin gelatine was located nearer within their clusters than the skin gelatine plot by PLS-DAAA. This result was evident in a narrower range of component 2 score of PLS-DAVIPAA than PLS-DAAA, i.e., porcine skin gelatine (-3 to 0), bovine skin gelatines (1 to 4) and fish skin gelatine (-1 to 1) in Figure 2 (b). In contrast, the component 2 score of the gelatine sources for PLS-DAAA was as follows: -4 to 0 for porcine skin gelatines, 0 to 5 for bovine skin gelatine, and -3 to 1 for fish skin gelatine (Figure 2 (a)). Nevertheless, comparing the clusters via these plots was prone to inaccurate interpretation due to macroscopic evaluation; hence, comparison via $R^2Y$, $R^2X$, $Q^2$, Fisher distance, p-value, percentage of correct classification in training, cross-validation, testing and verification datasets, and permutation test value was preferable. Westerhuis et al. (2008) also recommended against using skin gelatine plots to interpret the classification accuracy.

Of these AAs, Figure 2 (c) depicted the 17 AAs plot from PLS-DAAA with individual values of the correlation matrix (CMV) for each AA, where His, Hyp, Ser, Thr, Met, Pro and Leu had CMV of 0.95 – 0.88 and were followed by Val, Arg and Gly with CMV of 0.69 – 0.63 in component 1. The Ile, Phe, Tyr, Glu, Asp, Lys and Ala had the lowest CMV (0.47 – 0.064). For component 2, the Tyr and Phe had the highest CMV (0.85 – 0.83); Arg, Gly, Val, Lys and Ala had the moderate CMV (0.70 – 0.51); and Glu, Asp, Pro, Leu, Ile, Met, Thr, Ser, His and Hyp had the lowest CMV (0.38 – 0.04). Figure 2 (d) of PLS-DAVIPAA shows the CMV of 13 AAs where the CMV for each AA in components 1 and 2 had a similar value to the PLS-DAAA. Jannat et al. (2018, 2020b) carried out PLS-DA analyses to distinguish porcine, bovine and fish gelatines, but none explained the CMV of the detected compounds or AAs. Nevertheless, Ismail et al. (2021) classified the AAs into strong, moderate and weak factor loading for AAs according to the CMV of AAs from principal component analysis (PCA), not PLS-DA. The CMV was used to delineate the AA relationship among them and assign the AA to the gelatine sources (Ismail et al., 2021b).

Figure 2 (d) exhibits positive correlations based on AA direction proximities: His, Ser, Met and Thr; Gly and Arg; Tyr and Phe; and Leu and Pro. On the contrary, negative correlations of AAs were observed based on their opposite direction: His, Ser, Met and Thr against Hyp; Tyr and Phe against Ile; Val against Ile; and Leu and Pro against Ile. Arg and Gly did not correlate with Ile since their directions were at 90°.

Ismail et al. (2021) proposed that the AA's correlations were due to its polarity side chain; however, our study found that only Met has a non-polar side chain, although it had a positive correlation with His, Ser and Thr. Further generic grouping of AAs, e.g. basic, carboxylic, hydroxylic and hydrophobic, based on the chemical characteristics of Derkach et al. (2020), could not support the AA correlations. The opposite side chains of Gly and Arg and Tyr and Phe also signified that the correlations of AAs were independent of their polarity side chain and generic chemical characteristics. Nevertheless, the backbone of the chemical structure may suggest the reason for the positive correlations among the AAs and vice versa. For instance, Met, His, Ser and Thr, and Gly and Arg shared HO-CO-$CNH_2$-backbone; Tyr and Phe shared HO-CO-$CNH_2$-$CH_2$-benzene backbone while Leu and Pro shared HO-CO- backbone. Furthermore, Hyp and Pro, and Leu and Val were also positioned at close proximity that shared HO-CO-pyrrole and HO-CO-$NH_2$ backbones, respectively.

To assign the AAs to porcine, bovine and fish skin gelatines, the skin gelatines and AA plots shall be overlaid together where the PLS-DA feature of XLSTAT 2019 could not be provided in this study. However, PCA is preferable since AA and skin gelatine plots are available in the PCA feature that serves as exploratory chemometrics. Hence, this current study carried out the AAs assignment via PCA in the next section.

### 3.4 Exploring amino acid profile in skin gelatines

The PCA application aimed to (1) explain the distribution of 12 AAs by the PLS-DAVIPAA in porcine, bovine and fish skin gelatines and (2) determine the dominant, moderate and low AA content in each cluster and (3) verify the significant 12 AAs via comparing to the factor loading (FL) of each AA. Only 12 AAs were subjected to the PCA since these were AAs with VIP > 1 that the PLS-DAVIPAA determined. The skin gelatine plots in Figure 3 (a) – (d) had two principal components (PCs) with cumulative variability (CV) of 91.68% for Figure 3 (a) and Figure 3 (b), 88.75% for Figure 3 (c) and 88.88% for Figure 3 (d) that explained the 12 AAs distribution. However, our study could not determine the distribution of the 12 AAs in porcine, bovine, and fish skin gelatines since the clusters were mixed. Figure 3 (a) shows the overlapping of porcine and bovine skin gelatine clusters. Our study carried out the varimax rotation as suggested by Otto (2017), but the porcine and bovine clusters were still overlapping. Ayerdi & Graña (2014) proposed quartimax rotation instead to simplify the PC structure and achieve optimal clusters. Hence, our study applied quartimax rotation at two, four and six rotations to enhance the variance of factor loadings (FLs) of the PC, reducing dimensionality and facilitating the explanation of 12 AAs distribution in each skin gelatine (de Almeida et al., 2020). Of these rotations, the four quartimax rotations in Figure 3 (c) and six quartimax rotations in Figure 3 (d) achieved the correct reposition of all the skin gelatine into their clusters. Nevertheless, the six quartimax rotations had a higher CV at 88.88% than the four quartimax rotations; thus, our study selected the six quartimax rotations as the best one.

Figure 3 (e) assigned the AAs to the three clusters by overlaying the skin gelatine and AA plots to investigate their distribution in each skin gelatine. Figure 3 (e) also depicts the absent information in the PLS-DA, such as the dominant, moderate and low AA content in each cluster. The dominant AAs were as follows: Tyr, Phe and Val in porcine gelatine and Met, Thr, Ser, His, Arg and Gly in fish gelatine since these AAs and the clusters were in the same direction.

This finding aligned with Sani et al. (2021) result. Our study findings were also similar to Azilawati et al. (2015) on Tyr, Met, Thr and Ser distribution in porcine and bovine skin gelatines, respectively. The Pro, Leu and Hyp contents were moderate in porcine and bovine skin gelatines. This moderate content of

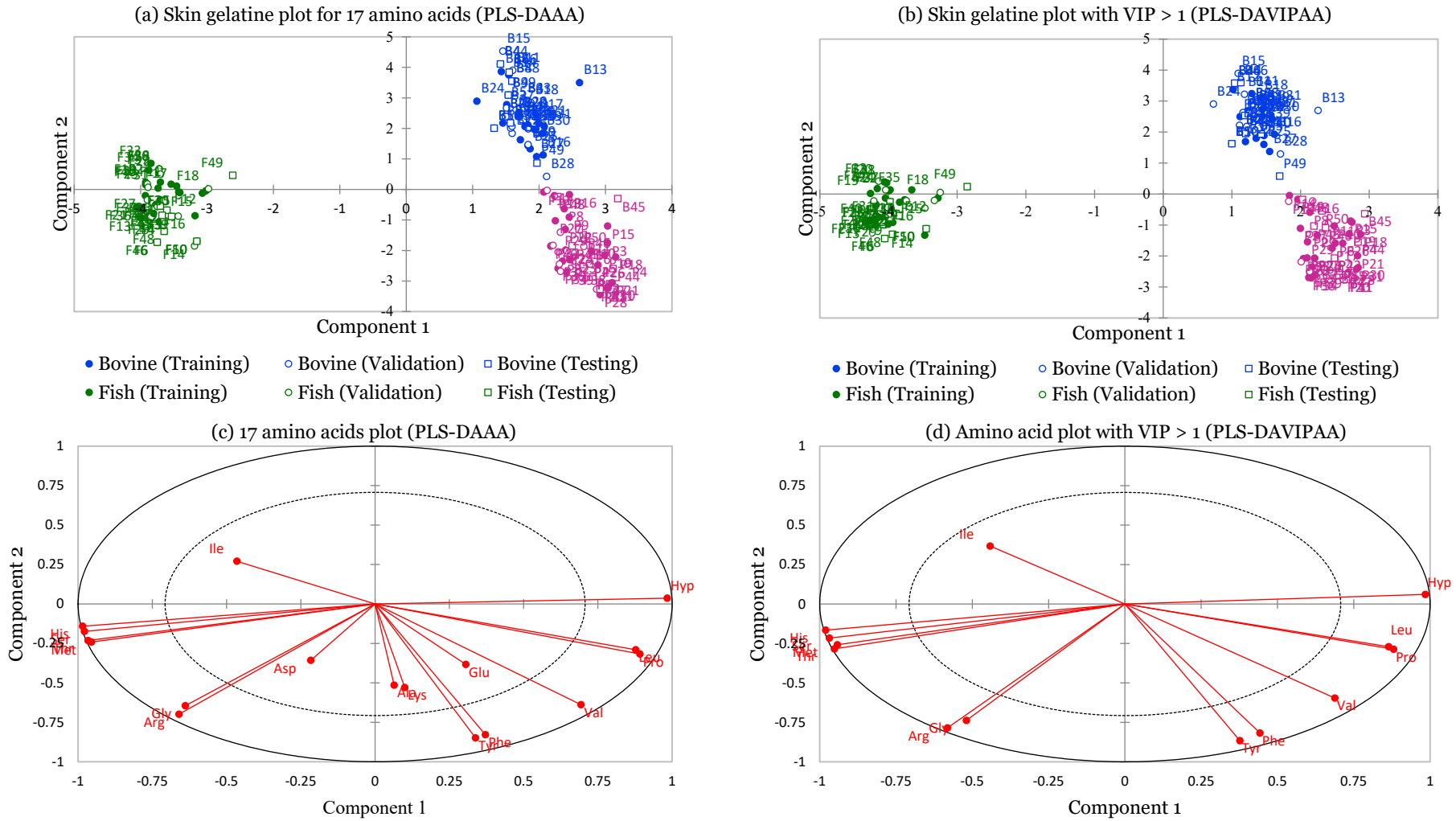


Figure 2: (a) Skin gelatine plot for 17 amino acids (PLS-DAAA), (b) skin gelatine plot with VIP > 1 (PLS-DAVIPAA), (c) 17 amino acids plot (PLS-DAAA) and (d) amino acid plot with VIP > 1 (PLS-DAVIPAA) via partial least square-discriminant analysis.

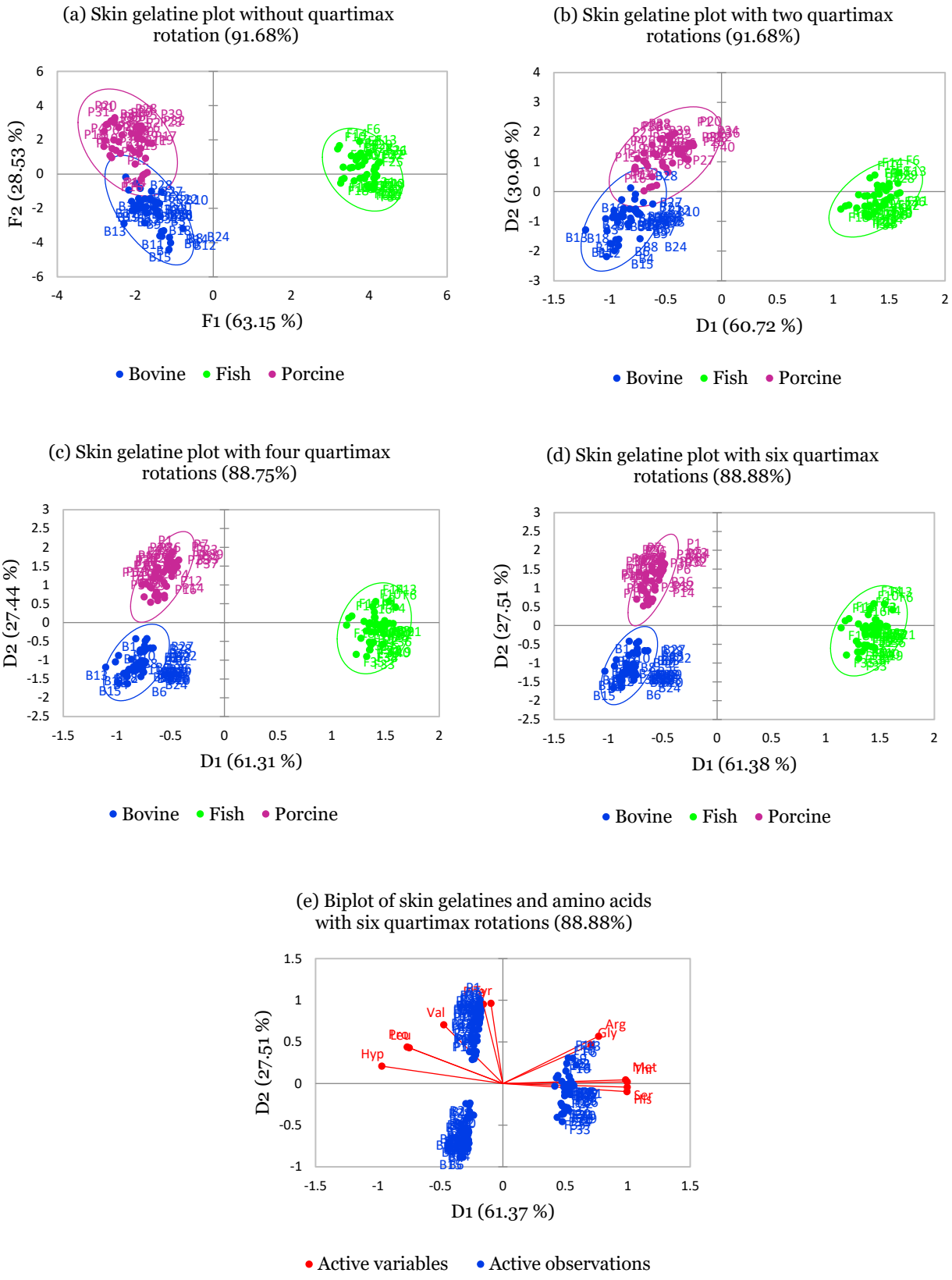


Figure 3: Skin gelatine plots with (a) no quartimax rotation, (b) two quartimax rotations, (c) four quartimax rotations and (d) six quartimax rotations, and (e) biplot of skin gelatines and amino acids with four quartimax rotations.

these AAs was due to their direction between these clusters. Since the Hyp was moderately distributed in porcine and bovine skin gelatines, our result may agree with Yuswan et al. (2021) study that proposed Hyp as one of the biomarkers for halal authentication in gelatine products. The Arg and Gly were low in bovine skin gelatine since their directions were opposite to this cluster. Likewise, fish skin gelatine had low Pro, Leu and Hyp.

Figure 3 (e) also provides information on significant AAs based on the FL of each AA; Hyp, His, Ser, Thr, Pro Tyr, Met, and Leu were the strongly contributing AAs (FL ≥ |0.750|) while Arg, Gly and Val moderately contributing AAs (|0.500| < FL < |0.749|) to the clusters. These results indicated that of the 12 AAs with VIP >1, only 9 were the most significant AAs in this study.

## 4. Conclusion

This study showed that putatively analysed AAs in skin gelatine via UHPLC-DAD incorporated with DA could discriminate the skin gelatine sources. The DA with a higher percentage of correct classification was superior to PLS-DA for distinguishing skin gelatine sources. The PCA with six quartimax rotations could also assign the skin gelatines to their clusters and provide the AA profile in each cluster. Further study on developing the diagnostic ratio for authentication of skin gelatine sources from this profiling is in the pipeline as a continuation of this study. This study focuses on the classification of skin gelatine sources only since gelatine from this source is the majority used in food industry manufacturing. Hence, a further study including other gelatine sources such as bone and variations, e.g., blooms, will also be carried out. Since this study did not undergo method validation and verification, it may expedite the authentication analysis with less cost and time. Based on this study, the authority may adopt and regulate a standard ad-hoc test to authenticate skin gelatine products.

## 5. Acknowledgement

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## 6. Conflict of interest statement

We declare no conflict of interest.

## 7. Research involving human participants and/or animals

We declare no human and/or animals involved in this study.

## 8. Informed consent and credit author statement

All authors have granted permission in full knowledge for the publication of this study. The credit author statement as follows: Azilawati Mohd Ismail for methodology; Muhamad Shirwan Abdullah Sani for conceptualisation, data curation and writing of original draft; Azman Azid for software and validation; Mohd Saiful Samsudin and Mohd Hafis Yuswan for visualisation and investigation.

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**HALALSPHERE**
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# Preparation and Characterisation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) Thin Film for Food Coating Application

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**Abstract**

Recognising the importance of preventing rapid food deterioration and prolonging the shelf life of fruits and vegetables from oxidation, we successfully created thin films composed of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) through a traditional blending technique. Fourier Transform Infrared Spectroscopy (FTIR) confirmed the successful preparation of nanolignin structures, with characteristic peaks observed at 513 cm-1 (C-C stretching in aromatic), 1222 cm-1 (phenolic OH), and 1107 cm-1 (Ar-H and syringyl group). Ultraviolet-visible spectroscopy (UV-Vis) revealed an absorption capacity within the 280 to 300 nm range. Film opacity increased with a greater nanolignin composition in the thin film, attributed to the presence of chromophore structures. Thermogravimetric Analysis (TGA) demonstrated a thermal degradation temperature exceeding 300°C. Differential Scanning Calorimetry (DSC) analysis unveiled two distinct glass transition temperatures (Tg) at approximately 60°C and 80°C, indicating microphase separation and immiscibility between gelatine and nanolignin particles. The nanolignin content significantly influenced solubility and water uptake, with higher nanolignin content leading to reduced solubility and water absorption. The application of NLGGCs film coatings on banana surfaces extended their shelf life compared to control samples after 10 days. Furthermore, NLGGCs underscore the pivotal role in enhancing the performance as a promising bio-based food coating alternative for future applications.

## 1. Introduction

Harmful microorganisms can pose a threat to fruits and vegetables, both in terms of reduced shelf-life and potential health risks. Additionally, food products can undergo degradation due to oxidation and exposure to artificial light. Wu *et al.* (2020) report that oxidation is a significant factor in the deterioration of harvested goods, causing decreased shelf life, unwanted sensory changes, and reduced food quality. To combat this problem, food coatings that exhibit resistance to UV radiation and antimicrobial properties have emerged as a viable solution for preserving food quality. Protective films can be applied to the surface of food items to extend their shelf life and maintain their integrity.

Moreover, there has been a notable surge in worldwide interest towards the halal food industry, owing to its potential to cater to a significant population of over 1.8 billion Muslim consumers. This industry's current value stands at $2.2 trillion and is poised to reach $4.7 trillion, primarily driven by the rapid expansion of the global Muslim population (Hafiz *et al.*, 2022). Several non-Islamic countries, such as China, Thailand, Brazil, New Zealand, Singapore, the Philippines, and Korea, have recognised its immense potential and are actively exploring opportunities within this industry (Najihah *et al.*, 2023). Consequently, the need to prioritise innovation in halal-based product manufacturing to stimulate national economies is rising.

The source of gelatine has sparked a contentious debate within the halal community due to its pivotal role in determining the halal status of products. This debate stems from the religious significance of adhering to Islamic dietary law, which is the fundamental basis of Islamic principles. Gelatine derived from non-halal sources, such as non-slaughtered animals in accordance with Islamic principles or, worse yet, from pigs, directly conflicts with these dietary regulations, rendering products containing such gelatine as haram. Approximately 326,000 tons of gelatine is produced annually, with pig skin as the highest contributor of gelatine source, making it 46 percent of the total production (Pradini D. *et al.*, 2018). In Europe, 80 percent of edible gelatine comes from porcine sources, making it harder for Muslim consumers to navigate the market confidently and select products aligned with Islamic dietary principles (Demirhan D. *et al.*, 2012). The significance of clarifying the halal status of gelatine and the innovation of dietary products from halal sources to replace non-halal source products will uphold the trust and confidence of Muslim

consumers toward the halal industry and ease them to make informed choices in line with their religious beliefs.

Besides that, significant headway has been made in developing food coatings with antioxidant properties for fruits and vegetables. However, using synthetic antioxidants has raised concerns due to potential health hazards. Synthetic phenolic antioxidants (SPAs), including butyl hydroxyanisole (BHA), dibutyl hydroxytoluene 2 (BHT), and tert-butyl hydroquinone (TBHQ), have been associated with adverse impacts on aquatic life and mammals, such as reproductive and developmental toxicity, as well as carcinogenic effects and disruptions to endocrine systems (Wang *et al.*, 2021). Since the requirements of manufacturing halal products entail the usage of halal and *toyyib* (safe, clean and nutritious) ingredients (Mohamad Asri *et al.*, 2022), it is necessary to encourage substantial innovation in creating halal-based products that could fulfil these requirements while stimulating economic growth.

Aadil *et al.* (2016) reported that lignin-gelatine film exhibits remarkable barrier properties, including effective UV light, high water uptake and thermal stability. El-Nemr *et al.* (2020) reported that the gelatine-lignin blend had shown high effectiveness against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* due to the presence of phenolic hydroxyl. However, a research gap exists regarding the impact of nanolignin composition in these films, with no prior studies on this specific aspect. Consequently, the factors influencing the physical properties of Nanolignin-Gelatine-Glycerol composites (NLGGCs), such as thermal stability, glass transition temperature, UV shielding capacity, solubility, and water uptake, remain unknown. According to Low *et al.* (2021), lignin nanoparticles exhibit superior antioxidant, antibacterial, and UV protectant applications compared to macro-sized lignin particles. The increased specific surface area enhances phenolic hydroxyl group distribution, contributing to superior UV protectant capabilities (Qian *et al.*, 2017). However, the performance of nanolignin in nanolignin-gelatine film is still unknown due to no previous research conducted. Comprehending these elements is critical in innovating outstanding food coating with exceptional features.

The current study has effectively acknowledged the importance of addressing specific bio-based thin film development issues. The study successfully produced a range of NLGGCs thin films using a conventional blending-casting method. The impact of nanolignin composition on NLGGCs thin film properties was evaluated through various analytical techniques, including FTIR Spectroscopy, UV-Vis Spectrophotometry, DSC, TGA, solubility assessments, and examinations of swelling properties. The results of this study are expected to help develop new and improved bio-based thin films with enhanced properties that can be utilised in various industries.

## 2. Methodology

### 2.1 Raw materials

Gelatine powder from a Bovine source was obtained from Take It Global Sdn. Bhd certified as a halal supplier, Refine Glycerol 99 %, sodium hydroxide pallet (NaOH), sulfuric acid ($H_2SO_4$, 95 - 97 %) and *Elais Guineensis* empty fruit bunch (EFB). The lignin Nanoparticles were extracted from *Elais Guineensis* EFB from the work reported by Sekeri *et al.* (2020) and Yaqoob *et al.* (2021).

### 2.2 Preparation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

NLGGCs thin film series was prepared by dissolving 0.5% w/v of nanolignin in 0.0125 N NaOH respectively in 100 mL of deionised water. After the nanolignin colour changed to black and a sign of homogeneity was shown, gelatine powder was dissolved into the solution according to Table 1. Next, 2% v/v of refined glycerol was added to plasticise the thin film solution and stirred for 1 hour at 60°C. A series of prepared NLGGCs thin films is summarised in Table 1.

Table 1: Ratio of Nanolignin: Gelatine: Glycerol in NLGGCs thin film series

| Sample Designation | Nanolignin (g) | Gelatine in g (ratio) | Refined Glycerol in g (ratio) | Nanolignin (%) |
|---|---|---|---|---|
| NLGGCs 59 | 0.5 | 1.6 (0.2) | 1.26 (0.5) | 58.82 |
| NLGGCs 50 | 0.5 | 4 (0.5) | 1.26 (0.5) | 50.00 |
| NLGGCs 43 | 0.5 | 6.4 (0.8) | 1.26 (0.5) | 43.48 |

Next, 26.5 mL of composite thin film solution was put into an 11 cm petri dish sprayed with mould release and dried for 16 hours at 60°C in the oven. The film was peeled and kept in desiccators with $P_2O_5$ at 25°C with 0% relative humidity. In this formulation, glycerol composition was set as constant to study the effect of nanolignin and gelatine composition in NLGGCs thin film.

### 2.3 Characterisation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed using a Perkin Elmer model system 2000 FT-IR (United Kingdom) to determine the functional group in a series of NLGGCs thin film according to *ASTM E168-06: Standard Practices for General Techniques of Infrared Quantitative Analysis* at a resolution of 4 $cm^{-1}$ from 400 to 4000 $cm^{-1}$ of wavenumber. UV-Vis spectroscopy analysis was performed using a Perkin Elmer Lambda 365 UV-Vis Spectrophotometer to determine the UV light absorbance according to ASTM E169-16: *Standard Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis* in the wavelength range of 200-800 nm. A wavelength of 600 nm was used for the determination of film transparency.

Thermogravimetric analysis (TGA) was performed using a Perkin Elmer Diamond STA-6000 analyser to determine the thermal stability according to ASTM E1131-08: *Standard Test Method for Compositional Analysis by Thermogravimetry* under a nitrogen atmosphere at a flow rate of 200 mL/min. About 5 mg of the film will be heated at room temperature to 700°C at 10°C/min. Differential Scanning Calorimetry (DSC) analysis was performed using Mettler Toledo DSC 822e to determine the glass transition temperature (Tg). About 5 mg samples were encapsulated tightly in aluminium pans and scanned under 50 mL/min dry nitrogen from 0°C to 300°C at 10°C/min.

The solubility was performed to determine the chemical stability of NLGGCs thin film using ASTM D5226-21: *Standard practice for Dissolving Polymer Materials*. About 10 mm x 10 mm were cut and soaked into 5 mL of sulfuric acid, acetic acid,

sodium hydroxide, ammonia solution, distilled water, dimethylformamide, and hexane. Water uptake analysis was conducted according to ASTM-D2765: *Standard Test Methods for Determination of Gel Content and Swell Ratio of Crosslinked Ethylene Plastics*. About 20mm x 20mm were cut, and initial mass was recorded.

Finally, the pre-coated banana shelf-life was investigated at room temperatures with moderate lighting and dry environments for 10 days. The appearance banana was selected, pre-cleaned and coated with a series of NLGGCs solutions for every banana. The control banana was prepared as a benchmark for banana ripening.

## 3. Results and discussion

### 3.1 Preparation of NLGGCs thin film series consideration

The choice of *Elais Guineensis sp*. (Empty Fruit Bunch) as a primary material is strategic. This resource was favoured due to its abundant and sustainable presence in Malaysia. Previous research findings have underscored its exceptional qualities, including its antioxidative attributes, antibacterial properties, UV resistance, and suitability for nano-enhancement applications (Aadil *et al*., 2016; Low *et al*., 2021; Yun *et al*., 2021). The properties displayed aligned well with the objective of crafting biobased food coatings.

In the realm of polymer matrices, the selection of bovine gelatine is a well-considered choice. This material boasts proven attributes that have been thoroughly documented in prior research. Notably, gelatine can be sourced from the halal livestock industry, effectively utilising resources that might otherwise be discarded. Furthermore, this choice carries significant potential within the halal industry, especially considering the projected growth of the global Muslim population to 29.7% by 2050 (Mohd *et al*., 2023). Gelatine is recognised for its strong resistance to oxygen permeation, although there is room for enhancement in its mechanical properties (Tyuftin & Kerry, 2021). Incorporating nanolignin and glycerol is justified to improve the properties of gelatine.

Glycerol plays a pivotal role as a plasticiser to counteract the inherent brittleness of dry gelatine. This addition effectively enhances the film's flexibility, elasticity, and extensibility, a phenomenon supported by Sanyang *et al.* (2015). It is important to emphasise that all materials employed in this study originated from natural sources, are renewable, and are deemed safe for use in food coatings. This ethical consideration aligned with the overarching goal of ensuring the safety of these materials when they are applied in food-related contexts.

### 3.2 Spectroscopy analysis

FTIR analysis was carried out to delve into the chemical composition of the NLGGCs thin films. It sought to reveal the unique characteristics of these films, with a particular focus on the lignin nanoparticles sourced from *Elaeis Guineensis* sp. EFB through the High Shear Homogenization method. Figure 1 provides visual evidence of the successful preparation of NLGGCs thin films, showcasing the absorption peak of nanolignin.

Within Figure 1, a broad absorption peak at 3334 $cm^{-1}$ signified the presence of aliphatic and aromatic O-H groups in the nanolignin. Additionally, moderate-intensity peaks at 1513 $cm^{-1}$ indicated C-C stretching within the aromatic structures of

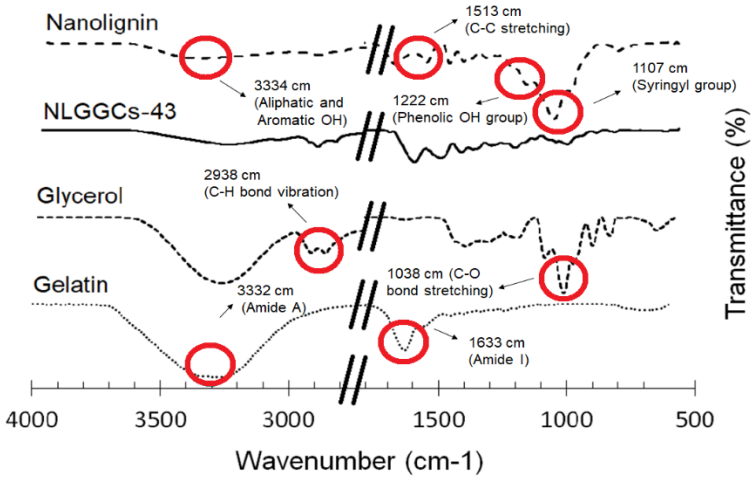


Figure 1: FTIR spectra of Nanolignin, Bovine Gelatine, Refined Glycerol and NLGGCs-43.

lignin. Furthermore, 1222 and 1107 $cm^{-1}$ peaks corresponded to phenolic OH and Ar-H and syringyl groups, respectively. These findings aligned with the report by Sekeri *et al.* (2020). The FTIR analysis of gelatine uncovered a broad peak at 3332 $cm^{-1}$ and another at 1633 $cm^{-1}$, suggesting the presence of Amide A and Amide I, respectively. These findings were consistent with prior research by Aadil *et al.* (2016). Notably, in the FTIR peak of NLGGCs-59, several distinctive peaks emerged. The most prominent band was a broad band at 3281 $cm^{-1}$, indicating the presence of O-H groups derived from nanolignin, glycerol, and gelatine's aromatic and aliphatic compounds. This observation was further supported by peaks at 2938 $cm^{-1}$, which imply the stretching vibration of C-H bonds within the aliphatic chain of glycerol, a correlation documented by Danish *et al.* (2016) in the context of refined glycerol. The thin film's absorption peaks at 3281 $cm^{-1}$ and 1633 $cm^{-1}$ were consistent with FTIR peaks identified in the gelatine raw materials. Furthermore, the 1038 $cm^{-1}$ band was attributed to the C-O stretch of glycerol. Notably, the peaks were associated with the Ar-H syringyl and phenolic OH groups in nanolignin that remained unchanged after the interaction. Figure 2 offers insight into the FTIR peaks across all NLGGCs thin film series, revealing a consistent absorption peak similar to that observed in NLGGCs-59.

However, intensity varied due to differing compound compositions. The O-H group at 3600-3200 $cm^{-1}$ exhibited variable intensities due to variations in glycerol composition. This phenomenon illustrated the plasticising effect of glycerol, with increasing glycerol content leading to a reduction in hydrogen bonding intensity. This outcome can be primarily attributed to glycerol's role in forming new hydrogen bonds within the gelatine network while disrupting internal hydrogen bonding within the gelatine protein network. These activities decrease internal forces and increase intermolecular spacing, as Chen *et al.* (2018) and Chen *et al.* (2018) explained.

The introduction of nanolignin plays a crucial role in shaping the physical and mechanical properties of the NLGGCs thin film series. According to Núñez-Flores *et al.* (2013), the inclusion of nanolignin leads to a reduction in the intensity of Amide I, Amide II, and Amide III in gelatine due to a "dilution effect," as nanolignin takes the place of gelatine. The mostnotable alterations occur in the 953-1633 cm-1 range, signifying substantial interference caused by nanolignin in the hydrogen bonding between water and imide residues. The addition of nanolignin has caused a reduction in the intensity

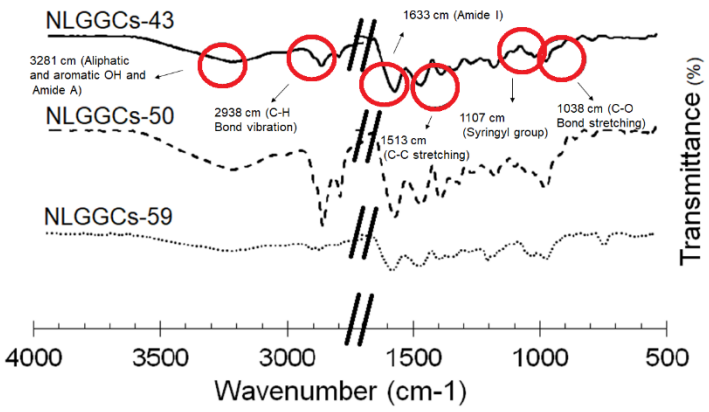


Figure 2: FTIR spectra of NLGGCs thin film series.

of Amide I, Amide II and Amide III of gelatine. The intensity reduction is due to the 'dilution effect' of gelatine replacement by nanolignin. The most notable changes in the thin film series range from 953-1633 $cm^{-1,}$ indicating strong intrusion caused by nanolignin in the hydrogen bonding between water and imide residues (Núñez-Flores *et al.*, 2013). This interaction arose from the hydrophobic group of polyphenols interacting with the hydrophobic region of the protein through hydrophobic interactions and hydrogen bonding between the phenolic hydroxy group of polyphenols and the polar group of the protein.

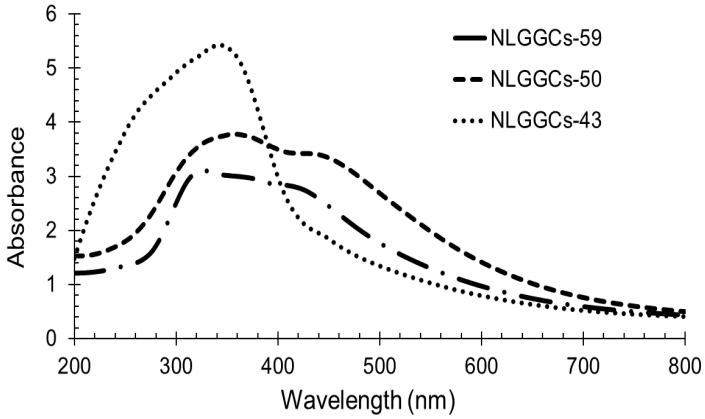


Figure 3: The effect of gelatine content on UV light absorption of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film.

UV-Vis was employed to assess the light barrier properties and film transparency, particularly within the 200-800 nm range, with a specific focus on transparency at 600 nm. Film transparency was inversely correlated with light barrier properties; as transparency decreased, the light barrier properties also increased. Figure 3 visually presents the opacity and light absorption of the NLGGCs thin film series across the 200-800 nm wavelength range.

Figure 3 illustrates that all NLGGCs series exhibit substantial UV absorption capacity within the 280-350 nm range. This phenomenon has been primarily attributed to chromophores within nanolignin, including phenolic and ketone groups (Qian *et al.*, 2015). These chromophores responsible for UV absorption have encompassed double bonds (CH=CH) conjugated with aromatic rings, quinone methide and quinones, chalcone structures, free radicals, and metal complexes with catechol structures (Zhang & Naebe, 2021). Additionally, the gelatine content has also contributed to high absorption within the 200-300 nm wavelength range, with absorption in the 200-250 nm range linked to peptide bonds in gelatine and absorption in the 250-300 nm range attributed to chromophore structures within gelatine, such as the phenylalanine and tyrosine amino acid aromatic groups (Etxabide *et al.*, 2015).

Table 2: The opacity of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

| Sample Designation | Film Thickness (cm) | $A_{600}$ | Opacity |
|---|---|---|---|
| NLGGCs 59 | 0.05 | 0.9667 | 19.34 |
| NLGGCs 50 | 0.31 | 1.4123 | 4.56 |
| NLGGCs 43 | 0.22 | 0.7958 | 3.62 |

Specifically, NLGGCs-59, with the highest nanolignin composition, exhibited the highest opacity (19.34), with opacity values decreasing as nanolignin composition decreases. This observation aligned with the findings of Yang *et al.* (2020), who noted that an increasing content of lignin nanoparticles in poly (lactic acid)-poly (ε-caprolactone)-nanolignin composite films have progressively reduced optical transparency. The opacity arises primarily due to the absorption of light in the visible range by chromophore structures within the 400-800 nm wavelength range, resulting in the dark brown colour of lignin that enhances UV shielding properties while potentially compromising the film's visual appeal in food coating applications (Aadil *et al.*, 2016). These properties hold significant importance in the food coating industry for addressing or mitigating lipid oxidation, a prominent concern in the food industry (Sá *et al.*, 2020).

### 3.3 Thermal properties

The study delved into the thermal behaviour of the NLGGCs thin films through TGA in a nitrogen environment, as shown in Figure 4.

The TGA curves unveiled four distinct decomposition stages common to all NLGGCs thin films. The initial stage witnessed a weight loss between 50°C and 180°C, attributed to the evaporation of water content absorbed within the films. These findings corroborated prior research by Benbettaïeb *et al.* (2016) and Inamura *et al.* (2013), which reported similar initial degradation occurring within the temperature ranges of 52°C to 220°C and 46°C to 140°C, respectively. The second stage of decomposition, occurring between 200°C and 280°C, corresponded to the volatilisation of glycerol and bound water within the films. This observation concurred with findings reported by Phreecha & Chinpa (2019), where glycerol decomposition occurred at approximately 250°C, proximate to its boiling point of 289°C. The most substantial degradation was noted between 280°C and 480°C, indicating the decomposition of gelatine and lignin structures, resulting in the highest weight loss percentage (35% to 55%). This decomposition was linked to the breakage of helical protein chain structures, rupturing of peptide bonds, and the breakdown of lignin C-C linkages. Remarkably, gelatine's primary and secondary structures remained unaffected by thermal degradation, aligning with the findings of Benbettaïeb *et al.* (2016). In the final stages, a minor weight loss occurred at temperatures ranging from 460°C to 550°C, attributed to the

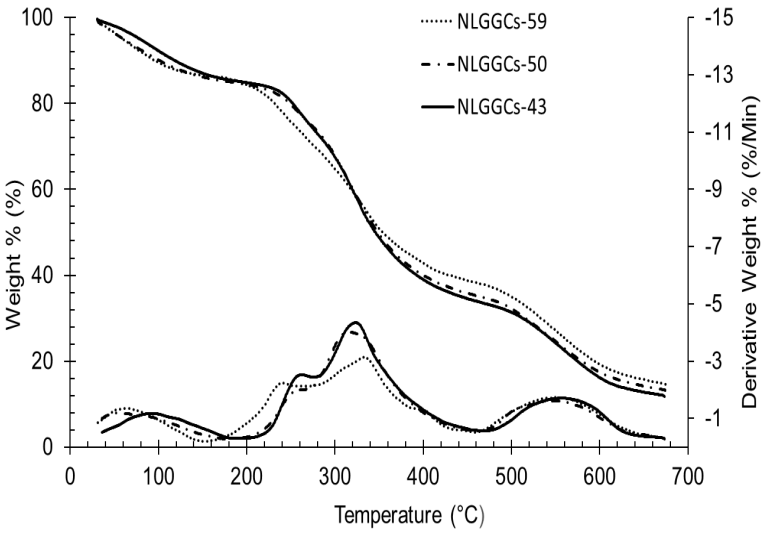


Figure 4: TG and DTG trace for thermal decomposition of a series of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) thin films in nitrogen gas atmosphere.

decomposition of more thermally stable compounds within the films.

Incorporating nanolignin played a pivotal role in elevating the thermal stability of the NLGGCs thin film series. As outlined in Table 3, all NLGGCs thin films exhibited maximum degradation temperatures surpassing 300°C, indicating robust thermal stability. This enhancement can be ascribed to the robust interaction between lignin nanoparticles and the gelatine polymer network in the NLGGCs thin film through hydrogen bonding, as elucidated by Huang *et al.* (2020). Similar results were achieved in the study conducted by Bian *et al.* (2018). Additionally, an escalation in nanolignin composition led to a substantial increase in the percentage of char yield at 800°C. This phenomenon of char formation might be linked to the condensation of lignin aromatic rings at temperatures exceeding 550°C, serving as a protective mechanism against polymer matrix degradation, as Monteiro *et al.* 2021 suggested.

DSC analysis was employed to scrutinise the impact of temperature on the properties of the NLGGCs thin film series, specifically focusing on determining the glass transition temperature (Tg), Figure 5.

Table 3: Results of TG/DTG traces of the Nanolignin-Gelatine-Glycerol Composites thin films in nitrogen gas atmosphere

| Sample Designation | $T_{10\%}$ (°C) | $T_{onset}$ (°C) | $T_{endset}$ (°C) | $\Delta T$ (°C) | $T_{max}$ (°C) | Char Yield at 800°C (%) |
|---|---|---|---|---|---|---|
| NLGGCs 59 | 88.03 | 154 | 461 | 307 | 334 | 14.509 |
| NLGGCs 50 | 92.67 | 179 | 463 | 284 | 315 | 13.146 |
| NLGGCs 43 | 110.33 | 194 | 470 | 276 | 324 | 11.85 |

Figure 5 visually encapsulates the heat flow within the NLGGCs series, revealing Tg values clustered around 80°C for all NLGGCs films. In comparison, bulk lignin showcased a Tg at 139.7°C, while mechanically sheared nanolignin exhibited a Tg at 156.6°C, as documented by Juikar & Vigneshwaran (2017). Nair corroborated these findings *et al.* (2014), where diverse lignin nanoparticles displayed Tg values ranging from 120°C to 150°C. However, including nanolignin within the thin film series reduced the Tg compared to the composite materials.

Furthermore, it was observed that certain series displayed an additional Tg at approximately 60°C. As per Núñez-Flores *et al.* (2013) report, the presence of two distinct Tg values might have stemmed from the immiscibility between lignin's hydrophobic structure and gelatine's hydrophilic nature, resulting in microphase separation that has enhanced gelatine mobility. As highlighted by Aadil *et al.* (2016), an escalation in nanolignin composition has led to a decrease in the Tg of the gelatine/glycerol thin film. This decline in Tg within the NLGGCs films can be attributed to interactions between nanolignin and gelatine, facilitated by hydrogen bonding and hydrophobic interactions, subsequently diminishing crystallinity and inducing changes in the helical structures of gelatine. In summary, this section provides an in-depth exploration of the thermal properties of the NLGGCs thin films, shedding light on their decomposition patterns, improved thermal stability due to nanolignin integration, and shifts in Tg.

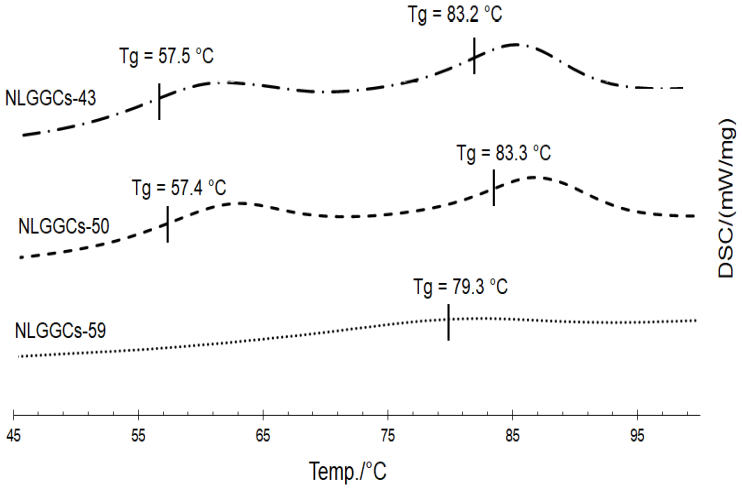


Figure 5: Heat flow of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film series.

### 3.4 Chemical stability

Table 4 elucidates the solubility of the NLGGCs thin film series across diverse solvents at 25°C to explore the chemical durability of NLGGCs thin films. It offered insights into their solubility across diverse solvents and swelling behaviour. The solubility of these thin films is profoundly influenced by the functional groups inherent in each constituent material within the film.

As outlined, all NLGGCs thin film series exhibited limited solubility in polar protic, polar aprotic, and non-polar solvents. The restricted solubility has arisen from differing chemical affinities between the solvents and the materials composing the film and the strength of interactions encompassing London dispersion forces, electrostatic bonds, and hydrogen bonding

(Zhao *et al.*, 2021). Remarkably, sulfuric acid, a potent degrading agent, has entirely dissolved all NLGGCs series.

Integrating nanolignin into the NLGGCs thin films is a pivotal factor influencing their solubility. Table 4 underscores that most NLGGCs thin films dissolved in strong (NaOH) bases and weak (ammonia solution), except for NLGGCs-59. This phenomenon stemmed from the substantial nanolignin content present in the thin film series. A plausible explanation was the interference of hydrophobic interactions between lignin nanoparticles and the gelatine network, hindering the dissolution of gelatine polymer chains in water (Aadil *et al.*, 2016). Núñez-Flores *et al.* (2012) have noted analogous observations in a study where the addition of lignin induced alterations in gelatine's helical structures, thereby influencing water solubility.

To investigate the swelling propensity of the NLGGCs thin film, a swelling analysis was conducted following immersion in distilled water for 30 minutes. The results, graphically represented in Figure 6, manifest a significant upsurge in swelling percentages during the initial phase, eventually reaching a saturation point where swelling degrees were stabilised and subsequently diminished, signifying an equilibrium in swelling. All NLGGCs thin films exhibited notable swelling, with percentages ranging from 241% to 249% after just 1 minute of immersion. The remarkable swelling properties are primarily attributed to the inherent hydrophilic properties of gelatine and glycerol, which facilitate water molecule binding via hydrogen bonding (Ciannamea *et al.*, 2018). Additionally, the porous structure within the gelatine network has contributed significantly to the high swelling ratios, allowing for enhanced water absorption (Kavoosi *et al.*, 2014). These augmented swelling properties hold promise for fruit coating applications, facilitating the complete removal of the thin film layer during fruit washing.

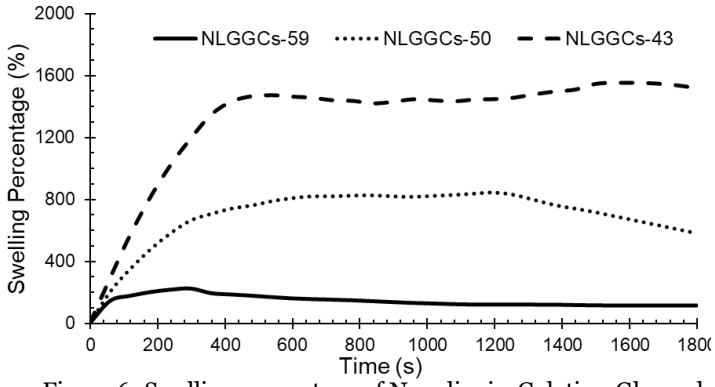


Figure 6: Swelling percentage of Nanolignin-Gelatine-Glycerol Composites in distilled water.

The influences of nanolignin composition on swelling properties proved noteworthy. The swelling graph depicted a declining water uptake trend with escalating nanolignin composition. NLGGCs-59 exhibited the lowest swelling percentage due to its elevated nanolignin content, while NLGGCs-43, characterised by the lowest nanolignin composition, registered the highest swelling percentage. This decrease in swelling propensity can be attributed to plausible interactions between the phenolic components of nanolignin and the amino groups within gelatine. These interactions have shielded the polar side chains of gelatine from water exposure, thus limiting interaction with water molecules (Bhat *et al.*, 2013). This section comprehensively explored the chemical durability of NLGGCs thin films, offering insights into their solubility across diverse solvents and swelling behaviour. These findings enhance our understanding of the films' chemical attributes and potential applications.

### 3.5 Pre-coated banana analysis

In this segment, the evaluation of pre-coating fruit shelf-life critically examined the NLGGCs thin film's potential utility in food coating applications. The results stemmed from a 10-day observation of bananas treated with the NLGGCs thin film solution, illustrated in Figure 7.

The outcome of this assessment demonstrated a noteworthy contrast between bananas coated with NLGGCs thin film series and the control sample. Following a 10-day storage period under room temperature conditions, the NLGGCs-coated fruit retained a fresh appearance devoid of any discernible juice leakage. In stark contrast, the control sample adhered to the standard banana ripening process expected in a non-coated scenario. This uncoated sample exhibited conspicuous mould growth, characterised by numerous black mould spots and a pronounced putrid odour

To summarise, this section provided compelling evidence of the potential advantages of utilising NLGGCs thin film in food coating applications, particularly in extending the shelf-life of coated fruits. The juxtaposition with a control sample enhanced the credibility of the observed effects. However, supplementing the qualitative observations with quantitative data, such as measurements of ripening rates, would further corroborate the findings and fortify the conclusions. Furthermore, these findings elucidated the practical implications and prospective applications within the food industry that would augment their overall impact.

To summarise, this section provided compelling evidence of the potential advantages of utilising NLGGCs thin film in food coating applications, particularly in extending the shelf-life of

Table 4: Solubility of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) thin film in various solvent mediums at 25 °C

| Sample Designation | Solvent | 1.0 M $H_2SO_4$ | | 1.0 M NaOH | | Distilled Water | Hexane | DMF |
|---|---|---|---|---|---|---|---|---|
| | Medium Type | Strong acid | Acetic acid Weak acid | Strong base | Ammonia solution Weak base | Polar protic | Non-polar | Polar aprotic |
| NLGGCs-59 | 88.03 | + | - | - | - | - | - | - |
| NLGGCs-50 | 92.67 | + | - | + | + | - | - | - |
| NLGGCs-43 | 110.33 | + | - | + | + | - | - | - |

(+): Soluble, (-): Insoluble

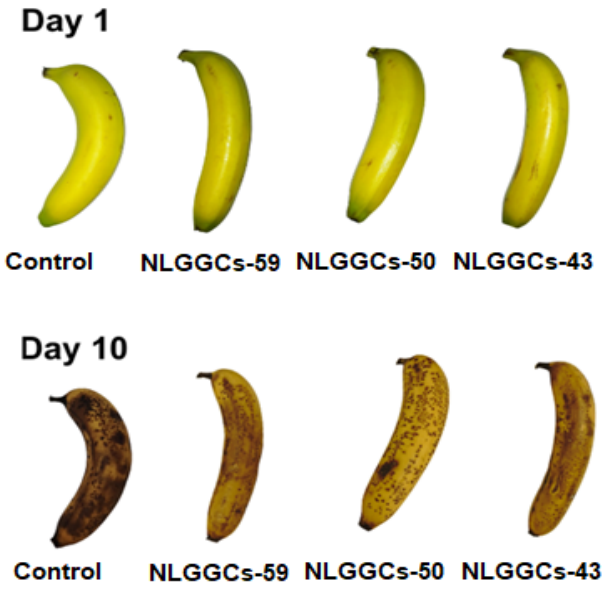


Figure 7: Pre-coated banana shelf-life analysis of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film after day 10.

coated fruits. The juxtaposition with a control sample enhanced the credibility of the observed effects. However, supplementing the qualitative observations with quantitative data, such as measurements of ripening rates, would further corroborate the findings and fortify the conclusions. Furthermore, these findings elucidated the practical implications and prospective applications within the food industry that would augment their overall impact.

## 4. Conclusion

In conclusion, this study successfully achieved its initial objective: creating thin films composed of the Nanolignin-Gelatine-Glycerol Composite (NLGGCs). Throughout this investigation, we thoroughly explored the impact of incorporating nanolignin into NLGGCs thin films using diverse analytical methods.

One notable discovery was the dark colouration exhibited by NLGGCs thin films, a direct result of nanolignin's presence. This characteristic was coupled with exceptional flexibility and elasticity within the film's textures. Furthermore, our FTIR characterisation unveiled the existence of crucial functional groups inherent to lignin nanoparticles. The peaks at 1222 and 1107 $cm^{-1}$ are particularly noteworthy, which confirm the presence of phenolic OH groups and Ar-H syringyl groups, respectively.

The UV-Vis analysis underscored nanolignin's remarkable UV shielding properties, which are attributed to the presence of chromophore structures and contribute to the films' high opacity. The films also exhibited impressive thermal stability, as evidenced by TGA, with all NLGGCs series showcasing maximum degradation temperatures surpassing 300°C.

DSC unveiled intriguing findings, manifesting as dual Tg at 80°C and 60°C in certain NLGGCs series. This phenomenon signifies microphase separation and immiscibility between gelatine and nanolignin particles, shedding light on their intricate interactions.

Our investigations into chemical stability highlighted nanolignin's role in rendering the thin films' poor solubility in weak acids, polar protic, polar aprotic, and non-polar solvents. Additionally, the reduction in water uptake with increasing nanolignin composition in NLGGCs can be attributed to its inherent hydrophobic nature.

Finally, the analysis of banana ripening following pre-coating with NLGGCs thin film demonstrated substantial enhancements in the shelf-life of the treated bananas compared to control samples over 10 days. This outcome underscored the promising potential of NLGGCs thin films as safe, bio-based food coatings with exceptional properties, paving the way for future advancements in the food industry.

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## 7. Author contributions

Muhammad Bisyrul Hafi Othman, Muhamad Shirwan Abdullah Sani, Mohamad Nasir Mohamad Ibrahim and Nur Najmina Rafiae: Conceptualisation and resources.
Nur Najmina Rafiae and Najwa Najihah Mohamad Daud: Formal analysis and investigation.
Muhammad Bisyrul Hafi Othman and Nur Najmina Rafiae: Data curation, writing, and original draft preparation.
Muhammad Bisyrul Hafi Othman, Mohamad Nasir Mohamad Ibrahim and Muhamad Shirwan Abdullah Sani: Validation, editing, visualisation, and supervision.
Muhammad Bisyrul Hafi Othman: Project administration.
All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

## 8. Conflict of interest

We declare no conflict of interest.

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# Clean Extraction of Pectin from Dragon Fruit Peels, Pomelo Peels, Okra, and Pineapple Peels Using Deep Eutectic Solvents and Ionic Liquids

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**Abstract**

Pectin is the main constituent of fruit peels that contributes to the fruit's solid and firm shape. Having wide applications in the food, pharmaceutical, and cosmetics industries, its extraction from bioresources would mark a sustainable advancement in biotechnology. The biomaterials for pectin extraction targeted in the study were dragon fruit peels (*Hylocereus costaricensis*), Pomelo peels (*Citrus grandis*), okra (*Abelmoschus esculentus*), and pineapple peels (*Ananas comosus*). Aqueous extractions of pectin from fruit peels were performed in a sono-reactor using deep eutectic solvents (DESs). Ionic liquids such as (Choline acetate ≥ 95%) [Ch][Ac] and (1-ethyl-3-methylimidazolium acetate ≥ 97%) [EMIM][Ac] were also employed as extraction solvents. Morphological screening with the electron microscope (SEM) and FTIR showed that the extracted pectin had a similar surface as commercial pectin. The extracted pectin can completely dissolve in water to form a homogenous suspension. The pectin yield from dragon fruit peels was 60±2.00 wt% with a degree of esterification at about 66-72%. This study introduces a clean extraction that can potentially substitute solvents in the pectin industry.

## 1. Introduction

Pectin is structurally the most complex polysaccharide abundant in plant cell walls (Mohnen, 2008). It is a natural polymer composed mainly of D-galacturonic acid molecules linked by α-(1,4)-glycosidic bonds with specific esterification levels (Liew *et al.*, 2018a). Thus, pectin is a family of polysaccharides rich in galacturonic acid (Mohnen, 2008). Pectin can be classified into two main types, i.e. high methoxyl (HM) and low methoxyl (LM). It has a degree of esterification (DE), ranges between 55-80% and forms gel at low pH in the presence of sugar (Barrera *et al.*, 2002) and a DE value > 50% (Liew *et al.*, 2018). Pectin is mainly employed in the food and pharmaceutical industries as a source of safe fibre in food additives, stabilizers, and gelling agents. It can also be added to strengthen orthopaedic casts and construction-grade cement.

A wide variety of edible fruits is produced and grown in Malaysia's tropical climate. Fruit consumption produces a lot of waste products, causing disposal problems. About 30-40% of certain fruit weight is the peel with good pectin supply (Liew *et al.*, 2018). For instance, pomelo (*Citrus grandis*), pineapple (*Ananas comosus*), jackfruit (*Artocarpus heterophyllus*), cempedak (*Artocarpus integer*), and dragon fruit (*Hylocereus costaricensis*) consumption leads to the production of fruit peels as waste, which are good sources of pectin. Commercial pectin extracted using acidic extraction agents (strong mineral acids) produces reasonable amounts of pectin and is a time-saving approach. However, the highly acidic process accelerates the corrosion and formation of rust in apparatus, entering the water hence causing health and environmental hazards. Although strong acids have various uses in food manufacture, their usage for extraction often generates negative attitudes among consumers due to the related dangers and safety issues. To address these issues, researchers have investigated alternate methods for extracting pectin. Recent studies emphasize the limitations of conventional techniques that employ abrasive mineral acids and underscore the necessity for more environmentally friendly approaches (Dao *et al.*, 2023). Pectin extraction technique has shown promise in utilizing organic acids and sustainable solvents such deep eutectic solvents (DES) and ionic liquids (ILs). (Turan *et al.*, 2024). These environmentally friendly solvents not only resolve safety issues but also provide improved efficiency in extracting pectin from different corps sources, such as dragon fruit peels, pomelo peels, okra, and pineapple peels. The transition towards more ecologically sustainable and secure techniques for pectin extraction is clearly apparent.

Table 1: Summary of several recent studies carried out for pectin extraction approaches, highlighting the solvents, methods, and yields obtained

| Fruit | Extraction Method | Conditions | Yield | Reference |
|---|---|---|---|---|
| Calamansi lime | Water-based extraction | Distilled water, pH 1-5, 80°C for 10 minutes centrifugation | 45.7% | (Zainudin *et al.*, 2021) |
| Grapefruit | Thermal treatment extraction | 90°C for 5 minutes, oven drying under vacuum at 50°C overnight | 56.84% | (La Cava *et al.*, 2018) |
| Pomelo peel | Deep eutectic solvent (DES) extraction | Lactic acid–glucose–water DES (6:1:6), pH 1.80, 88°C for 141 minutes | 39.72% | (Liew *et al.*, 2018b) |
| Apple pomace | Enzymatic extraction | Enzyme Celluclast, doses 20-60 µL/g, 40-60°C, 12-24 hours | 97.46% | (Dranca & Oroian, 2019) |
| Pomelo peel | Sonication and water bath extraction | Sonication at 60°C for 120 minutes, water bath at 75°C for 120 minutes | 96.37% | (Elgharbawy *et al.*, 2019) |
| Lemon peel powder | Ohmic Heating-Assisted extraction | pH 2, 90°C, 30 minutes, solid: liquid ratio of 1:40 g/mL | 16.91% | (Tunç & Odabaş, 2021) |

As an example, Liew *et al.* (2018) demonstrated this change by isolating pectin from *Citrus grandis* by the utilization of both citric acid and deep eutectic solvents (DESs). The extraction process, carried out at a temperature of 88 °C for a duration of 141 minutes, resulted in a pectin yield of 39.72% with a DE% value of 57.56%.

Considering the reported promising yields, the properties of the extracted pectin would be worth investigating into to ascertain their applications. Hence, we aim to identify properties of extracted pectin from pomelo peel, pineapple peel, dragon fruit peel, and okra using different extraction solvents: ionic liquid (IL), deep eutectic solvent (DES), HCl, water assisted-ultrasonication and heating. The use of various extraction solvents and conditions can identify the potential solvents and optimize the extraction process condition while reducing energy input and devising a cleaner extraction without the involvement of a large quantity of chemicals.

## 2. Materials and methods

The biomaterials used in this study were dragon fruit peels (*Hylocereus costaricensis*), Pomelo peels (*Citrus grandis*), okra (*Abelmoschus esculentus*), and pineapple peels *(Ananas comosus*). They were obtained from a local market in Selangor, Malaysia. All collected feedstocks were of the same ripeness and same batch (to avoid inconsistency due to variations). After washing them with water, all the fruit peels were cut except for the okra. The samples were dried at 50 °C until a constant weight was achieved. The dried peel was ground into powder (<500 µm) and stored in an airtight container in a dry atmosphere. All solvents and chemicals used in this study were of analytical grade. Ionic liquids (Choline acetate ≥ 95%) [Ch][Ac], and (1-ethyl-3-methylimidazolium acetate ≥ 97%) [EMIM][Ac], used in this study were purchased from Merck, Malaysia. Commercial pectin was purchased from a local baking supplier in Kuala Lumpur, Malaysia.

### 2.1 Deep Eutectic Solvent (DES) preparation

Choline chloride and glycerol with a molar ratio 1:2 was employed to prepare the DES. The optimal preparation ratio of the DES is selected according to previous literature (Hayyan *et al.*, 2013); (Tommasi *et al.*, 2017). Choline chloride (ChCl) and glycerol (gly) were mixed at the specific molar ratio, heated at 80°C and stirred (350 rpm) using a hot stirrer plate for 2 hours until the sample was homogenised, and a clear solution was obtained. The water content of the DES varied between 0.43–1.23% (Karl-Fischer titration).

### 2.2 Pectin extraction

The experiment was conducted at a constant condition in the pectin extraction, and the solvents were screened to determine their capacity in the extraction process. The solvent with the highest extractive capability was used for characterization. A 3 g sample of dried powder was mixed with each ILs, HCl, and DES (ChCl:gly) at a ratio of 1:3 (water). The mixture was incubated at 60°C for 120 minutes using a sonication bath. The sonoreactor aided in the extraction of the pectin. On the other hand, heat extraction of pectin using a water bath was conducted using the same

conditions and ratio but at a temperature of 75°C, Applying the stated techniques (Dranca & Oroian, 2018).

### 2.3 Pectin yield determination

After the sonication cycles were complete, the tubes containing the mixture were centrifugated for 5 minutes in 1900 ×g (8 x 50 mL Fixed Angle Rotor). A 1.5 volume of absolute ethanol was added to the obtained filtrate for coagulation and left to set overnight. Fabric filters were used to strain the formed pectin, and it was subsequently washed with distilled water followed by 96% ethanol. The obtained precipitate was freeze-dried in a freeze dryer for 48 hours. The pectin yield was calculated using Equation (1) (Raji *et al.*, 2017):

$$Yield\ of\ Pectin\ (\%) = \frac{w}{w_0} \times 100 \qquad (1)$$

where dried pectin weight is expressed as w in grams, and the initial powder's weight is $w_0$ in grams.

### 2.4 Characterisation of pectin

#### 2.4.1 Degree of esterification (DE)

The degree of esterification DE is determined by measuring the presence of esterified carboxyl groups in the galacturonic acid groups. Liew *et al.* (2018) described the titration method to measure the pectin DE. Ethanol was added to 0.2 g of the obtained pectin and incubated in a 45°C water bath, with intermittent shaking until complete dissolution was observed. The phenolphthalein was added to the mixture and titrated against 0.1 N NaOH to initiate the titration. The final volume was measured once the titration reached the endpoint. Next, the solution was completely neutralized by adding 10 mL of 0.1 N NaOH. Pectin was de-esterified by shaking the sample and allowing it to sit for two hours at room temperature. NaOH was neutralized with 10 mL 0.1 N HCl until its pink tint disappeared. Phenolphthalein was added, and the second reading was recorded with 0.1 N NaOH when a pink hue first appeared. The percentage of DE was calculated using the following formula:

$$DE\ (\%) = \frac{Final\ titration\ volume\ (ml)}{Initial\ titration\ volume\ (ml) + Final\ titration\ volume\ (ml)} \times 100 \qquad (2)$$

#### 2.4.2 Fourier Transform Infrared (FTIR) spectroscopy chemical structure

Chemical structures of extracted pectin were analysed using Fourier Transform Infrared (FTIR) Spectroscopy. FTIR absorbed electromagnetic radiation that interacted with a substance and transmitted, reflected, scattered, or had photoluminescence (PL), which provided significant information on the substance's molecular structure and energy level. In this study, the infrared spectra of the functional groups of extracted pectin were measured with a Thermo Scientific FTIR spectrometer. The absorbance of the different extracted pectin was acquired over the wavenumber range of 4000 to 1000 $cm^{-1}$.

#### 2.4.3 Raman Spectroscopy

To compare their chemical structures, Raman spectroscopy was performed on extracted and commercialised pectin. inVia™ Qontor® confocal Raman microscope at 20× magnification was used with an extended Raman range of 734 – 1787 $cm^{-1}$ and an exposure time of 10 seconds. Laser power was adjusted at 1% and 785 nm edge.

#### 2.4.4 Differential Scanning Calorimetry (DSC) thermal analysis

Differential Scanning Calorimetry (DSC) was used to measure the samples' melting and crystallisation points. Thermal characteristics of extracted pectin were performed on a Mettler Toledo Flash DSC equipped with a Freon intercooler maintained at -105°C and nitrogen gas purge of 20 mL/min. All extracted pectin was scanned between 25°C to 500°C.

#### 2.4.5 Analysis of pectin samples using Scanning Electron Microscope (SEM)

The extracted pectin and the commercial sample were analysed using scanning electron microscopy (SEM) to determine their surface morphological properties, such as porous pattern and surface structure. The dried pectin samples were observed under a scanning electronic microscope (SEM) by Hitachi SU1510 SEM at the magnifications of X500, X1000 and X5000 at BSE mode.

## 3. Results and discussion

### 3.1 Screening of the co-solvents for pectin extraction

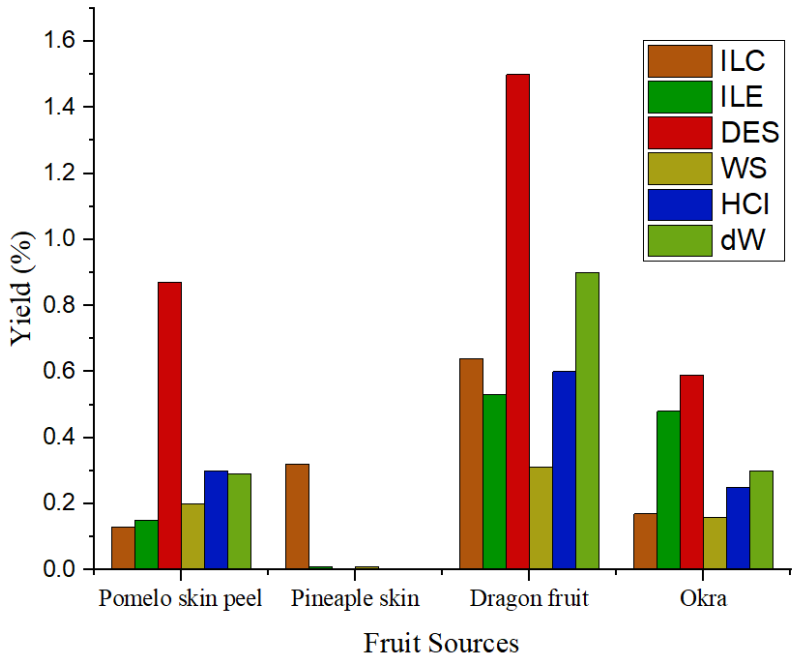
Heat-assisted extraction with water resulted in a higher pectin yield than the DES used for the dragon fruit sample. Water-assisted extraction reduces the viscosity and consequently improves the extraction of pectin (Kalhor & Ghandi, 2019). Likewise, ultrasonication-assisted extraction with water also produced pectin with a higher yield than DES. The results obtained proved that water can be useful as an alternative extraction solvent because the hydrodynamic property of the pectin molecular chains is significantly improved by the addition of water and enhancing the mass transfer as a result of the dilution of the pectin aggregates (Migliori *et al.*, 2011). Meanwhile, the extraction using both ionic liquids recorded the highest pectin yield, 29.33% and 35.33%, respectively. This concords with the findings that ionic liquids are better extraction solvents for higher extraction yield (Guolin *et al.*, 2012). As explained by (Xiao *et al.*, 2018), the good extraction ability of ionic liquids is due to their chemical compositions, which consist of organic cations and inorganic or organic anions, thus making them able to extract compounds better (Montalbán *et al.*, 2018) at low vapour pressure. Likewise, DES acts in a similar way to the ILs.

### 3.2 Degree of esterification of extracted pectin

To classify the type of pectin and their potential industrial application, the degrees of esterification were evaluated for pectin extracted from pomelo peel, pineapple peel, dragon fruit peel and okra and the results were summarized in Figure 1(b).

The current study found that pectin extracted from dragon fruit recorded a high degree of esterification, i.e., above 50%, but not for other fruit peels; it is worth noting that only significant pectin DE% values were consistent using different extraction techniques for dragon fruit peel. Another interesting finding is

a)




b)

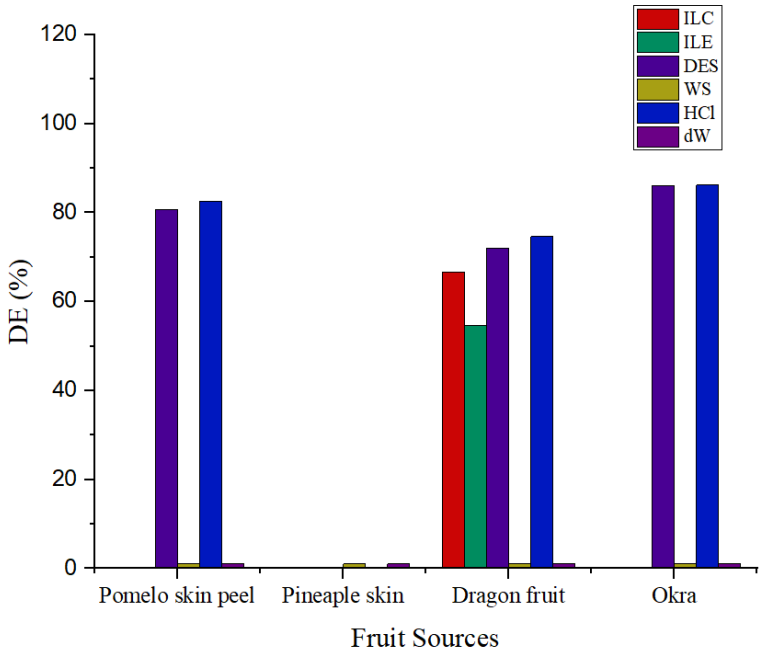



Figure 1: (a) Bar-graph of Pectin yield from various fruit sources using different co-solvents (b) Degree of esterification (DE%) of extracted pectin derived for diverse biomaterials
*the value is too small and almost neglectable. HCl- hydrochloric acid, WS- water-ultrasonication-assisted extraction and WH-water-heated assisted extraction

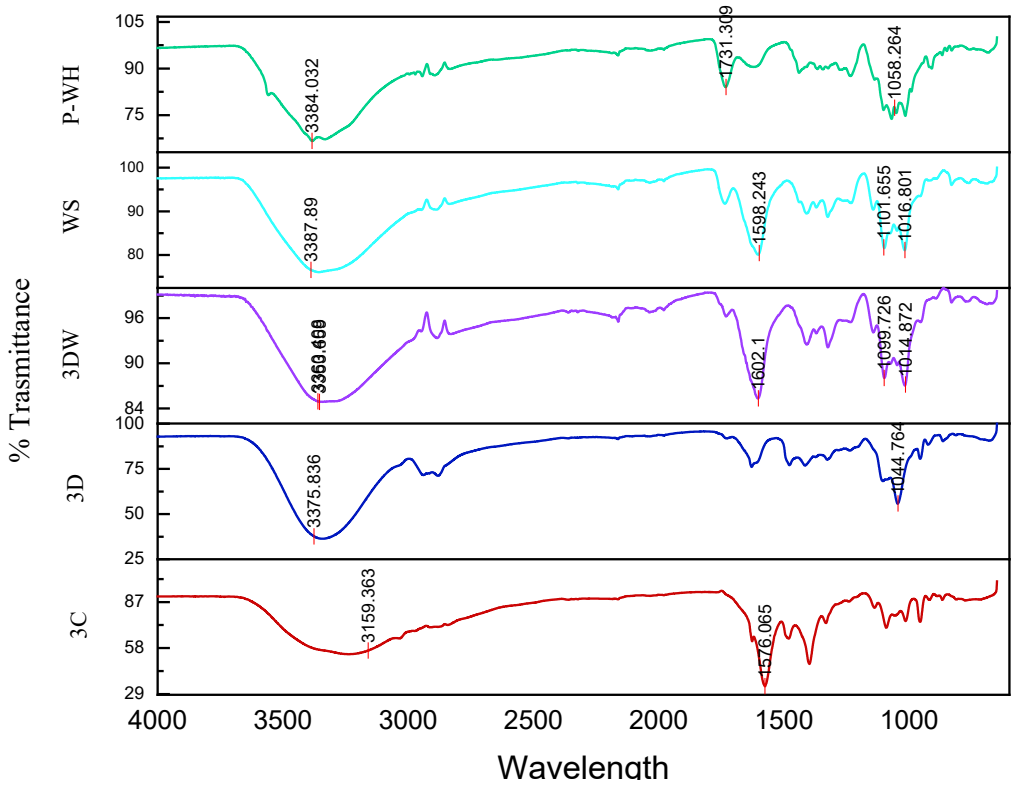



Figure 2: Stacked result of FTIR Spectra of extracted pectin using different treatment methods.
Note*: 3C- Commercial pectin, 3D-Ionic Liquid [EMIM][Ac] assisted extraction, 3DW- DES (ChCl: gly 1:2) assisted extraction, 31- WS: Water-ultrasonication assisted extraction, P- WH: Water-heat assisted extraction at the following wavenumbers: 3500 cm-1 to 1000 cm-.

on a similar pectin yield was obtained from the pomelo peel, and the dragon fruit peel registered a comparable yield of DE above 70%. The overall average value of pectin esterification was 66.32% for all samples (Figure 1b). The highest DE was attained when the fruit peels were treated with hot water. Both ILs showed a lower degree of esterification than water heat-assisted extraction, although they produced the highest pectin yield. Meanwhile, DES showed a promising result for the esterified carboxyl group, indicating that it can be an alternative solvent to hot water for pectin extraction. According to Zanella and Taranto (2015), a good grade pectin, as specified by the Food and Agriculture Organization (FAO), has a Galacturonic acid level greater than 65%. The dragon fruit peel is a potential source of HM pectin and is feasible to extract by applying different extraction techniques and solvents.

Table 2. FTIR Spectra of the stacked extracted pectin using different treatment methods at the following wavenumbers: 3500 $cm^{-1}$ to 1000 $cm^{-1}$

| Wavelength number /$cm^{-1}$ | Peak Vibration |
|---|---|
| 3238.39 - 3383.03 | -OH stretching |
| 1603.68 -1627.02 | Carbonyl group of carboxylate ion (COO–) |
| 1575.54 | Diketones |
| 1481.12 | O=C–O stretching |
| 1324.56 -1398.39 | –OH bending |
| 1098.99 - 1102.59 | C–O–C stretching |
| 1021.69 - 1070.53 | Alkyl amine |
| 1015.74 - 1018.09 | –CH–O–CH stretching |

### 3.3 Observation of pectin sample's chemical structure

From the FTIR spectrum analysis depicted in Figure 2, the chemical structure of pectin extracted using ionic liquid-assisted extraction and water heat-assisted extraction showed similar results as compared to commercial pectin in which the absorption bands for both solvents indicated carboxylic acid at 3341.41 $cm^{-1}$ and 3356.35 $cm^{-1}$ respectively. Both extraction techniques produced several peaks (Table 2) indicating the presence of aliphatic hydrocarbons and primary aliphatic alcohol at absorption bands of 1603.68 $cm^{-1}$ and 1099.39 $cm^{-1}$ for ionic liquid extraction and 1604.01 $cm^{-1}$ and 1098.99 $cm^{-1}$ for water heat-assisted extraction.

The extracted pectin contained mainly carboxylic acid and secondary alcohol at absorption bands of 3356.35 $cm^{-1}$ and 1099.39 cm-1 compared to commercial pectin at 3383.03 $cm^{-1}$ and 1102.59 $cm^{-1}$ respectively. The FTIR spectra within 3500 $cm^{-1}$ to 3000 $cm^{-1}$ indicating O-H stretching vibration due to free and bound hydroxyl group of carboxylic acid (van Tran *et al.*, 2019). Meanwhile, Bichara *et al.*, (2016) reported that the absorption band of commercial pectin in between 2000 $cm^{-1}$ to $1000^{-1}$ was due to the deformation vibration of -OH group as well as due to C-O stretching which explained the result obtained. Furthermore, Rahmati *et al.*, (2019) revealed that FTIR spectra ranging from 800 $cm^{-1}$ to 1300 $cm^{-1}$ were attributed to the pectin fingerprint indicating the presence of pure pectin.

Meanwhile, it was observed that pectin extracted using DES-assisted extraction exhibited carboxylic acid at 3238.39 $cm^{-1}$ absorption spectra whereas the existence of anhydride group was at 1045.74 $cm^{-1}$ for water ultrasonication-assisted

extraction. This suggests that ionic liquid is more suitable to be used in extracting pectin compound whereby it provides similar characteristics and structure as the commercial pectin. The finding is substantiated by a study on ionic liquid being an excellent solvent in extracting and separating organic materials (Guolin *et al.*, 2012).

Table 2 shows the tabulated form of the vibrational peaks of the FTIR spectra of commercial pectin and the other extracted pectin using the different types of solvents. The strongest vibrational band observed in the FTIR spectra is at 3383.03 $cm^{-1}$ which is attributed to the -OH stretching functional group of the pectin. The presence of this -OH stretching region in the pectin is due to the presence of hydrogen bonds in the galacturonic acid polymer. This vibrational band was seen to be shifted to 3238.39, 3342.36, 3341.41, and 3356.35 cm-1 for the Commercial pectin, [EMIM][Ac], (ChCl: gly 1:2), and Water-ultrasonication, respectively. On the other hand, the vibrational band at 1102.59 $cm^{-1}$ was assigned to C–O–C stretching of the Water-heat assisted extraction of the pectin. This vibration peak has been shifted to wavenumbers of 1098.99 and 1099.39 $cm^{-1}$ for ChCl: gly and Water-ultrasonication, respectively. The band observed at 1070.53 and 1021.69 $cm^{-1}$ were related to the Alkyl amine functional groups and this vibration peak is found to be shifted to 1045.74 $cm^{-1}$ in the [EMIM][Ac]. The peak at 1015.74 $cm^{-1}$ was ascribed to the –CH–O–CH stretching, and this peak shifted to 1017.38 and 1018.09 $cm^{-1}$ for ChCl: gly and Water-ultrasonication, respectively. Furthermore, in the commercial pectin showed peaks at 1627.02, 1575.54, 1481.12 and 1398.39 $cm^{-1}$ which were not consequently present in the Water-heat assisted extraction (WH) and belonged to the functional groups of carbonyl group of carboxylate ion (COO–), Diketones, O=C–O stretching, and –OH bending, respectively. The difference of vibration peaks in the pectin structure demonstrates the presence of complex pectin structures and the various depth of extraction ability using different solvent systems.

The results based on the FTIR spectra analysis were further supported by an analysis conducted using RAMAN in which chemical structures of extracted pectin using ionic liquid-assisted extraction and commercial pectin have a close resemblance. Both extracted and commercialized pectin produced several peaks at the wavelength ranging from 100 $cm^{-1}$ to 650 $cm^{-1}$. The results obtained from the RAMAN spectra are shown in Figure S1.

The interpretation of Raman spectra can be categorized into two sections based on their wavenumbers where the region less than 1600 $cm^{-1}$ are sensitive to the structures of cellulose backbone and the region above 2700 $cm^{-1}$ are sensitive the structure relating to hydrogen bonds (Szymańska-Chargot *et al.*, 2011). Since, most of the spectral range of the peaks varies between 100 $cm^{-1}$ to 1650 $cm^{-1}$. Hence, the pectin extracted are more influenced by the structures of cellulose backbone. The Raman images reveal that most of the changes in the pectin is related to the cell corner zones, implying the release of pectin in the junction corner during fruit harvesting contribute to the mechanical resistance of tissue. (Szymańska-Chargot *et al.*, 2016). The different proportion of the vibration peaks indicates the different capacity of the solvents in penetrating the different functional groups and composition present inside the pectin apart from the commercial pectin. The wavelength peak close at 700 $cm^{-1}$ indicates the presence of highly mixed complex molecule of C-C bonds.

### 3.4 Thermal properties of pectin samples

Thermal properties of pectin were analysed using Differential Scanning Calorimeter (DSC) in the study. Pectin extracted using water-ultrasonication assisted extraction shows similar result as that of the commercial pectin in which it had only an endothermic peak (decomposition temperature) at 118.73°C which is slightly lower compared to 235.74 °C for the latter; and both are without an exothermic peak (degradation temperature). The decomposition temperature for commercial pectin reported by Ruano *et al.*, (2019) was 234.73°C. The pectin molecules in commercial pectin is tightly bound to water due to the high degree of esterification and galacturonic acid content (Wang & Lü, 2014). Thus, the decomposition temperature of commercial pectin was higher as more heat or energy is required to break the bond.

Extracted pectin has lower decomposition temperature due to having a different chemical structure from commercialized pectin (Cava *et al.*, 2018). This is evident from the FTIR result for the pectin extracted using water-ultrasonication assisted extraction with anhydride group at 1045.74 $cm^{-1}$. Anhydride is a compound which has two acyl groups bonded to the same oxygen atom forming van der Waals dispersion and dipole-dipole attractions (Buttkus *et al.*, 1965). The bonds are weaker than hydrogen bonds which reduces the decomposition temperature of the pectin structure.

Meanwhile, pectin extracted using ionic liquid-assisted extraction, water-heat assisted extraction and DES-assisted extraction recorded both endothermic and exothermic peaks. The endothermic peaks for the three extraction techniques were observed at 137.13°C, 179.42°C and 302.81°C while exothermic peaks were observed at 243.65°C, 249.33°C and 227.45°C, respectively. The high degradation temperature indicated that the pectin samples had enough energy to form ordered arrangements and undergo crystallization. This further indicates that pectin extracted using DES was more stable compared to pectin by other methods as it requires higher decomposition temperature to break down the bond.

The evaluation of the DSC for the thermal properties is a result of the measure of the glass transition temperature ($T^g$), which measures the heat capacity when the polymer matrix changes from glass to rubber state due to interactions of the intermolecular, molecular weight, cross-linking density, etc (Perumal *et al.*, 2018). The figure below shows similar patterns of the endothermic peaks visible at time 21 minutes for commercial pectin while the extracted pectin similar peak at 28 minutes, 23 minutes, 24 minutes, and 16 minutes for [EMIM][Ac], (ChCl: gly 1:2), WS and WH, respectively. Commonly, the endothermic peak indicates the capability of the pectin polysaccharide to retain the water content, and this is connected to the hydrophilic properties of the pectin sample. Hence, the lower and higher endothermic peak reflects the low and high content of galacturonic acid and the degree of methylation ($D_w$) (Wang & Lü, 2014). While the exothermic peak was observed only for the Water-heat assisted extraction at 22 minutes, which is usually related to the degradation property of the pectin sample and consequently, it reflects the sample's chemical profile.

### 3.5 Morphology of pectin samples

To gain insight into the morphological structure of the extracted pectin, the morphology of pectin extracted from dragon fruit peels was examined and compared with commercial pectin

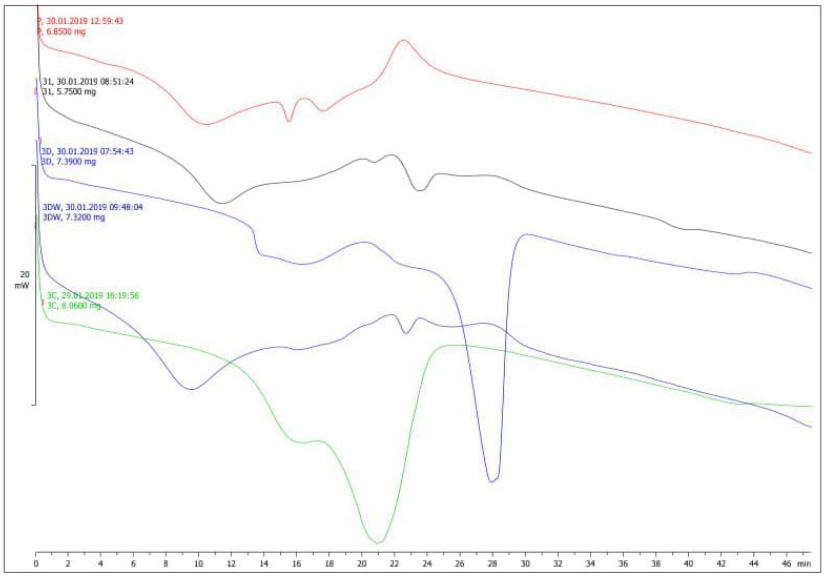



Figure 3. Thermal properties of extracted pectin using different treatment methods shown in a differential scanning calorimeter (DSC).

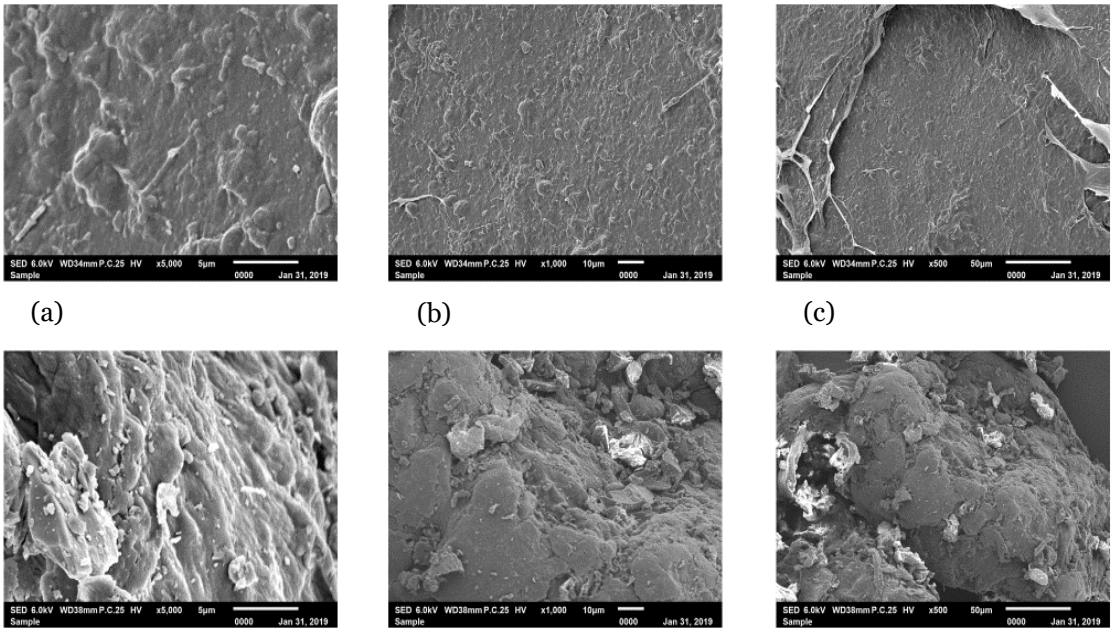



Figure 4: (a) water-heat assisted extraction pectin, mag: X500, (b) water-heat assisted extraction pectin, mag: X1000, (c) water-heat assisted extraction pectin, mag: X5000, (d) commercial pectin, mag: X500, (e) commercial pectin, mag: X1000, (f) commercial pectin, mag: X5000.

(shown in Figure S2(c)). The morphology of the extracted pectin from water heat-assisted extraction and commercial pectin was observed using Scanning Electron Microscopy (SEM) as shown in Figure 4. Figure S2 (a) and (b) are the images of the extracted pectin before and after filtration (for Figure S1, S2, and Table S1, please refer to Supplementary Figures and Tables). The image of the extracted pectin in Figure S2 (b) manifests a jelly-like structure after filtration. This shows the capability of pectin to be used in the designing of food products and their versatile gelling properties. These properties are used in the industry to make jellies, fruit juice, jams, confectionery products etc. Furthermore, the degree of methylation (DM) is the parameter to observe the gelling capabilities using the infrared spectroscopy, which will further allow to understand and apply their use in the food industry. The different gelling properties were related to the different charged carboxyl groups (Gawkowska *et al.*, 2019).

Figure 4 (a) - (c) are the micrographs of extracted pectin from water-heat assisted extraction and Figure 4 (d) - (f) are the micrographs for commercial pectin at magnifications of X500, X1000 and X5000, respectively. Both the extracted and commercial pectin samples showed better images at magnification of X5000. The extracted pectin showed smooth and less aggregate surfaces as compared to the commercial pectin which had more aggregate surfaces. Nevertheless, the extracted pectin contained relatively less porous surfaces compared to the commercial pectin. Moreover, the extracted pectin is not as dense and less compact in structure comparatively. The observation was supported by Rahmati *et al.* (2019) in which pectin extracted from dragon fruit peel exhibited a uniform, compact, and dense structure.

A similar pattern was observed by Benassi *et al.*, (2021) in the orange peel for the surface structure under the SEM giving a similar morphological distinction between the commercial pectin and the extracted pectin. The commercial pectin showed a granular shape while the extracted pectin was seen as flat surface. The stressed surface with deep cavities reflected the use of harsher conditions while the milder conditions are reflected by its smoother surface. On the other hand, the micrograph images from a passion fruit peel revealed similarly that the nano structure of the extracted wet pectin was smooth and compact plus the surface showed little wrinkles (Liew *et al.*, 2014). Thus, the images of the extracted pectin were shown to have structural surface that were smooth with little mound-shaped pellets present.

## 4. Conclusion

Using ionic liquid for extracting pectin from dragon fruit resulted in much higher yields compared to other solvents. More precisely, the extraction of pectin employing ionic liquid resulted in a yield that was 60±2.00 wt% greater than the extraction obtained from other fruit sources examined in this work. Furthermore, the use of ionic liquid resulted in an esterification degree of approximately 66-72%, demonstrating a desirable combination of a substantial yield and a moderate level of esterification. The results demonstrate that ionic liquid is the optimal solvent for extracting pectin from dragon fruit due to its superior performance in terms of both yield and esterification levels. Furthermore, water is an effective solvent capable of generating a pectin structure that closely resembles commercial pectin, which is composed of carboxylic acid and secondary alcohol. On the other hand, DES showed more stable thermal properties which is an important aspect for extraction solvent. These findings conclude that both ionic liquid and DES can be potential extraction solvents attributing to higher yield, degree of esterification, better chemical structure, and thermal properties compared to commercial solvent.

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## 7. Conflict of interest

The authors declare no conflict of interest.

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**Supplementary Figures and Tables**

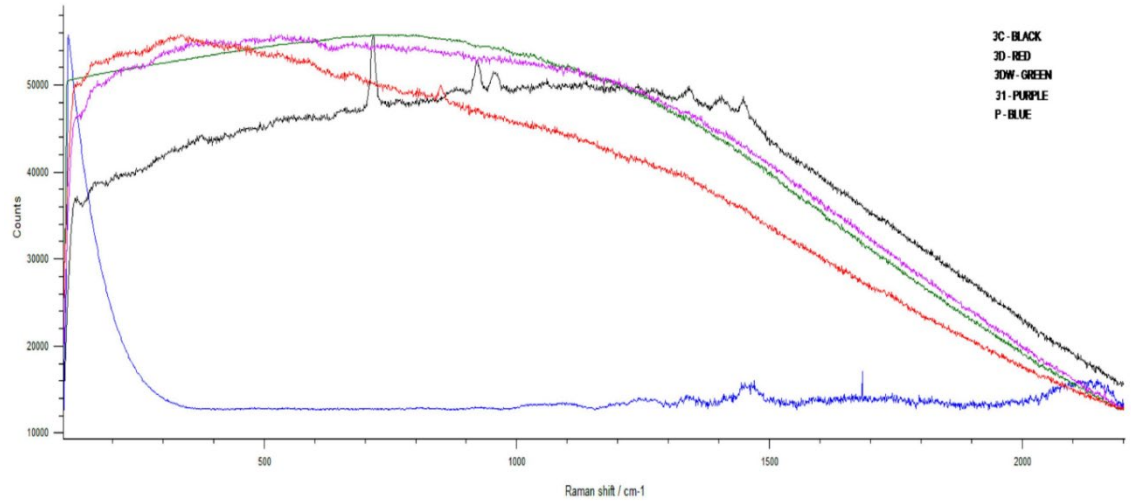


Figure S1. RAMAN Spectra of extracted pectin using different treatment methods: (a) Commercial pectin, (b) Ionic Liquid (1-ethyl-3-methylimidazolium acetate ≥ 97%) [EMIM]OAc assisted extraction, (c) Deep Eutectic Solvent (Choline chloride and glycerol, 1:2) assisted extraction, (d) Water-ultrasonication assisted extraction, (e) Water-heat assisted extraction at the following conditions: 20× magnification, extended Raman range : 734 – 1787 $cm^{-1}$, exposure time: 10s, laser power: 1% 785 nm edge.

(a) (b) (c)

Figure S2. (a) Water-heat assisted extraction pectin before filtration (b) Commercial Pectin in powdered form. (c) Water-heat assisted extraction pectin after filtration.

Table S1: Thermal properties of the extracted pectin using different treatment methods. Note: Ionic Liquid - [EMIM]OAc assisted extraction, DES - (ChCl: gly 1:2) assisted extraction, WS- water-ultrasonication assisted extraction and WH- water-heated assisted extraction. at the following wavenumbers: 3500 $cm^{-1}$ to 1000 $cm^{-1}$

| Method of Pectin Extraction | Glass Transition Temperature ($T^g$)(˚C) |
|---|---|
| Commercial Pectin | 179.16˚C – 235.74˚C |
| [EMIM][Ac] | 137.13˚C – 258.31˚C |
| (ChCl: gly 1:2) | 162.37˚C - 302.81˚C |
| WS | 118.73˚C - 251.02˚C |
| WH | 126.34˚C – 249.33˚C |

**HALALSPHERE**
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# Impact of Date Powder, Sacha Inchi Oil, and Moringa Powder in a Novel Cognitive-Enhancing Health Bar: An Evaluation of Physicochemical Properties and Functional Benefits

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**Abstract**

Recent trends show a growing preference for healthy snacks in diets, particularly among health-conscious consumers, with snack bars gaining popularity among youth. This study aimed to develop and analyse a nutritious health bar to enhance cognitive performance. The health bar's formulation was created using design expert software. Comprehensive testing included proximate analysis, fatty acid profiling, and vitamin, mineral, and antioxidant evaluations, complemented by texture and colour assessments. Findings reveal these bars to be nutritionally rich, featuring excellent physical characteristics. They are notably abundant in essential fats (omega-3 and omega-6), vitamins, and minerals. The bars' high essential fat content, varied vitamin and mineral composition, and strong antioxidant properties align with the Recommended Nutrient Intakes (RNI) for Malaysian children and adolescents. This composition suggests that these health bars could effectively boost cognitive performance in this group.

## 1. Introduction

Recent shifts in consumer behaviour indicate a gradual movement towards more intelligent and health-conscious eating and snacking patterns. Emphasis on maintaining overall health and wellness is not just limited to exercise and fitness; it significantly encompasses the practice of balanced dietary habits. This shift is partly spurred by health promotion campaigns and the implementation of nutritional guidelines in hospitals and public settings, notably increasing consumer health awareness (Curtain & Grafenauer, 2019). Consequently, healthier snacking options have been integrated into everyday diets, providing consumers with an extensive array of choices that align with their perception of healthy eating. Among these options, snack bars have emerged as popular, especially among children and adolescents. Recent trends have seen snack bars increasingly occupy a prominent place in grocery store aisles (Andrew, 2018; Mohd Noor, Hazahari & Shahidan, 2022). Their appeal spans a diverse consumer base, ranging from athletes to the working population, owing to their convenience, immediate energy boost, nutrient content, and satiety.

Integrating functional ingredients into the bars has significantly added value to them. Functional ingredients are bioactive compounds that can manufacture food products to give consumers extra health benefits beyond basic nutrition (Syed, Akram & Shukat, 2019). In response to this demand, manufacturers are fortifying snack bars with functional ingredients that provide essential nutrients like vitamins, minerals, antioxidants, and fibre, addressing the growing need for genuinely 'healthy bars' (Amerikanou *et al.*, 2023; Hess, Rao & Slavin, 2017). This nutritional enhancement promises a sustainable, global market appeal. The evolving consumer expectation towards low-sodium, low-calorie, and low-sugar products challenges manufacturers to innovate while maintaining the bars' original taste and appeal. Such innovation holds the potential to capture a wider consumer segment, particularly young people, who significantly drive global snack bar sales (Curtain & Grafenauer, 2019).

Furthermore, the link between a balanced diet and cognitive performance, especially in children and adolescents, is becoming increasingly evident. Studies show that adequate and nutritious food intake optimises brain functions like focus, comprehension, and application in learning contexts (Salam *et al.*, 2019; Correa-Burrows *et al.*, 2016). In Islamic dietary practices, the concept of *halalan toyyiban* — promoting balanced nutrition for both the body and the soul — is integral (Abd Razak, Ramli, and Jamaludin, 2019). Foods that are both halal and *toyyib* (wholesome) are believed to be beneficial for

brain health due to their high nutrient content (Wahju Dyah *et al.*, 2018).

Given this backdrop, this study was initiated with dual objectives: first, to create a *halalan toyyiban* health bar tailored to enhance cognitive performance in children and adolescents as a part of their daily snack intake; second, to analyse its physicochemical properties. This involved conducting comprehensive proximate, vitamin, and mineral analyses, assessing antioxidant activity and determining the bars' texture and colour to underline their nutritional and sensory qualities. The remarkable results obtained from the first two objectives will be used for a later stage of this study by conducting an acceptance test of the health bar and investigating the cognitive performance of school children and adolescents before and after the health bar intervention in the diet while testing the suggested theory in this paper.

## 2. Materials and methods

### 2.1 Materials

Several components were included in the health bar formulation, notably the functional ingredients. Date powder, Sacha inchi *(Plukenetia volubilis)* oil, and Moringa (*Moringa oleifera)* powder were obtained from local manufacturers in Selangor. The primary structure of the health bar, comprising bubble rice, pumpkin seeds, and oats, was acquired from a manufacturer in Klang, Selangor. Additionally, Beryl's dark chocolate compound and sugar alcohols (maltitol and isomalt) were utilised as coatings for the health bar.

### 2.2 Methods

*2.2.1 Development of a health bar recipe*

Optimisation of the functional ingredients (Date powder, Sacha inchi *(Plukenetia volubilis)* oil, and Moringa (*Moringa oleifera)* powder) was conducted based on the simplex lattice design (Ying *et al.*, 2018), capping at a maximum of 25% for the 25.00 g health bar recipe. The quantities of other ingredients, including dark chocolate compound, maltitol, isomalt, bubble rice, oats, and pumpkin seeds, were kept constant throughout the study. A previous study had established that the upper limits for date powder, Sacha inchi *(Plukenetia volubilis)* oil, and Moringa *(Moringa oleifera)* powder were 12.5%, 10.0%, and 7.5%, respectively, while their lower limits were set at 7.5%, 5.0%, and 2.5%, respectively (Sarifudin *et al.*, 2020). These limits were inputted into Stat Ease Design Expert Version 12, yielding 14 different health bar formulas.

*2.2.2 Assembling the health bar*

All ingredients were measured according to the recipe as the initial step in preparing the health bar. The pumpkin seeds and oats were then toasted in an oven at 160℃ for about 5 minutes (Ho *et al.*, 2016), followed by mixing with bubble rice and Sacha inchi *(Plukenetia volubilis)* oil. Subsequently, the sugar alcohols (maltitol and isomalt) along with date powder were melted at 150℃ (Hadjikinova & Marudova, 2016) and then combined with the main components of the health bar (bubble rice, pumpkin seeds, and oats). The dark chocolate compound was melted using a double boiler method and then mixed with Moringa *(Moringa oleifera)* powder, ensuring thorough stirring to prevent clumping. The health bar was then formed by pressing the mixture into a mould measuring 20.0 cm x 20.0 cm x 3.0 cm. The melted dark chocolate compound was evenly drizzled over the health bar and then stored in the refrigerator before analyses.

*2.2.3 Composition assessment with proximate analysis*

The proximate composition of the 14 health bars, including moisture, ash, protein, fat, fibre, carbohydrate, and energy content, was determined following the AOAC methods 18th ed. 2005, 984.25, 923.03, 981.10, 991.36, 962.09, respectively. These measurements were conducted in triplicate.

*2.2.3.1 Moisture content*

The moisture content of all health bar samples (14 formulations) was analysed using a moisture analyser (METTLER TOLEDO, United States). Around 2.0 g of the health bar from each recipe was weighed using a weighing scale (Sartorius, Germany) and individually placed in the moisture analyser. The samples were run in the moisture analyser until the optimum moisture content was reached. The final moisture content readings for each sample were recorded in the logbook.

*2.2.3.2 Ash content*

The total ash content of all health bar samples was determined following AOAC (1990), Method 923.03. The percentage of crude ash was calculated using the following equation:

$$Crude\ ash\ (dry\ basis)\ (\%) = (W1 \div W2) \times 100$$

W1 is the weight after ashing, and W2 is the weight before.

*2.2.3.3 Protein content*

All health bar samples' protein and nitrogen content were analysed using the Kjeldahl Method (AACCI, 1995), Method 46-11.02. The samples underwent processes of digestion, distillation, and titration. Upon completion of titration, the instrument displayed the final percentage of protein and nitrogen, which was recorded in the logbook. The percentage of crude protein was expressed from the total nitrogen percentage, multiplied by a factor of 6.25 – the nitrogen-protein conversion factor for grain samples. The measurement of crude protein was calculated using the following equation:

$$Crude\ protein\ (\%) = Nitrogen\ (\%)\ in\ sample \times 6.25$$

*2.2.3.4 Fat content*

The fat content of all health bar samples was analysed using the Automatic Soxhlet extraction method (Gerhardt Soxtherm® extractor, Germany). Approximately 2.0 g of samples from each recipe were weighed on filter papers. Each filter paper containing the sample was folded and placed into a pre-dried extraction thimble, topped lightly with glass wool. Then, the thimble was positioned into the extraction beaker containing three boiling stones and filled with 130 mL of petroleum ether. The instrument was programmed according to Gerhardt's manual. Finally, the extracted residue was dried overnight in an oven at 105°C. The percentage of fat content was calculated using the following equation:

$$Fat\ (\%) = [(W1 - W2) \div W0] \times 100$$

W1 is the total weight of the extraction beaker with boiling stones and extracted fat, W2 is the total weight of the extraction beaker and boiling stones, and W0 is the weight of a healthy bar sample.

*2.2.3.5 Crude fibre content*

The crude fibre content of all healthy bar samples will be determined according to the AOAC (1990) Method. The crude fibre will be determined by the digestion of samples with sulphuric acid. Moreover, sodium hydroxide is on a hot plate (Favorit, Malaysia), as Adamu, Ajayi and Oyetunde (2016) claimed. The crude fibre content will be calculated using the following equation:

$$Crude\ fibre\ (\%) = [(C - A) - (D - E)] / B \times 100$$

C is the weight of the crucible and dried fibre bag after digestion, A is the weight of the fibre bag, D is the weight of the crucible and ash, E is a blank value for the empty fibre bag, and B is the weight of the healthy bar sample.

*2.2.3.6 Carbohydrate content*

The carbohydrate content of all health bar samples was determined using the following equation:

$$Carbohydrate\ (\%) = 100 - [Moisture\ (\%) + Ash\ (\%) + Protein\ (\%) + Fat\ (\%)]$$

*2.2.4 Fatty acid composition*

Referring to the proximate analysis results, only two health bar formulations were selected for further analysis. The fatty acid profiling of these selected formulations was conducted using the AOAC method 20th edition 2016, 996.06. The measurements were carried out in triplicate.

*2.2.5 Evaluation of mineral, vitamin and antioxidant levels*

The mineral content (magnesium) of the selected health bar formulations was determined using the Atomic Absorption Spectroscopy (AAS) Method No: STP/Chem/A13-AAS. In addition, the vitamin E (alpha-tocopherol) content in these formulations was determined using the High-Performance Liquid Chromatography (HPLC) Method No: STP/Chem/A11-HPLC. Antioxidant activity was evaluated by monitoring the inhibition (%) of the compound 2,2-diphenyl-1-picrylhydrazyl (DPPH). These measurements were conducted in triplicate.

*2.2.6 Assessment of texture and colour*

Two of the selected health bar formulations were tested for hardness using Texture Analyzer TA-XT Plus (Stable Micro System, London), referring to the method as previously described (Puangjinda, Matan & Nisoa, 2016). To evaluate the colour of the selected health bar, the Hunter Lab Colorimeter (LabScan XE Spectrophotometer, Hong Kong) was used as described in the previous study (Prazeres *et al.*, 2017). The measurement was carried out in triplicate.

*2.2.7 Quantitative data analysis*

The statistical analyses for this study were conducted using the SPSS software, version 20.0 from IBM. Utilising analysis of variance (ANOVA), relationships among various variables were tested. Measurements for this study were taken thrice, and the significance of differences in the mean values of the samples was determined, with a significance level set at $p < 0.05$.

## 3. Results and disussion

### 3.1 Fundamental nutrient composition of health bar

The nutrient composition of 14 different health bar formulations was determined using proximate analysis, focusing on moisture, ash, protein, fat, fibre, carbohydrate, and energy content. The data depicted in Table 1. This table illustrate the nutrient levels per 100 g servings of these health bars.

For moisture content, our health bars showed a range of 5.15 to 7.89 g per 100 g servings, a markedly lower range than date paste snack bars (15.73% - 26.25%), as Parn *et al.* (2015) reported. This reduction is attributed to our use of powdered ingredients over the paste, a shift that Cuq *et al.* (2013) noted typically leads to decreased moisture and, consequently, longer shelf lives.

In contrast, the ash content in our health bars was notably higher, ranging from 2.17 g to 2.85 g per 100 g servings, compared to the 1.88% - 1.93% in date paste bars. Nevertheless, our bars showed lower ash content than oat-date paste bars (1.4% - 4.5%), as Munir *et al.* (2018) reported. Such results suggest our bars may be rich in certain micronutrients, especially magnesium and vitamin E, as inferred from their ingredients like dates, Moringa *(Moringa oleifera)*, and pumpkin seeds (Syed, Akram & Shukat, 2019; Gopalakrishnan, Doriya & Kumar, 2016). Besides, these studies found that magnesium and vitamin E are pivotal in enhancing cognitive performance since they transport energy to brain cells.

Protein levels in our bars ranged between 9.33 g and 10.96 g per 100 g servings. These figures surpass those in prior studies (Ho *et al.*, 2016; Parn *et al.*, 2015) and nearly meet the European Consumer Health and Consumers Directorate-General's (2012) minimum guideline of 10 g of protein in health bars. Our ingredient selection, including protein-rich Moringa *(Moringa oleifera)* powder and pumpkin seed, led to several formulations achieving the desired protein content. Furthermore, these bars potentially provide 21% to 26% of the Malaysian Recommended Nutrient Intake (RNI) protein in children and adolescents.

Fat content was another area where our bars excelled, showing higher values (15.06 g - 22.76 g per 100 g servings) than previous studies by Munir *et al.* (2018) and Parn *et al.* (2015). The bars incorporate omega-3 and omega-6, high in essential fatty acids from sources like Sacha inchi *(Plukenetia volubilis)* oil and Moringa *(Moringa oleifera)* powder. Ya-Nin & Phakkharawat (2017) research indicated that such inclusion could enhance cognitive functions in children and adolescents. By Malaysian health standards, these bars could contribute around 25.0% to 30.8% of the total recommended fat intake for the youth.

Although the fibre content was relatively modest (1.38 g - 3.47 g per 100 g servings), it aligns with the findings by Saini *et al.* (2021) and Javed *et al.* (2020). Meeting the European Union's

Table 1. Nutrient composition of the health bar

| Formulation | Test Parameter / Nutrition facts (per 100 g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Moisture (g) | Ash (g) | Protein (g) | Fat (g) | Crude fibre (g) | Carbohydrate (g) | Energy (kcal) |
| 1 | 6.09 ± 0.09$^{b}$ | 2.76 ± 0.03$^{b}$ | 9.38 ± 0.45$^{c}$ | 17.80 ± 0.22$^{c}$ | 2.30 ± 0.29$^{b}$ | 63.97$^{a}$ | 453.61$^{b}$ |
| 2 | 7.10 ± 0.27$^{a}$ | 2.49 ± 0.03$^{b}$ | 9.67 ± 0.43$^{c}$ | 18.17 ± 0.73$^{c}$ | 3.47 ± 0.24$^{a}$ | 62.56$^{a}$ | 452.45$^{b}$ |
| 3 | 7.69 ± 0.08$^{a}$ | 2.37 ± 0.03$^{b}$ | 9.80 ± 0.09$^{c}$ | 19.68 ± 0.29$^{b}$ | 2.34 ± 0.09$^{b}$ | 60.48$^{a}$ | 458.21$^{b}$ |
| 4 | 7.87 ± 1.51$^{a}$ | 2.43 ± 0.02$^{b}$ | 9.51 ± 0.13$^{c}$ | 19.59 ± 0.49$^{b}$ | 2.75 ± 0.04$^{b}$ | 60.60$^{a}$ | 456.73$^{b}$ |
| 5 | 6.80 ± 0.16$^{b}$ | 2.49 ± 0.06$^{b}$ | 9.41 ± 0.06$^{c}$ | 16.83 ± 0.16$^{c}$ | 1.67 ± 0.35$^{c}$ | 64.47$^{a}$ | 446.98$^{b}$ |
| 6 | 6.41 ± 0.11$^{b}$ | 2.85 ± 0.07$^{b}$ | 9.82 ± 0.51$^{c}$ | 15.06 ± 0.23$^{c}$ | 2.55 ± 0.25$^{b}$ | 65.87$^{a}$ | 438.25$^{b}$ |
| 7 | 6.02 g ± 0.07$^{b}$ | 2.57 ± 0.01$^{b}$ | 10.03 ± 0.11$^{c}$ | 17.86 ± 0.31$^{c}$ | 1.49 ± 0.29$^{c}$ | 63.52$^{a}$ | 454.92$^{b}$ |
| 8 | 5.97 ± 0.51$^{c}$ | 2.44 ± 0.05$^{b}$ | 9.43 ± 0.06$^{c}$ | 18.73 ± 0.90$^{c}$ | 1.38 ± 0.11$^{c}$ | 63.43$^{a}$ | 460.01$^{b}$ |
| 9 | 5.23 ± 0.15$^{c}$ | 2.36 ± 0.06$^{b}$ | 9.33 ± 0.08$^{c}$ | 21.01 ± 0.95$^{b}$ | 2.17 ± 0.32$^{b}$ | 62.07$^{a}$ | 474.67$^{b}$ |
| 10 | 5.15 ± 0.12$^{c}$ | 2.35 ± 0.011$^{b}$ | 10.38 ± 0.18$^{b}$ | 20.22 ± 0.27$^{b}$ | 2.14 ± 0.19$^{b}$ | 61.90$^{a}$ | 471.10$^{b}$ |
| 11 | 5.25 ± 0.12$^{c}$ | 2.31 ± 0.007$^{b}$ | 10.31 ± 0.08$^{b}$ | 22.76 ± 0.13$^{b}$ | 1.92 ± 0.04$^{c}$ | 59.37$^{a}$ | 483.58$^{b}$ |
| 12 | 5.81 ± 0.09$^{c}$ | 2.33 ± 0.009$^{b}$ | 10.86 ± 0.16$^{a}$ | 19.88 ± 0.64$^{b}$ | 3.36 ± 0.22$^{a}$ | 61.12$^{a}$ | 486.83$^{b}$ |
| 13 | 5.43 ± 0.35$^{c}$ | 2.17 ± 0.006$^{b}$ | 10.96 ± 0.04$^{a}$ | 20.91 ± 0.56$^{b}$ | 2.42 ± 0.43$^{b}$ | 60.53$^{a}$ | 474.13$^{b}$ |
| 14 | 5.62 ± 0.03$^{c}$ | 2.29 ± 0.005$^{b}$ | 10.51 ± 0.09$^{a}$ | 21.31 ± 0.43$^{b}$ | 3.34 ± 0.23$^{a}$ | 60.28$^{a}$ | 474.92$^{b}$ |

Values are means ± SD. Values with the same letters within the same column are not significantly different ($p < 0.05$).

(EU) definition of a source of fibre, the bars promise a longer satiety duration, a claim supported by Ho *et al.* (2016).

Lastly, our health bars could provide a significant percentage of the daily energy requirement for active adolescents and children, between 16.60% and 22.86%. Carbohydrate levels, deduced through basic calculations, ranged from 59.37% to 65.87%, akin to the findings of Parn *et al.* (2015).

### 3.2 Polyunsaturated fatty acid composition of health bar

Following the proximate analysis results presented in Table 1, we chose two health bar formulations, Formulation 12 and Formulation 14, for advanced assessments due to their superior nutritional profiles. These formulations were further analysed for their polyunsaturated fatty acid composition, mineral and vitamin contents, antioxidant properties, and physical attributes like colour and texture. The polyunsaturated fatty acid composition for both formulations is provided in Table 2.

The analysis of polyunsaturated fatty acids (PUFAs) in Formulation 12 and Formulation 14 aimed to detail the fat composition noted in the proximate analysis results. In Formulation 12, each 100 g sample contained 5.74 g (80.88%) of Linoleic (cis) acid and 1.28 g (18.07%) of α-Linolenic acid. For Formulation 14, the composition per 100 g included 6.80 g (68.90%) of Linoleic (cis) acid (omega-6) and 3.01 g (30.52%) of α-Linolenic acid (omega-3). The statistical analysis showed no significant differences in these acids' quantities between both formulations. Given the critical role of these PUFAs in enhancing cognitive functions, their quantification was crucial. The high levels of these fatty acids can be attributed to the substantial inclusion of Sacha inchi *(Plukenetia volubilis)* oil in both formulations. Prior research on Sacha inchi *(Plukenetia volubilis)* oil revealed that it contains about 39.86% Linoleic (cis) acid and 42.71% α-Linolenic acid, accounting for nearly 83.00% of its total fat composition. Omega-3 fatty acids, particularly α-Linolenic acid, are essential for transporting ions and neurotransmitters across nerve cell membranes. Dong *et al.* (2020) noted that a deficit in omega-3 fatty acids could lead to other fats compensating, potentially reducing membrane fluidity, impairing synapse and dendrite functions, and altering neurotransmitter concentrations. This alteration can significantly affect brain performance. Furthermore, Dighriri *et al.* (2022) indicated that incorporating omega-3 and omega-6 fatty acids into diets enhances oxygen saturation and concentration in haemoglobin, improving cerebral blood flow. This enhancement is particularly beneficial in correcting early memory and learning challenges, especially in young children.

Table 2. Polyunsaturated fatty acid composition of the health bar

| Formulation | Fatty acid profile (g /100 g sample) | |
|---|---|---|
| | Linoleic (cis) | α-Linolenic |
| 12 | 5.74 [a] | 1.28 [a] |
| 14 | 6.80 [a] | 3.01 [a] |

Values with the same letters within the same column are not significantly different ($p < 0.05$).

Table 3. Mineral, vitamin and antioxidant levels of the health bar

| Formulation | Analysis | | |
|---|---|---|---|
| | Magnesium (mg/kg) | Vitamin E as alpha-tocopherol (mg/100g) | DPPH (% inhibition) |
| 12 | 1166[a] | 43 [a] | 7.37 [a] |
| 14 | 1152 [a] | 80 [a] | 8.91 [a] |

Values with the same letters within the same column are not significantly different ($p < 0.05$).

### 3.3 Mineral, vitamin and antioxidant levels of health bar

Table 3 displays the content of magnesium, vitamin E as alpha-tocopherol, and the percentage of DPPH inhibition in the chosen health bar formulations (Formulation 12 and Formulation 14).

The magnesium levels for Formulation 12 and Formulation 14 were measured at 1166.0 mg/kg and 1152.0 mg/kg, respectively. These figures are marginally above those found by Munir *et al.* (2018), who reported about 1074 mg/kg. The Recommended Nutrient Intakes (RNI) indicate that children and adolescents of both sexes should consume at least 410.0 mg and 360 mg of magnesium, suggesting these bars could meet these needs. Compared to the RNI, both bars fell short in vitamin E content, with Formulation 12 providing 0.43 mg/100 g and Formulation 14 at 0.80 mg/100 g, against the advised 10.0 mg/100 g for males and 7.5 mg/100 g for females. Therefore, the bars could offer only about 4.3% (Formulation 12) to 5.7% (Formulation 14) of the RNI for vitamin E to both male and female adolescents.

DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition measured antioxidant activity was 7.37% for Formulation 12 and 8.91% for Formulation 14. This activity is notably less than in previous studies, where DPPH inhibition ranged from 12.40% to 26.04% (Yasinta, Yao & Chang, 2021). The DPPH assay, employing a stable radical to evaluate antioxidant scavenging capacity, signifies the antioxidant's ability to neutralise free radicals potentially harmful to brain function. Crucial micronutrients for enhancing cognitive abilities, such as magnesium, potassium, zinc, vitamin E, and B-complex vitamins, have been identified in earlier research (Tardy *et al.*, 2020). These nutrients are vital for cognitive health as they participate in the energy metabolism of brain cells, neurotransmitter synthesis, receptor binding, and maintenance of membrane-ion pumps (Huskisson, Maggini and Ruf, 2007). Hence, it is reasonable to suggest that the current formulations of these health bars can provide key nutrients beneficial for cognitive enhancement.

### 3.4 Texture and colour assessment of health bar

Evaluating texture and colour is crucial as they significantly influence consumer acceptance of health bars. The texture measurements for Formulation 12 and Formulation 14 revealed hardness values of 24.03 ± 2.49N and 23.59 ± 2.67N, aligning closely with earlier findings of 24.27 ± 0.29N and 25.14 ± 0.69N by Yasinta, Yao & Chang (2021). The firmness of these health bars is attributed to the solidification of sugar alcohol and its integration with moisture-absorbing date powder, leading to a denser structure. Nevertheless, this level of hardness falls within a desirable range, indicating ease of chewing (Parn *et al.*, 2015). In terms of colour, the health bars showed L*, a*, and b* values of 48.97 ± 0.84 and 44.02 ± 0.14 for lightness, 4.79 ± 0.11 and 6.62 ± 0.14 for redness, and 20.82 ± 0.60 and 24.70 ± 0.40 for yellowness, respectively. In colourimetric terms, L* values range from 0 to 50 for darker shades and 51 to 100 for lighter ones. Thus, both bars exhibited darker hues, primarily due to the caramelisation of sugar alcohol during their production (Lucas *et al.*, 2019). Furthermore, positive a* and b* values indicate the dominance of red and yellow tones, imparting an orange tint to the bars (Parn *et al.*, 2015).

### 4. Conclusion

The simplex lattice mixture design method effectively identified the optimal blend of date powder, Sacha inchi *(Plukenetia volubilis)* oil, and Moringa *(Moringa oleifera)* powder for crafting the health bar. Optimal formulations were achieved with two specific combinations: one comprising 12.50% w/w date powder, 5.00% w/w Sacha inchi *(Plukenetia volubilis)* oil, and 7.50% w/w Moringa *(Moringa oleifera)* powder, and the other consisting of 12.50% w/w date powder, 10.00% w/w Sacha inchi *(Plukenetia volubilis)* oil, and 2.50% w/w Moringa *(Moringa oleifera)* powder. These health bars not only stand out nutritionally — featuring low moisture but high in ash, protein, fats, carbohydrates, energy, and dietary fibre — but also excel in their content of crucial fatty acids, significant levels of magnesium and vitamin E (alpha-tocopherol), and notable antioxidant properties. As far as producing this health bar, it was challenging to find the best ingredients to cater for the nutrition needs whilst keeping its good taste, finding the best method to assemble the health bar

and experimenting with the bar's flavour to be accepted in the market. Therefore, several suggestions to improve the health bar experiment can be made for future research, including finding other suitable ingredients to coat and bind the health bar, testing the shelf life of the health bar and carrying out detailed nutrition analysis on the health bar. Nevertheless, given these attributes of the produced bars, they are theoretically primed to enhance cognitive abilities in children and adolescents. They are poised to redefine the snack bar market, which traditionally prioritises energy provision over nutritional richness.

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HALALSPHERE
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# Exploring the Potential of 5 Commercial Essential Oils to Inhibit the Proliferation of A549 Lung Cancer Cells

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**Abstract**

Globally, lung cancer stands as the primary cause of cancer-related fatalities, securing a spot among the top 10 in newly reported cases. The late detection of lung cancer is often attributed to its indication through commonplace symptoms like coughing. Seeking alternatives, this research investigated the medicinal potential of certain essential oils (EOs) with a rich history in traditional medicine. The study aimed to assess the effectiveness of 5 EOs — Clary Sage, Frankincense, Marjoram, Myrrh, and Thyme as a halal alternative therapy for lung cancer A549 cell line. Notably, based on these investigations' findings, Myrrh and Thyme emerged as promising candidates, displaying significant capability to inhibit lung cancer cell expansion. Myrrh had the lowest $IC_{50}$ value, 19 µg/mL, followed by Thyme, 45 µg/mL. In optimisation research, Myrrh resulted in 85% inhibition after 72 hours of exposure to 800 µg/mL concentration. Myrrh demonstrated reduced cell generation and growth rates for cytokinetic study and increased cell death rate. In conclusion, this research was designed to explore the cytotoxic effects of EOs on lung cancer cells using the A549 cell line, leading to the identification of a potential alternative source of halal-compliant pharmaceuticals.

## 1. Introduction

Lung cancer is the leading cause of death worldwide, along with prostate and breast cancers for both genders. Non-small cell lung cancer (NSCLC) is the most common case reported among the cancer cases, accounting for about 85% overall, while the remaining 15 cases belong to small cell lung cancer (SCLC). There are 3 sub-types of NSCLC: (a) adenocarcinoma, (b) squamous cell carcinoma, and (c) large cell carcinoma (Li & Liu, 2023). To this day, the statistical analysis reports that the 5-year relative survival rate for lung cancer is approximately 22%. Overall, from the lung cancer cases, NSCLC's survival rates stand at 26%, while SCLC's is lower, at only 17%. As only 24% of lung cancer diagnoses happen during the localised stage, early detection of lung cancer is rare. If lung cancer could be detected earlier, the 5-year survival rate could improve to 60%. When talking about earlier detection, the symptoms of lung cancer are normally present only at advanced stages. Not only are the symptoms present late, but they are always misinterpreted as normal symptoms or other types of disease just because they are quite common, such as recurrent lung infections, difficulty breathing, chest pain, persistent coughing, and blood in the sputum (Siegel *et al.*, 2022).

The optimal treatment for lung cancer, whether NSCLC or SCLC, is conditional upon factors such as tumour type, stage, and molecular characteristics. In cases of otherwise healthy individuals diagnosed with early-stage NSCLC, the standard approach involves surgery, possibly complemented by chemotherapy, targeted medications, immunotherapy, and/or radiation therapy (Ahern *et al.*, 2021). For advanced-stage NSCLC, common treatments include chemotherapy, targeted medications, and/or immunotherapy (Guo *et al.*, 2022). Early-stage SCLC is typically addressed with chemotherapy, either alone or in conjunction with radiation. In certain instances of early-stage SCLC, prophylactic cranial radiation may be administered to reduce the risk of brain metastases. Individuals with advanced-stage SCLC may undergo chemotherapy with or without immunotherapy (Hiddinga *et al.*, 2021).

Beyond the conventional approaches to lung cancer treatment mentioned earlier, phytochemicals are developing as promising additions to therapeutic agents (Nguyen *et al.*, 2021). Phytochemicals, derived from plants and employed in traditional medicine for an extended period, are gaining attention among researchers for anticancer therapies. Notable examples of plant-based anticancer medications in the market include vinplastine, etoposide, paclitaxel, and camptotecin (Omara *et al.*, 2020). Essential oils (EOs), considered secondary metabolites produced by plants for protection against environmental stresses, are another approach of interest. These aromatic essences, volatile or ethereal oils, can be extracted from various plants (Sharmeen *et al.*, 2021). With a history rooted in traditional medicine, EOs have been attributed to various biological properties over the centuries. Long-term studies suggest their potential beneficial impact in the fight against cancer. Researchers are growing interested in

natural chemicals and plant-based products with halal status, fascinated by the potential anticancer properties these substances may possess. Unlike synthetic pharmaceuticals, these substances maintain their natural identities, demonstrate comparable efficacy, and often have fewer adverse effects (Abd-Rabou & Edris, 2022).

As the global Muslim population continues to rise, there is a corresponding increase in the demand for halal food and products, crucial for devotees of Islam. Pharmaceuticals developed to address specific diseases, particularly cancer, are vital to these requirements. Recognising the necessity for halal products in the Muslim community, researchers have explored alternative substances that can serve as safe raw materials. While certain fundamental components, such as plants, are inherently considered halal, the utilisation of plant species varies due to potential toxicity in some. Hence, experts are increasingly focusing on natural substances, particularly those derived from plants, as potency halal medical regimens for varied ailments and lung cancer. Essential oils emerge as potentially valuable complementary medicinal sources, drawing from their extensive historical use in traditional medicine. Numerous earlier studies have highlighted the anticancer effects of EOs, positioning them as promising candidates in the pursuit of halal medical treatments. This exploration aligns with the growing awareness of the diverse applications of natural substances, emphasising their potential role in meeting the unique needs of the Muslim community.

Remarkably, there is an increasing focus on the pharmacological applications of EOs, particularly their ability to induce apoptosis in various cancer cell lines. The potent antibacterial effects against various pathogens and well-documented anticancer and antioxidant capabilities, especially in EOs derived from medicinal herbs, underscore their potential therapeutic value. Existing literature strongly supports the broad spectrum of anticancer, anti-inflammatory, and antimicrobial attributes associated with EO (Mahboubi, 2020). Clary Sage (*Salvia sclarea*) has been observed to inhibit cancer cell growth and exhibit antioxidant activities (Mahboubi, 2020). Frankincense (*Boswellia sacra*), highlighted in research by Abd-Rabou & Edris (2022), induces apoptosis in lung cancer A549 cell lines. Marjoram (*Origanum majorana*) displays noteworthy potential by suppressing the proliferation of human HT-29 colorectal cancer cells and impeding their ability to form new colonies (Athamneh *et al.*, 2020). Myrrh (*Commiphora myrrha*) showcases cytotoxicity against breast cancer, as Shehata & Elsewedy (2022) reported. Thyme (*Thymus vulgaris*) exhibits a dose-dependent inhibition of cell proliferation, as evidenced by Niksic *et al.* (2021).

This research aims to investigate the promising potential of these EOs as halal alternative therapeutic sources for lung cancer. The diverse effects observed across these oils underscore their complex nature and the broad spectrum of potential applications in cancer treatment.

## 2. Materials and methods

### 2.1 Selection of essential oils

The study utilised 5 commercially obtained EOs for evaluation. The assortment included oil from Clary Sage (*Salvia sclarea*), Frankincense (*Boswellia species*), Marjoram (*Origanum majorana*), Myrrh (*Commiphora myrrha*), and Thyme (*Thymus vulgaris*). These EOs were specifically chosen for their reported therapeutic properties.

### 2.2 Cultivation and maintenance of human cancer cell lines

Human lung carcinoma cell lines (A549) were acquired from the UPM-MAKNA Cancer Research Laboratory (CANRES) and carefully stored in a cryogenic tank equipped with a liquid nitrogen supply. To ensure their viability, the cell lines were cultivated and consistently maintained in a humidified incubator at 37°C within an environment containing 5% $CO_2$. The subculture process involved utilising a media culture combination comprising Roswell Park Memorial Institute-1640 (RPMI medium) supplemented with 10% fetal bovine serum (FBS). Following the methodology outlined by Abdik (2021), several chemicals, including accutase (cell detachment solution), dimethyl sulfoxide (DMSO), and phosphate buffer saline (PBS), were systematically included within the subculture process.

### 2.3 Cell proliferation assay

The cytotoxicity of the A549 cell line was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay, a yellow water-soluble tetrazolium salt. This specific assay assessed the viability of cells following exposure to EOs, determining whether they could induce cell death or inhibit cell growth. The MTT assay, adapted to suit the experimental conditions, followed the procedure outlined by Fithrotunnisa *et al.* (2020). Its primary objective was pinpointing the optimal $IC_{50}$ value among the 5 EOs tested on lung cancer cells. The EOs exhibiting the lowest $IC_{50}$ values, calculated based on the percentage of cell viability, were singled out for further optimisation testing. Conducted 3 times, each assay involved 4 replicates for each type of EO. This experiment's positive and negative controls were 0.5% DMSO and untreated cells. The parameters utilised in the test were kept constant across repetitions. The most favourable result among the repeated tests was carefully selected and incorporated into the dataset, ensuring that the data presented reflects the most promising outcome from this experimental series.

In a flat-bottomed 96-well plate, 100 µL of A549 cells were seeded at a density of $5 \times 10^4$ cells/well. Before seeding the cells in the 96-well plate, the cells were first counted using a trypan-blue staining assay to ensure the cells' density was fixed to the desired amount, $5 \times 10^4$ cells/well. The 96-well plate was then incubated for 24 hours to ensure the cells grew familiarly in the plate and detached to the wall.

After 24 hours of incubation, the EO with a concentration of 4 mg was added to the 96-well plate with 10-fold dilution and triplicated in the plate. The negative control, 0.5% DMSO, was seeded in the same 96-well plate. The final working amount for each well was 200 µL after adding the EO and negative control, DMSO. The cell was treated and incubated for 48 hours in the incubator before being tested with the MTT reagent. After 48 hours, 10 µL of MTT reagent assay was added to the all well in the 96-well plate. After the assay was added, a vibrant purple colour precipitate was formed. The plate was then again incubated in the incubator for 4 hours. After 4 hours of incubation, 100 µL was discarded, and 100 µL of DMSO was added to each well. The plate was then wrapped with aluminium foil and shaken on the 96-well plate shaker for 5 minutes to ensure the solution was mixed properly. All the process handling of the MTT assay part was done under dark conditions. The absorbance was measured and recorded at 570 nm using a microplate spectrophotometer after 5 minutes of

shaking in the shaker. The data of the reading was recorded for analysis.

The optical density (OD) value directly relates to the number of viable cells in the well. As a result, the OD reading from the MTT test will be used to count the number of cell viability after treatment, evaluate how these cells fare compared to the untreated cells, and further count $IC_{50}$. In other words, this is essential for determining the relationship between cell survival and cell inhibition, which are inversely related.

### 2.4 Optimisation of essential oils

Following identifying the most promising EO among the 5 types, characterised by the lowest $IC_{50}$ value, treatment parameters were optimized to determine the optimum conditions for the EO's efficacy. The '2 Factorial Design' was employed for this optimisation process, focusing on two key parameters: EO concentration (µg/mL) and incubation time (hour). The Experiment (DoE) design for this optimisation study was meticulously constructed using Design-Expert software (version 13). The factors included "[A] concentration of EO µg/mL" and "[B] incubation time h," while the response variable for the experiment was "cell viability %."

This optimisation study specifically targeted 2 types of EO: Myrrh and Thyme. The concentration of EOs investigated during the optimisation ranged from 200 µg/mL to 800 µg/mL, and the incubation time for treating cancer cells spanned 24 to 72 hours. The cell viability in the percentage of lung cancer cells was quantised as the response in this optimisation study. It was evaluated using the MTT assay, following the methodology presented by Larsson *et al.* (2020), with necessary adjustments regarding the media use and the range of the parameters to suit the experimental conditions. The systematic exploration of these parameters aimed to define the most effective conditions for maximising the EO's impact on lung cancer cells.

The Analysis of Variance (ANOVA), employing a collection of statistical models to examine the variation and statistical significance in the data, was conducted using Design-Expert software (version 13). This analysis allows for assessing the model's overall significance and the significance of its terms. A "Prob > F" value less than 0.05 indicates that the model terms are statistically significant. Additionally, ANOVA enables the assessment of the lack of fit in the model, providing valuable insights into the model's overall reliability and applicability to the experimental data.

### 2.5 Cytokinetic study

For the cytotokinetic study, both treated and untreated cells were counted to obtain the cell growth profile. For a start, the A549 from cryopreservation was thawed and grown into the culture plate until confluent. The lung cancer cell was subcultured at least two passaging before being inoculated into the T-25 flask with 5 mL of fresh culture media. Fourty (40) flasks were prepared, 20 flasks for treated cells and 20 flasks for untreated cells. At 8 hours of incubation, one flask containing treated cells and one flask containing untreated cells were harvested from the monolayer and proceeded to cell counting with the trypan-blue staining assay. Following the same process, where 8 hours intervals after that, one flask each was harvested and proceeded to cell counting. The cell collection was triplicated for the cell counting process. The numbers of viable and non-viable cells were recorded to plot the growth curve for this growth kinetics study.

## 3. Results and discussion

### 3.1 Assessment of the cytotoxicity

The cytotoxic effect of 5 different types of EOs — Clary Sage, Frankincense, Marjoram, Myrrh, and Thyme was studied against the lung cancer A549 cell line. The MTT assay was employed in this investigation to identify the anti-proliferative and cytotoxic capabilities of these selected EOs. The MTT assay was a valuable tool to evaluate cell inhibition and determine the $IC_{50}$ values, figuring out these EOs' potential to influence lung cancer cells' growth and viability.

As represented in Figure 1, increasing the concentration of the EOs resulted in a noticeable adverse impact on cell viability over all examined EOs. Each EO exhibited a distinct pattern in the graph's drop, reflecting variations in cell growth inhibition. As cell vitality lessened, the inhibition of cell proliferation increased. Notably, the cell viability of both Frankincense and Thyme exhibited a similar decreasing trend, although the extent of reduction differed. This decline was gradual, with the overall count remaining consistent.

In contrast, Myrrh demonstrated a more substantial decrease in cell viability than Frankincense or Thyme, followed by a subsequent reduction aligning with both EOs. Clary Sage and Marjoram exhibited a slower decline in cell viability than the other EOs, yet the final count of viable cells reached approximately the same level. At the highest concentration of EOs, both Myrrh and Thyme proved effective in significantly suppressing the proliferation of cell cultures.

The fact that the EO inhibited the maximum number of cells was demonstrated by the lowest number of cell viability, according to the results of these MTT assays. This implies that the higher the number of inhibited cells, the lower the number of viable cells. According to Table 1, the EOs of Frankincense and Thyme showed the lowest percentage of cell viability, 6.1 ± 1.2% for Frankincense and 6.5 ± 0.7% for Thyme. It was shown that the Frankincense inhibited around 93.9 ± 1.2% of the A549 cells, whereas the Thyme inhibited approximately 93.5 ± 0.7% of the A549 cells. The other 3 EOs showed good inhibitory effects as well. Clary Sage inhibited around 88.7 ± 0.6 of the cells, Marjoram inhibited approximately 86.6 ± 1.7%, and Myrrh inhibited approximately 84.2 ± 1.4% of the cells. After being treated with EOs, the cells were inhibited with a positive result; the proportion of inhibited cells was greater than 80%, demonstrating a good cytotoxic impact. The EOs ranked from the highest to lowest cell inhibition: Frankincense, Thyme, Clary Sage, Marjoram, and Myrrh. This ranking is based on their respective effectiveness in inhibiting the proliferation of the A549 lung cancer cell line, with Frankincense demonstrating the highest inhibition and Myrrh showing the lowest among the selected EOs.

In pharmacological research, the $IC_{50}$ serves as a crucial metric, indicating the amount of a drug required to inhibit a biological process by half. This measurement provides insight into the relative effectiveness of an antagonist agent. Schaduangrat *et al.* (2022) discussed a correlation between the $IC_{50}$ value and the potency of EOs. A lower $IC_{50}$ value signifies greater potency, indicating that a smaller substance is needed to produce the desired effect.

The findings from the previous study by Khalil *et al.* (2020) revealed that the extract of *Commiphora molmol* (Myrrh)

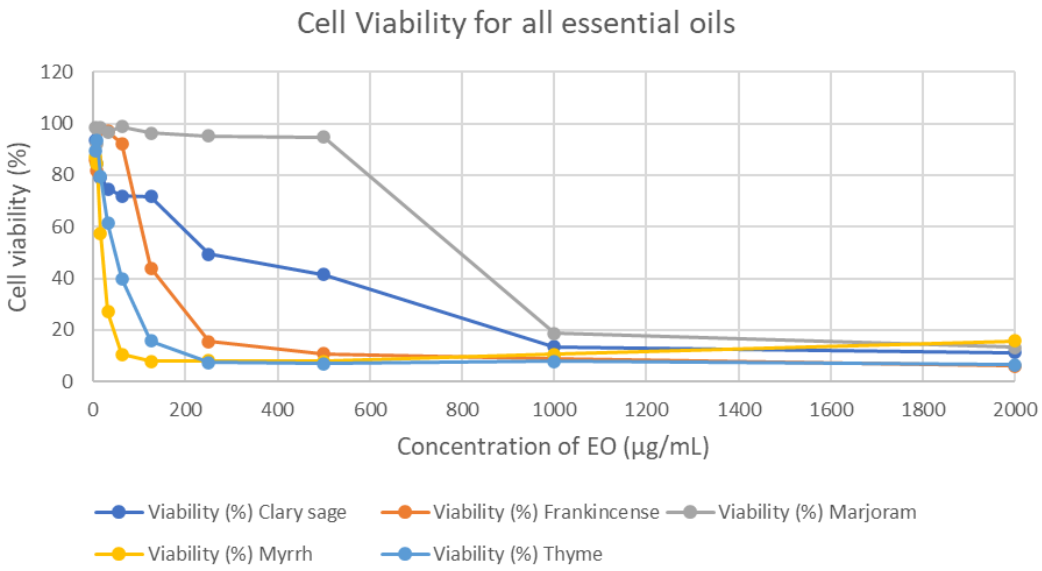


Figure 1: The cell viability (%) graph of lung cancer after being treated with the EO.

Table 1: Cell viability (%), cell inhibition (%), and $IC_{50}$ values for each EO towards lung cancer cell A549 cell line

| Essential oil | Cell viability (%) | Cell inhibition (%) | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| Clary Sage (*Salvia sclarea*) | 11.3 ± 0.6 | 88.7 ± 0.6 | 563 ± 14.4 |
| Frankincense (*Boswellia sacra*) | 6.1 ± 1.2 | 93.9 ± 1.2 | 119 ± 11.4 |
| Marjoram (*Origanum majorana*) | 13.4 ± 1.7 | 86.6 ± 1.7 | 752 ± 13.5 |
| Myrrh (*Commiphora myrrha*) | 15.8 ± 1.4 | 84.2 ± 1.4 | 19 ± 3.3 |
| Thyme (*Thymus vulgaris*) | 6.5 ± 0.7 | 93.5 ± 0.7 | 45 ± 7.5 |

exhibited inhibitory effects on human liver cancer (Hep G2), human breast cancer (MCF-7), and colon cancer cell lines (HCT-116). The reported $IC_{50}$ values were 41.52 µg/mL, 10.93 µg/mL, and 19.71 µg/mL, respectively. In the present study, Myrrh demonstrated a similar inhibitory effect on the A549 cell line with an $IC_{50}$ value of 19 µg/mL. Notably, this $IC_{50}$ value falls within the range observed in the previous research studies, highlighting the consistency of Myrrh's inhibitory potential across different cancer cell lines.

Similarly, the previous study by (Niksic *et al.*, 2021) documented the inhibitory effects of *Thymus vulgaris* (Thyme) on non-small cell lung cancer cells (H460) and breast cancer (MCF-7), with $IC_{50}$ values of 68.59 µg/mL and 52.65 µg/mL, respectively. In the current study, Thyme demonstrated an $IC_{50}$ value of 45 µg/mL for treating the A549 cell line. The results indicate that the $IC_{50}$ values are nearly equivalent to those observed in the previous research study, reinforcing the consistent inhibitory impact of Thyme, particularly on lung cancer cells.

Referring to Table 1, Myrrh exhibited the lowest $IC_{50}$ value, specifically 19 µg/mL. Following closely, Thyme had the second-lowest $IC_{50}$ value at 45 µg/mL. Consequently, Myrrh and Thyme were selected for the optimisation study. This step aimed to identify the optimal parameters for the most favourable outcomes in suppressing lung cancer cell proliferation. The selection was supported by their impressive inhibitory effects, as denoted by their low $IC_{50}$ values. To summarise, the EOs that showed the highest cell inhibition to the lowest rank were the Frankincense, Thyme, Clary Sage, Marjoram, and Myrrh.

### 3.2 Optimisation of essential oils

The optimisation was conducted with two experiments: Myrrh and Thyme. Table 2 shows the result from the design-expert software for both EOs. The 2-factor design generated 16 runs experiment, and based on the results from all the runs, the lowest percentage of cell viability for the Myrrh was 15% with 800 µg/mL for 72 hours, and the highest percentage was 24% with 200 µg/mL for 24 hours. For the Thyme, the lowest percentage of cell viability was 16% with 500 µg/mL for 48 hours, and the highest percentage was 72% with 200 µg/mL for 72 hours.

Based on the data presented in Table 3, the F-values for the models generated by the software are 39.68 for Myrrh and 249.81 for Thyme. As mentioned earlier, P-values lower than 0.05 indicate the significance of the model terms. In this case, both Myrrh and Thyme have P-values less than 0.05, affirming the significance of both EOs in the model. The model components A and B are identified as crucial in this analysis. A significant lack of fit indicates a substantial deviation between predicted and actual values, considered non-significant to attain a fit model. Examining the data in Table 3, the p-values for the lack of fit generated by the software are 0.0755 for Myrrh and 0.1829 for Thyme. As recommended, these p-values are greater than 0.05, signifying that the lack of fit is statistically insignificant. The non-significant lack of fit suggests that the predicted values align well with the actual data, affirming the adequacy and reliability of the model for both Myrrh and Thyme.

Table 2: Cell viability (%) of lung cancer cells treated with Myrrh and Thyme

| Run | Factor 1 A:Concentration (µg/mL) | Factor 2 B:Incubation time (hours) | Response 1 Cell viability (%) (Myrrh) | Response 2 Cell viability (%) (Thyme) |
|---|---|---|---|---|
| 1 | 200 | 72 | 20 ± 0.5 | 72 ± 1.2 |
| 2 | 200 | 24 | 24 ± 0.3 | 59 ± 1.1 |
| 3 | 200 | 72 | 17 ± 0.2 | 69 ± 1.4 |
| 4 | 200 | 24 | 24 ± 0.2 | 51 ± 1.1 |
| 5 | 200 | 24 | 23 ± 0.3 | 61 ± 1.3 |
| 6 | 200 | 72 | 18 ± 0.1 | 66 ± 1.5 |
| 7 | 500 | 48 | 18 ± 0.3 | 19 ± 0.9 |
| 8 | 500 | 48 | 17 ± 0.4 | 19 ± 1.1 |
| 9 | 500 | 48 | 20 ± 0.3 | 19 ± 1.1 |
| 10 | 500 | 48 | 19 ± 0.2 | 16 ± 0.6 |
| 11 | 800 | 24 | 18 ± 0.2 | 19 ± 1.2 |
| 12 | 800 | 24 | 18 ± 0.1 | 21 ± 1.1 |
| 13 | 800 | 72 | 16± 0.2 | 30 ± 1.4 |
| 14 | 800 | 72 | 15 ± 0.1 | 24 ± 0.7 |
| 15 | 800 | 72 | 15 ± 0.2 | 26 ± 0.4 |
| 16 | 800 | 24 | 19 ± 0.3 | 19 ± 0.8 |

Table 3: Analysis of Variance (ANOVA) for both Myrrh and Thyme

| | Myrrh | | Thyme | |
|---|---|---|---|---|
| | F-Value | p-Value | F-Value | p-Value |
| Model | 39.68 | < 0.0001 | 249.81 | < 0.0001 |
| A | 39.68 | < 0.0001 | 472.73 | < 0.0001 |
| B | 39.68 | < 0.0001 | 26.89 | 0.0002 |
| Lack of Fit | 3.85 | 0.0755 | 2.02 | 0.1829 |

A: concentration of (µg/mL); B: incubation time (hours)

Table 4: Validation of the experimental design

| Essential oil | Incubation time (hours) | Concentration EO (µg/mL) | of Predicted cell viability (%) | Actual cell viability (%) |
|---|---|---|---|---|
| Myrrh | 72 | 800 | 14.75 | 15 ± 0.1 |
| Thyme | 24 | 800 | 18.25 | 19 ± 0.8 |

The experimental design generated by the Design-Expert software provided optimum values for incubation time and EO concentration to achieve the lowest percentage of cell viability in the treatment of lung cancer cells. Table 4 presents Myrrh's and Thyme's suggested parameter values. For Myrrh, the actual cell viability value was 15%, closely matching the predicted value of 14.75%, resulting in a difference of 0.25%. Similarly, for Thyme, the actual cell viability was 19%, with a predicted value of 18.25%, yielding a difference of 0.75%. Due to the fact that the difference between the 2 EOs' numbers is only <1, it is regarded to be significant. When comparing the 2 EOs, the percentage value for Myrrh is notably lower than that of Thyme. Myrrh inhibited cell growth by approximately 85%, while Thyme inhibited around 81%. This indicates a substantial inhibitory effect of Myrrh on lung cancer cell viability compared to Thyme in the experimental conditions.

### 3.3 Cytotoxicity of essential oils

After 24 hours of incubation, the Myrrh was introduced to the cells as a treatment once the lung cancer cell had reached a steady condition. The treated cells began to demonstrate the effect, and their numbers started to decline immediately after 24 hours, which is when the exponential phase of the cell cycle takes place. During this phase, cells that were either untreated or treated had reached their maximum growth between 80 and 88 hours. According to the findings, Myrrh successfully inhibited cell proliferation as the number of cell generations was reduced from 4.358 to 3.954.

The process has entered the stationary phase when no growth in the cell concentration exists. When the population is in the stationary phase, the rate of death will be equivalent to the rate of increase. At this stage, the proliferation of cells is restricted

either because there is an insufficient supply of nutrients at a level that cannot support further cell growth or because of an accumulation of metabolic by-products that may have reached a level that is inhibitory to the growth of cells. When the A549 cells were allowed to grow untreated, they reached the maximum cell volume of 3.075 x $10^6$ cells/mL. However, when the cells were treated with Myrrh, the maximum cell volume only survived at 2.325 x $10^6$ cells/mL.

According to the findings, the growth rate fell at the exponential phase from 0.107 $h^{-1}$ to 0.106 $h^{-1}$. Even though Myrrh only slowed down growth rates by a modest number, the kinetics analysis conducted as part of this investigation demonstrated that Myrrh possesses inhibitory properties. The majority of chemotherapeutic medications work by interfering with the processes of cell division, making them particularly effective against tumours that are growing. From this experiment, this can be demonstrated as proof when the doubling time (td) is increased from 2.81 to 2.84 hours. When the normal proliferation of cancer cells is interrupted by therapeutic interference, a compelling therapeutic agent can lower the cancer's growth rate. This is the summarised concept of doubling time. This is proven by inspecting any capable therapeutic agent drives to boost the doubling time of cancer cells. In essence, the doubling time serves as a measurable indicator of the impact of a therapeutic intervention on the growth dynamics of cancer cells, highlighting the potential of effective treatments to slow down the rate of cancer cell proliferation.

The final phase of cell growth, known as the death phase, happens naturally due to cell death. During this phase, the viability of cells is at its lowest point. The findings demonstrated that Myrrh caused an increase in the rate of cell death in A549 cells. Compared to untreated cells, which had a death rate of only 0.016 $h^{-1}$, the death rate was boosted to 0.022 $h^{-1}$. Drugs that usually cause necrosis in the growing cell can sometimes cause the natural death of cells to be halted or sped up. Necrosis is a non-active process typically occurring due to sudden and extreme stress on cells. According to (Boise & Collins, 2001), this is distinguished by a breakdown of the plasma membrane, which causes the cell to enlarge, ultimately leading to the rupture of the cell.

## 4. Conclusion

By investigating the potential of EOs as a halal alternative therapeutic source for lung cancer, we have uncovered important findings that advance our understanding that EOs can inhibit the growth of lung cancer. The key findings of this study include the anticancer properties of EOs. The main goal of this study is to prove that EO possesses anticancer abilities by experimenting to check their cytotoxicity effect on lung cancer. All of the EOs in this study inhibited the A549 lung cancer cells. Frankincense and Thyme showed the lowest percentage of cell viability, 6.1% for Frankincense and 6.2% for Thyme. Myrrh and Thyme showed the lowest $IC_{50,}$ possessing cytotoxic activities toward the A549 cell line. Myrrh had the lowest $IC_{50}$ value, 19 µg/mL, followed by Thyme, 45 µg/mL. Based on this optimisation study, Myrrh showed a very good ability to inhibit lung cancer compared to Thyme. The consistency and the percentage number of inhibitions for Myrrh are better than those for Thyme. The design of the experiment (Design-Expert) showed that both the factors, incubation time and concentration of EOs, are important for the cytotoxicity study of cancer cell treatment. A growth kinetics study of A549 cells treated with Myrrh showed reduced cell generation numbers. Even though Myrrh was only responsible for a modest reduction in growth rate and an increment in death rate, the treatment shows the effects, which indicates that Myrrh possesses anticancer activity at least partially through inhibition of cell growth.

Products derived from plants are gaining recognition as promising choices for halal products. Essential oil, extracted from plants and known for their historical use in traditional medicine, has piqued the interest of experts. This research suggests that EO can successfully treat various ailments, including certain types of cancer, such as lung cancer. Essential oil not only offers a halal alternative to pharmaceutical items but also provides a complementary option to chemical products that may have potential negative impacts on consumers. However, it is crucial to conduct further research, including clinical trials, to enhance the efficacy of these treatments and ensure their safety and effectiveness in diverse populations.

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# The Impacts of Supply Chain Integration on Halal SMEs Supply Chain Performance: the Mediating Role of Innovativeness

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**Abstract**

This paper proposed a new conceptual framework for examining the impact of halal SMEs' Supply Chain Integration (SCI) on their Supply Chain Performances (SCP). In the model, SCI positively impacts the SCP of halal SMEs, while innovativeness mediates the effect. There is little research on halal SMEs' innovativeness and supply chain integration in Asia or Malaysia. This conceptual paper is unique for introducing innovativeness into studying the relationship between halal SMEs' SCI and their SCP. Since COVID-19-induced supply chain disruptions emerged, SCI has increasingly attracted attention as an integrated business strategy for achieving business supply chain performance, competitiveness, and sustainability. Also, considering the crucial roles of Malaysian halal SMEs in the country's economic development and the increase in demand for halal products worldwide, appropriate utilisation of innovativeness and SCI must be examined to achieve their supply chain performance. Bearing all this in mind, the researchers have proposed this framework. After reviewing the literature and citing relevant studies conducted on the subject matter, a tentative research agenda and directions for future studies are suggested. This aims to achieve efficient and competitive Supply Chain Performance (SCP) for halal SMEs through Supply Chain Integration (SCI) with effective interactions and innovativeness.

## 1. Introduction

Supply Chain Integration (SCI) has been established as critical to the Supply Chain Performance (SCP) of SMEs in ensuring sustainable competitive advantage and effective responses to strategic, operational, and technological challenges (Birhanu *et al.*, 2022; Jagan Mohan Reddy *et al.*, 2019; Kalyar *et al.*, 2020). However, despite its significance in boosting SMEs' competitiveness and resilience, implementing SCI has become a big challenge for small businesses (Chin *et al.*, 2012; Hakim *et al.*, 2018; Palomero & Chalmeta, 2014). Undoubtedly, SCI awareness as an element in supply chain management has increased, but its application in SMEs is rarely understood or explored in academia and industry (Hakim *et al.*, 2018; Msimangira & Venkatraman, 2014; Qurtubi & Kusrini, 2018). Also, according to Gonen (2022), the academic literature on halal supply chain and logistics is new, considering that studies focusing on these areas started in the last decade. For these reasons and many more, SCI relationships and their impacts on supply chain performance have attracted considerable attention in academia and industry across various economies (Kalyar *et al.*, 2020).

In addition, establishments are progressively realising that their capacity to innovate (innovativeness) is essential to their successes, resiliencies, performances, and long-term survival (Claudino *et al.*, 2017; Kalyar *et al.*, 2020; Kim & Chai, 2017; Rampersad *et al.*, 2020). However, there is a dearth of systemic analyses of SCI from an innovation perspective in emerging markets in the existing literature (Kalyar *et al.*, 2020). The concept is scarcely examined to ascertain its impacts on the supply chain performance of firms and halal SMEs, in particular through the mediation of innovativeness. More specifically, there is very few research on halal SMEs' innovativeness and supply chain integration in Asia or Malaysia, which is the context of this study (Qurtubi & Kusrini, 2018). The positive impacts of innovativeness on firm supply chain integration (SCI) and sustainable performance have been investigated and established empirically by works of literature (Espino-rodríguez & Taha, 2022; Walder *et al.*, 2019).

Considering their huge size and economic impacts, halal SMEs, like other SMEs, have a significant role in Malaysia's economic development. The contribution of halal SMEs to Malaysian economic development can be enhanced by boosting their supply chain performance with appropriate measures of SCI and innovativeness. Despite this, little or none is known to have been researched on the mediating role of innovativeness in the relationship between SCI and the supply chain performance of halal SMEs in Malaysia. Given the existence of empirical literature that has established the positive impacts of SCI and innovativeness on SCP, it is conceptualised by the researchers thus: SCI effectively and positively impacts halal SMEs' SCP, mostly when innovativeness mediates the relationship. Thus, we propose this conceptual framework where halal SMEs' innovativeness plays a mediating role in the relationship between the impacts of SCI on their SCP.

The conceptual framework of this paper is proposed by building on theories adopted in studies on innovativeness, SCI, supply chain performance, and related fields. Such theories include the Resource-Based View (RBV) of a firm, Dynamic Capability Theory (DCT), and Contingency Theory, which have been found suitable for research on innovativeness and supply chain integrations (Al-Habahbeh, 2022; Alraja *et al.*, 2022; Celtekligil & Adiguzel, 2019; Piprani *et al.*, 2020). As a dynamic capability, the resources of an enterprise, including its "innovation capability" (innovativeness), affect its ability to relate to external opportunities, increasing its innovation and performance (Celtekligil & Adiguzel, 2019). RBV is appropriate for developing supply chain strategy taxonomy and has been widely used in studies of SMEs' sustainability and competitive advantage (Alraja *et al.*, 2022; McKone-Sweet & Lee, 2009). SCI is an integrated and collaborative effort between suppliers and customers within a business (Flynn *et al.*, 2010; Hamdana *et al.*, 2022). Benefits accruing from inter-firm collaboration include increased knowledge creation capabilities, revenue enhancements, cost reductions, and operational flexibility to cope with high demand (Skippari *et al.*, 2017). Contingency theory (Lawrence and Lorsch, 1967; Thompson, 1967) argues that organisations should match their structures and processes to their environment to maximise performance, while customers and suppliers are regarded as an important part of a firm's environment (Flynn *et al.*, 2010). Significantly, these three theories are well connected and relevant to this study's three variables: supply chain integration, innovativeness, and performance.

In conclusion, this paper suggests examining the impacts of halal SMEs' SCI on their supply chain performance and how innovativeness mediates the relationship. This aims to improve halal SMEs' performance by developing a conceptual framework that illustrates this relationship, its impact, and its significance in the halal industry sector. The framework assumes that SCI has a positive relationship with the supply chain performance of halal SMEs when innovativeness mediates the relationship. The conceptual framework will provide impetus to conduct further empirical research on these variables and related ones that can aid SCI, innovativeness, and supply performance of halal SMEs, hence their competitiveness. It will also aid policymakers and other stakeholders in identifying specific potentials and enable the halal SMEs sector's environmental, human, and material resources required for its growth and sustainable performance. Also, despite the vast majority of firms worldwide being SMEs and providing 60–70% of the jobs in some countries, most studies on SCI focus mainly on large firms (Claudino *et al.*, 2017; Latifi *et al.*, 2021). The present conceptual study focuses on SMEs to remedy this research gap.

## 2. Literature review

The paper conducts a literature review on Supply Chain Integration (SCI), innovativeness, and Supply Chain Performances (SCP). The review explores these variables' definitions, types, significances, and benefits and their possible relationships. The authors draw insights and deductions from the reviewed literature to present a conceptual framework that defines the impacts of the three elements of halal SMEs' SCI on their SCP. The literature review also cites relevant studies conducted on the subject matter.

### 2.1 Supply Chain Integration (SCI)

Supply chain integration (SCI), the strategic collaboration between companies and their supply chain partners, has become increasingly critical (Hendijani & Saeidi Saei, 2020). This is due to SCI's crucial role in leveraging internal and external resources across the whole supply value chain, keeping the firm in the global market and improving firm performances and competitiveness (Birhanu *et al.*, 2022; Hendijani & Saeidi Saei, 2020; Jagan Mohan Reddy *et al.*, 2019). Internal integration, considered a forerunner of supply chain integration (Hakim, 2020), refers to integration processes within an organisation. External integration refers to inter-organisational collaboration, which primarily involves customers and suppliers and is hence classified into Customer Integration (CI) and Supplier Integration (SI) (Cao *et al.*, 2015).

Studies have shown that internal, product, and process integration positively affects a firm's operational and financial performance (Hendijani & Saeidi Saei, 2020). Also, works of literature have acknowledged that harmonising an organisation's interior and exterior environments positively impacts the performance of the businesses (Du *et al.*, 2022). These are due to the capacity of SCI to facilitate effective management, information dissemination, and physical flows along the supply chain (Kalyar *et al.*, 2020). For example, Supply chain (SC) networks can be characterised by uncertainty leading to inventory destabilisation, supply chain shock, or business mortality, as witnessed during the COVID-19 pandemic (Rozhkov *et al.*, 2020). SMEs, which often encounter uncertainty in the business environment and a high rate of competition (Hakim *et al.*, 2018), are also known to be more vulnerable to supply chain shocks than higher businesses (Adam & Alarifi, 2021; Du *et al.*, 2022; Sha *et al.*, 2020; Sonobe *et al.*, 2021; UNCTAD, 2022). Nevertheless, with the adoption of SCI, SMEs are better positioned to overcome these constraints by getting information on price and quality that will give them competitive advantages, having the capacity to predict the number of sales obtained through customer database, reduced operating cost which is vital in setting the final price; and increased efficiency through integrations of stakeholders final price (Hakim *et al.*, 2018).

Likewise, studies investigating the impacts of collaborative innovations on a firm's supply chain performance show a positive relationship between collaborations between organisations and innovative performance (Skippari *et al.*, 2017). It has been argued that to ensure the effective dissemination of innovations within existing systems, other players must access the advantages of existing innovations (Celtekligil & Adiguzel, 2019). This is viewed from the perspective that one firm's innovation may require other firms to innovate simultaneously; hence, firms may depend on counterparts' innovation ability in their supply chain network (Skippari *et al.*, 2017). According to Skippari *et al.* (2017), empirical results indicate that the more suppliers are involved, the more benefits to innovation and the firm's financial performance.

In addition, information sharing and collaboration in supply chain integration can reduce uncertain outcomes, enhancing sustainability performance (Wong *et al.*, 2020). Close collaborations and information exchange within and between companies aided by information systems are necessary to grow supply chain performance (Du *et al.*, 2022; Manzaneque-Lizano *et al.*, 2019). Impacts of external activities of an organisation, e.g., regulations imposed by environmental

bodies, are important parts of the supply chain that affect it and have become an important factor in environmentally friendly supply chain practices (Du *et al.*, 2022). According to Manzaneque-Lizano *et al.* (2019), it has been identified that business performance and sustainability are closely related to the capability to collaborate with stakeholders. This is because the business environment includes external parties to the company, including customers, other industry businesses, sellers, and government regulations that interfere with its operations (Du *et al.*, 2022). They also indicated that qualities exhibited by external forces, such as intricacy, lethargy, and generosity, are emphasised as part of external environmental aspects influencing firm performance.

**2.2 Innovativeness**

Two perspectives of innovativeness exist in the literature: innovativeness as the measurement of frequencies of innovations that are turned out and as the potential or propensity to innovate or innovative culture of an organisation based on its human capital, resource capability, etc. (Kalyar *et al.*, 2020; Kamaruddeen *et al.*, 2010; Seo *et al.*, 2014). Some studies define innovativeness as the rate and quality of usage of technology or new ideas by a firm compared to its competitors in order to gain competitive advantages in terms of cost, time, and value effectiveness (Du *et al.*, 2022; Kamaruddeen *et al.*, 2010). It refers to the capacity of a firm to innovate or influence its existing human resources, capabilities, strategy, marketing, and technological resources through innovation (Du *et al.*, 2022; Seo *et al.*, 2014). Some literature describes innovativeness as a firm's organisational characteristics, capability, and culture to transform opportunities into realities, which facilitates the implementation of innovations with the complement of adequate resources (Kalyar *et al.*, 2020; Seo *et al.*, 2014). According to them, innovativeness depends on the new knowledge embedded in a firm or its innovative culture as motivating factors for workers to adopt innovative behaviours. Innovativeness is much related to having appropriate potential in the form of highly-qualified human resources (Skibiński & Sipa, 2015).

Irrespective of the adopted view, innovativeness is fundamental and essential for the long-term performance, competitiveness, and survival of supply chain firms and SMEs in emerging markets (Adam & Alarifi, 2021; Du *et al.*, 2022; Kalyar *et al.*, 2020). A couple of studies suggested that innovativeness strengthens supply chain management and is thus significant for firms' performances (Kalyar *et al.*, 2020), while a high level of innovativeness positively impacts Supply Chain Integration (SCI) (Seo *et al.*, 2014; Skippari *et al.*, 2017). However, innovation requires an effective mixture of resources and organisational characteristics at different levels (Halim *et al.*, 2021). A higher level of innovativeness is indicative of openness to change, tendency and willingness to introduce or execute new ideas, products, processes, and solutions as an entrepreneur's value-system and organisational culture (Adam & Alarifi, 2021; Halim *et al.*, 2021; Seo *et al.*, 2014; Walder *et al.*, 2019). Also, the resource capabilities of an enterprise impact its ability to identify external opportunities to increase its innovation and performance (Celtekligil & Adiguzel, 2019). Hanifah *et al.* (2017) note that studies have found that more innovative organisations are managed by richly educated, skilful or knowledgeable human capital, increasing the organisations' innovativeness. These all follow the resource-based view (RBV), which is one of the underpinning theories of this conceptual paper.

As critical contributors to Malaysia's economy (Yusoff *et al.*, 2018), halal SMEs must be able to make innovation, access and deploy resources required for their performances (Du *et al.*, 2022). World Bank Surveys showed that innovation and technology adoption were Malaysian SMEs' most important performance boosters, having the highest impact on productivity and employment growth (National SME Development Council, 2012). Over the years, the introduction of astounding innovations has significantly transformed supply chain business operations, while digital technology has completely changed the operational process for SMEs (Hussain *et al.*, 2022; Talib *et al.*, 2022). Some of these innovations in the supply chain include containerisation, Electronic Data Interchange (EDI), Radio Frequency Identification (RFID), big data, blockchain technology, and electric vehicles (Talib *et al.*, 2022). Taking advantage of revolutionary innovations like Artificial Intelligence (AI), robotic engineering, 3D printing, etc, would lower production costs, improve the quality of goods, and ultimately improve firm competitiveness (Hussain *et al.*, 2022).

**2.3 Supply Chain Performance (SCP)**

Due to the different performance metrics adopted by various writers, constructs of Supply Chain Performance (SCP) are diverse and vary (Kalyar *et al.*, 2020). Traditional SCP measures such as cost, activity time, customer responsiveness, and flexibility are incomplete based on inclusiveness, universality, measurability, and consistency criteria (Seo *et al.*, 2014). Hence, Gunasekaran *et al.* (2004) widely referenced study proposes a comprehensive SCP measurement framework categorised as strategic, financial, operational, and tactical performance (Kalyar *et al.*, 2020; Seo *et al.*, 2014). The framework is further divided into metrics for order planning, evaluation of supply link, measures and metrics at the production level, evaluation of delivery link; measuring customer service and satisfaction; and supply chain and logistics (Gunasekaran *et al.*, 2004; Seo *et al.*, 2014).

Kalyar *et al.* (2020) divide the Supply Chain (SC) operational metric into SC efficiency and SC effectiveness. According to Kalyar *et al.*, SC efficiency refers to evaluating the time needed to respond to unexpected supply requirements without additional cost and SC cycle time spent on value-adding activities. SC effectiveness refers to order fulfilment lead time - the average amount of time between order entry and order delivery- and perfect order fulfilment (Kalyar *et al.*, 2020). Perfect order fulfilment refers to orders delivered completely on the date requested by the customer, in perfect condition, with the correct documentation, all relative to the total number of orders (Tsanos *et al.*, 2014). Significant SCP metrics chiefly aided by Supply Chain Integration (SCI) are improved pipeline and demand visibility - the visibility of each partner's supply chain activities (Palomero & Chalmeta, 2014). Other key Supply chain performance (SCP) metrics classifications identified in the literature include service dimension - a measure of how well or not customers have been served; assets - monetary value and inventory turns; and speed dimensions - quoted customer response time, supply chain cycle time and cash conversion cycle time (Hausman, 2005).

Generally, effective and efficient SCP are indicated by improvement of business operations, achievement of value added to the customer, efficiency when inbound and outbound supply are integrated, and achievement of firm best performance (Hakim, 2020). Other indicators, as articulated by Palomero & Chalmeta (2014), include cost reduction; increased revenue; improved quality, customer satisfaction,

and on-time delivery; reduced operational and process time; standardised product, automated processes, and production; improved distribution and payment process; and improved global competitiveness.

To conclude this section, the researchers have so far demonstrated how Supply Chain Integration (SCI) and innovativeness positively impact the supply chain performance of SMEs. The reviewed literature and studies' outcomes show that SCI and innovativeness are essential to modern-day business entities' survival, competitiveness, and supply chain performance. Likewise, they establish that the SCI and supply chain performance exhibit positive relationships, especially when mediated by innovativeness. Hence, the researchers have incorporated innovativeness into the model as a mediating variable, as shown in Figure 1 below, to ascertain whether it mediates the impacts of SCI on the supply chain performance of halal SMEs. Also, innovativeness was incorporated into the model to reveal whether the impacts of SCI on supply chain performance will be different for a more innovative halal SME.

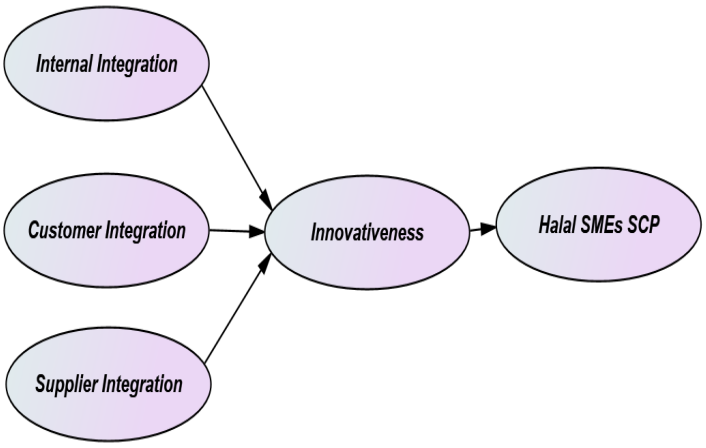


Figure 1: Conceptual Framework.

## 3. Context of the study - Malaysian halal industry

Malaysia, which is ranked the world's top halal industry country (Dinar Standard, 2021) with over 40 years of experience in the halal industry, has realised the huge economic potentials that still exist in the halal industry. By 2030, the global Halal market is expected to grow to USD 5.0 trillion, while domestic growth is estimated to reach USD 113.2 billion. However, according to the report by Halal Development Corporation (2020), there is an estimated 80% gap between the demand for and production of global halal products. With a comprehensive Halal ecosystem, Malaysia has a competitive advantage to fully capitalise on this timely opportunity (Halal Development Corporation, 2020). Significantly, SMEs have always been identified to be central to the country's national development, while the government has given significant concerns to halal SMEs in particular (Tahir *et al.*, 2016). SMEs account for 97.4% of the country's overall establishments, contributing to 47.8% of total employment and 37.4% of GDP (DOSM, 2022). Also, the Malaysian Twelfth National Development Plan has been designed to boost the halal industry. According to the government, the industry's development will be accelerated through the Halal Industry Master Plan (HIMP) 2030). This plan outlines seven strategic thrusts focusing on producing high-quality products and services along the halal supply chain (Marketing-Interactive.com, 2021). These undoubtedly show the strategic, social, and economic importance of halal SMEs that operate in the Malaysian MSME sector.

However, despite the strategic importance of SMEs to Malaysian development plans, the sector is still vulnerable to economic constraints, uncertainty, and supply chain shocks and is threatened by the volatile competitive business environment experienced during the COVID-19 pandemic. Meanwhile, studies have identified SCI and innovativeness as crucial to halal SMEs' survival, competitiveness, and supply chain performance. Therefore, the researchers have proposed a model through this study to effectively understand the impacts of SCI on halal SMEs supply chain performance. It also includes the mediating role of innovativeness in the relationship.

## 4. Research design for future research

In alignment with the proposed model, it is suggested that future investigations be conducted using a positivist approach or research paradigm. This approach is recommended to enable future research to objectively test causal relationships among the variables, as it is widely accepted in quantitative research (Khaldi, 2017). Positivism research paradigm is based on the ontological assumption of a reality that is independent of the observer (Dahler-Larsen, 2015): realities of the world are objective and knowable in their entirety; a researcher can be separated from the research's object; hence his/her task is to describe and analyse this reality neutrally (Khaldi, 2017). Also, the ethical procedural implication of the positivism paradigm, such as confidentiality, informed consent, and avoidance of coercion, should be followed by future research (Dahler-Larsen, 2015).

In addition, future research is expected to develop a questionnaire based on the existing literature that would consider all variables in the model. Such a questionnaire would be employed to collect data from employees in halal SMEs businesses to investigate these variables. The procedure for data collection could be a probability or non-probability sampling approach using a valid instrument (Likert scale) that measures the impacts of SCI on the Supply Chain Performance (SCP) of halal SMEs. The sample population to be studied by future research would be adults with at least three years of experience and currently working in the halal SME sector. A sample size between 100 and 500 participants is recommended for structural equation modelling, which is appropriate for the proposed model. Based on responses from the research participants, conclusions would be made on the impacts of SCI on SCP, the relationship between them, and the mediating and possible moderating variables. In addition, various recommendations would be sought to enrich the study.

## 5. Conclusion and research outcome

The researchers have proposed a model and conceptual framework where the assumption is that the SCI of halal SMEs positively impacts their SCP while innovativeness mediates the relationship. While there are limited empirical studies that investigate these relationships, we have built on the existing literature to show that, amongst other proofs, SCI impacts positively on SMEs' SCP. Furthermore, innovativeness plays a mediating role between SCI and SMEs' SCP. Based on this model proposed by the researcher and various empirical findings, we assume Supply Chain Integration (SCI) will positively impact halal SMEs' Supply Chain Performance (SCP). In addition, it is also assumed that these relationships would be mediated by innovativeness– SCI might have a more significant positive impact on SCP if innovativeness is

introduced as a strategic element of SMEs operation. Hence, the impacts of SCI on SCP may vary according to the quantity and quality of innovativeness introduced.

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**HALALSPHERE**
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# Unlocking the Halal Food Industry: Embracing Halal L-Cysteine and the Importance of Halal Certificates

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**Abstract**

The global demand for halal food has experienced unprecedented growth, elevating the halal food industry into a substantial economic and cultural entity. This manuscript offers an in-depth exploration of the halal food industry, focusing on two key elements: halal L-Cysteine as a food additive and the indispensable role of halal certificates. While the manuscript highlights the industry's robust growth and the unique attributes of halal L-Cysteine, it also addresses the complexities and challenges associated with its use. These include ethical sourcing dilemmas, potential allergic reactions, stringent quality control requirements, and the environmental impact of production. Furthermore, the manuscript emphasizes the critical function of halal certificates in ensuring compliance with Islamic dietary laws, enhancing transparency, and facilitating market access. By examining the industry from the perspectives of sourcing, production, and consumer empowerment, this manuscript provides a nuanced understanding of the multifaceted dynamics shaping the contemporary halal food industry.

## 1. Introduction

The halal food industry has witnessed an incredible surge in popularity, propelled by a growing Muslim population and the demand for ethical and religiously permissible food choices (Radzuan *et al.*, 2017; Suleiman, 2018). Within this dynamic industry, the significance of halal L-Cysteine as a vital additive cannot be understated. This review article delves into the importance of the halal food industry, sheds light on the significance of halal L-Cysteine, and emphasizes the pivotal role and power of halal certificates in ensuring compliance with Islamic dietary guidelines. However, the use of L-Cysteine is not without its challenges. Ethical concerns arise, particularly when L-Cysteine is sourced from human hair, duck feathers, or hog hair, which may not align with Islamic dietary laws. Allergic reactions, although rare, are another issue that cannot be ignored. These can range from mild skin irritations to severe anaphylactic reactions, necessitating proper labelling and consumer education.

Quality control is another critical aspect that needs attention. The purity and efficacy of L-Cysteine can vary depending on its source and manufacturing process, thereby affecting the overall quality of the food product. This necessitates stringent quality control measures, which can be time-consuming and costly. Additionally, synthetic L-Cysteine, although ethically more acceptable, is generally more expensive to produce, impacting the overall affordability of halal food products.

Environmental considerations also come into play, especially in producing synthetic L-Cysteine, which may involve hazardous chemicals. This has implications for environmental sustainability and adds another layer of regulatory complexity. Agencies like the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have stringent guidelines that manufacturers must adhere to, making regulatory compliance challenging. Consumer perception, particularly among vegan or vegetarian consumers, can also influence the market dynamics of L-Cysteine-containing products. Transparency in sourcing and production methods is crucial for maintaining consumer trust.

In conclusion, while halal L-Cysteine plays a vital role in the halal food industry, it comes with its own set of challenges that need to be addressed. Halal certification serves as a cornerstone in ensuring that these products meet Islamic dietary laws, but it is equally important for both consumers and industry stakeholders to be aware of the complexities involved in its use. This article aims to provide a nuanced understanding of these issues, providing a comprehensive guide for consumers and industry professionals.

## 2. Methodology

The methodology for this review article involved a comprehensive literature search primarily conducted through the Scopus database, supplemented by additional sources where necessary. The search was designed to capture articles, reviews, and reports that specifically address the role of L-Cysteine as an additive in the halal food industry and the importance of halal certification in ensuring compliance with Islamic dietary laws. Keywords such as 'L-Cysteine,' 'halal food,' 'additive,' and 'halal certification' were used in various combinations to maximize the retrieval of relevant literature.

The search was further refined by applying filters for peer-reviewed articles, publication dates, and relevance to the topic at hand. Articles were then selected based on their contribution to understanding the complexities, ethical considerations, and consumer perceptions surrounding using L-Cysteine in halal food products. This rigorous approach ensured that the literature included in this review is current and highly relevant, providing a nuanced understanding of the subject matter.

## 3. The growing influence and significance of the halal food industry

The halal food industry has experienced an exponential rise, catering to the global Muslim population's dietary needs and cultural preferences (Hossain *et al.*, 2013; Rehman *et al.*, 2019). As individuals seek to align their consumption with Islamic principles, the demand for halal products has surged. This industry encompasses various sectors, including food production, processing, certification, and distribution (Ahmed & Zafar, 2018). The market has expanded to include halal-certified food products, restaurants, tourism, and even financial services (Rauf & Mat Som, 2020).

### 3.1 Unveiling the essence of halal L-Cysteine

At the heart of the halal food industry lies the significance of halal L-Cysteine—an amino acid with exceptional functional properties often used as an additive in food production (Ahmad *et al.*, 2016; Fernando, 2006). The importance of halal L-Cysteine stems from its compliance with Islamic dietary guidelines, ensuring that it meets the stringent requirements outlined in Islamic principles (Halim *et al.*, 2021). Halal L-Cysteine is meticulously sourced from halal-compliant origins and manufactured in accordance with Islamic standards, alleviating concerns regarding animal sourcing and production processes (Ding *et al.*, 2018). Its versatile properties make it a sought-after ingredient, adding flavour, texture, and stability to various food products (Hassan *et al.*, 2018).

As an essential amino acid, L-Cysteine offers functional properties that enhance various food products' quality, taste, and texture. It is a sought-after ingredient in the food industry, widely used in bakery goods, processed meats, and savoury snacks (Adil *et al.*, 2017; Rahman *et al.*, 2018). By embracing halal L-Cysteine, food manufacturers can cater to the dietary needs and preferences of the Muslim population while adhering to halal standards. Its usage extends across various food products, providing specific benefits and enhancing the overall quality and sensory characteristics. In the bakery industry, halal L-Cysteine serves as a crucial dough conditioner. It helps improve the dough's extensibility, making it easier to handle during processing. By strengthening the gluten network, halal L-Cysteine contributes to increased dough elasticity, resulting in improved volume, texture, and crumb structure in baked goods like bread, cakes, and pastries (Shobirin *et al.*, 2020; Al-Tamrah *et al.*, 2021).

Processed meats also benefit from the usage of halal L-Cysteine. It acts as a tenderizer by breaking down the protein structures, enhancing tenderness and juiciness in products such as sausages, ham, and cured meats. Including halal L-Cysteine in meat formulations helps improve the overall eating experience and ensures a more enjoyable texture (Rahim *et al.*, 2013; Djenane *et al.*, 2007). One of the notable aspects of halal L-Cysteine is its ability to enhance flavour profiles. It contributes to developing savoury and umami tastes, which are highly desirable in snacks, seasonings, and ready-to-eat meals. Adding halal L-Cysteine enhances the perception of richness and depth in flavour, making food products more appealing to consumers (Owusu-Apenten, 2018; Ma & Liu, 2019).

Furthermore, halal L-Cysteine is valued for its antioxidant properties. It acts as a natural antioxidant, helping to inhibit oxidation processes and extend the shelf life of food products. This makes it a sought-after ingredient in functional food products that promote health and well-being by offering added nutritional benefits (Ashiqueali & Choudhury, 2019; Othman *et al.*, 2017).

Halal L-Cysteine also finds application in producing nutritional supplements and protein blends. Its inclusion in these products provides a valuable source of amino acids, supporting muscle growth, recovery, and overall body maintenance (Ashraf *et al.*, 2014; Ramezanzadeh *et al.*, 2021). It is important to note that the usage of halal L-Cysteine in food products must adhere to halal standards and regulations. This ensures that the source of L-Cysteine is halal-certified and that the production processes align with Islamic dietary requirements, assuring consumers seeking halal food options (OIC/SMIIC, 2019; JAKIM, 2014).

Overall, the usage of halal L-Cysteine in the food industry plays a significant role in improving product quality, enhancing sensory attributes, and meeting the demands of consumers seeking halal-certified and ethically permissible food choices.

Halal Sources of L-Cysteine: To ensure compliance with Islamic dietary guidelines, halal L-Cysteine must be sourced from halal-certified origins. There are various sources of halal L-Cysteine available in the market:

1. Plant-Based Sources: Plant-based sources of L-Cysteine provide a halal option for manufacturers. These sources include garlic, onions, and other vegetables (El-Adawy, 2003). They undergo rigorous certification processes to ensure they are halal-compliant.

2. Microbial Fermentation: Microbial fermentation is widely used to produce halal L-Cysteine. Specific strains of bacteria or yeast are cultured in controlled environments, enabling them to produce L-Cysteine as a byproduct of their metabolic processes (Yalcin *et al.*, 2008). This method ensures that the L-Cysteine produced is free from any non-halal components.

3. Synthetic Production: Synthetic production of L-Cysteine offers an alternative source for halal L-Cysteine. Through chemical synthesis, L-Cysteine can be manufactured in laboratories using halal-compliant starting materials (Wu & Abu-Hashim, 2015). This synthetic production method provides a consistent and reliable source of halal L-Cysteine.

### 3.2 The power of halal certificates: upholding compliance and fostering transparency

Halal certificates play a paramount role in the halal food industry by establishing and verifying compliance with Islamic dietary guidelines while providing transparency and assurance to consumers (Kartina *et al.*, 2015). These certificates are issued by esteemed halal certification bodies, following meticulous inspections, audits, and rigorous verification processes (Yaacob *et al.*, 2018). By acquiring a halal certificate, producers demonstrate that their products, including halal L-Cysteine, adhere to the rigorous requirements specified by Islamic principles (Aziz *et al.*, 2019). Halal certificates inspire

consumer confidence by assuring them that their food aligns with their religious beliefs and meets halal standards (Razak *et al.*, 2015).

### 3.3 Safeguarding sourcing and production through halal certificates

Halal certificates act as a safeguard, validating the sourcing and production practices within the halal food industry, including the manufacturing of halal L-Cysteine (Rekik *et al.*, 2015). These certificates ensure that animals in L-Cysteine production have undergone halal-slaughter practices and that manufacturing facilities and equipment comply with stringent halal standards (Mohd Nasir *et al.*, 2019). This thorough verification process upholds the integrity and authenticity of halal L-Cysteine, providing consumers with unwavering confidence in its compliance with Islamic guidelines (Lim, 2021). The traceability ensured by halal certificates enhances accountability and ensures that the entire supply chain—from sourcing to manufacturing—adheres to halal principles (Ramli & Ghani, 2020).

### 3.4 Empowering consumers and facilitating market access

Halal certificates empower consumers by instilling confidence in their food choices, as they can rely on the presence of halal certificates to ensure compliance with their religious beliefs (Jin *et al.*, 2020). With the assurance provided by halal certificates, consumers can make informed decisions, confident that their products, including halal L-Cysteine, align with their dietary requirements (Saleh, 2017). Halal certificates also facilitate market access for producers, opening doors to the rapidly expanding global halal market and enabling them to cater to the specific needs of Muslim consumers (Helble *et al.*, 2019).

## 4. Conclusion

The halal food industry serves as a cornerstone for Muslims seeking ethically and religiously permissible food options. Within this realm, halal L-Cysteine stands as a crucial additive, meticulously selected to adhere to Islamic dietary guidelines. The power of halal certificates cannot be underestimated, as they provide the necessary transparency and assurance for consumers and uphold compliance with Islamic principles. As the halal food industry continues to flourish, the global standardization of halal certification fortifies its integrity and promotes harmonization within the market. Ultimately, the halal food industry, along with the significance of halal L-Cysteine and halal certificates, facilitates the fulfilment of dietary and religious obligations while fostering ethical and sustainable consumption practices. The growth and continued advancement of the halal food industry contribute to a more inclusive and diverse global marketplace that embraces religious and ethical considerations, catering to the needs and preferences of a wide range of consumers.

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# *Halalan Toyyiban* Concept as Religious-Based Intervention for Healthy Diet among Youth

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*Halalan toyyiban*; Diet; Faith-based intervention; Digital-based intervention

**Abstract**

Healthy youth eating habits are crucial for optimal growth and development. It helps build a strong immune system and reduces the risk of chronic diseases. In Islam, there is a focus on maintaining a healthy lifestyle, including a healthy diet, guided by the *Qur'an* and the teachings of Prophet Muhammad(ﷺ). The article uses a narrative review to highlight the potential applicability of the *halalan toyyiban* concept (as a faith-based intervention) to promote a healthy diet among youth. Faith-based intervention involves integrating religious beliefs into the intervention process. To provide context, this article explores the application of interventions such as multimodal nutrition education and digital approaches to improve dietary habits among youth. Various interventions targeting university students' dietary habits yielded mixed results. This highlights the necessity for multifaceted intervention approaches. The article posits combining the *halalan toyyiban* concept with digital technology to enhance university students' healthy dietary practices.

## 1. Introduction

The intersection of religious principles and dietary practices holds profound significance, particularly within Islam. Embedded in the daily lives of Muslims, the *halalan toyyiban* concept elucidates the holistic approach Islam takes towards food consumption. This comprehensive approach encompasses not only the permissibility (halal) of food but also emphasises its quality, cleanliness, and benefits. Grounded in *Qur'anic* verses, the concept of *toyyib* underscores the importance of consuming not only permissible (halal) but also clean, safe, pure, and health-promoting food. This introduction sets the stage for exploring the *halalan toyyiban* concept to promote a healthy diet among youth. It first discusses the *halalan toyyiban* concept, the emphasis on a healthy diet in Islam, and the integral role of good etiquette in shaping Muslim dietary behaviours.

### 1.1 *Halalan toyyiban* concept

Muslims do not confine their religious practices solely to weekends or festive occasions; religion is integral to their daily lives. As a comprehensive religion, Islam has laid out guidelines encompassing all aspects of human life, including food consumption. The *Qur'an* contains numerous verses encouraging believers to take advantage of pure and righteous food. According to Ayatollahi (1992), verses related to food and beverages in the *Qur'an* can be divided into the following four groups:

i) The verses that indicate the permissibility principle (see: *Al-Qur'an* 2:168-170; 3:50)
ii) The verses that forbid some comestibles (see *Al-Qur'an* 2:173; 6:145)
iii) The verses concerning alcoholic beverages and prohibiting vinosity (see *Al-Qur'an* 2:219, 5: 90-91, 4:43)
iv) The verses that stipulate the permissibility of some food items, in particular *Al-Qur'an* (see *Al-Qur'an* 6:143-145)

Muslims, in general, are advised to eat permissible (halal), good, and pure things and not indulge in impure, bad, and harmful things, thus following their open enemy, *Satan*:

> *"O People! Eat of what is lawful and good on the Earth and do not follow the footsteps of Satan, for he is your open enemy." (Al-Qur'an 2:168)*

The *Qur'an* provides further clarification on the types of food that are prohibited. It explicitly states:

> *"He (Allah) has only forbidden you (from eating) dead animals, blood, the*

> *flesh of swine, and that (animal) over which the name of other than Allah has been invoked." (Al-Qur'an 2:173)*
> "And the cattle, He has created them for you, in them, there is warmth (clothing) and numerous benefits, and of them you eat." (Al-*Qur'an* 16:5)

When discussing halal food, it is imperative to invoke the term "*halalan toyyiban*," rooted in the *Qur'anic* verse:

> "O you Messengers! Eat of the clean and pure (*toyyib*) and act righteously" (*Al-Qur'an* 23: 51).

The concept of halal and *toyyib* has been extensively explored in various publications, including the works of Arif & Ahmad (2011) and Arif & Sidek (2015). A prevailing consensus suggests a close association between "*toyyib*" and food safety, as Neio Demirci, Soon, and Wallace noted in 2016. However, it is important to recognise variations in scholars' understanding and interpretations of the *toyyib* concept. Imam Malik interprets the term "*toyyib*" as "good." In contrast, scholars like Arif & and Ahmad (2011) take a broader approach, defining "*toyyib*" to encompass concepts such as "clean," "pure," and "*Shari'ah*-compliant." Additionally, Ibn Katheer provides a nuanced explanation, stating that the use of "good" (*toyyib*) in verse 168 of Al-Baqarah signifies something "delicious" for humans. Such food not only gratifies the senses but also refrains from causing harm to the body or mind. Furthermore, *toyyib* can be interpreted as food and drink containing beneficial nutrients for health, avoiding detrimental side effects or harm to the body (Chalil, 2019). These multiple interpretations reflect the multifaceted nature of halal food.

### 1.2 *Halalan toyyiban* food and health

*Halalan toyyiban* encompasses a comprehensive approach to food choices beyond mere permissibility. It encompasses safety, quality, nutritional value, and their impact on wellbeing. Within Islam, adherents are encouraged to make dietary choices that support physical and spiritual health. Scholars, such as those cited by Khattak *et al.,* (2011), have expounded upon the manifold benefits of halal food, particularly its positive implications for health. Islam highlights the significance of good health as a valuable blessing; as Prophet Muhammad (ﷺ) once said:

> "There are two blessings that many people are deceived into losing: health and free time." (*Sahih al-Bukhari* 6412)

Maintaining a balance in dietary habits is emphasised in Islam, cautioning against overeating. In a *hadith* narrated by Jabir (RA), the Prophet Muhammad (ﷺ) advised moderation:

> Narrated Abu Huraira (RA): the Prophet Muhammad(ﷺ) said: "The food for two persons is sufficient for three, and the food of two persons is sufficient for four persons." (*Sahih al-Bukhari* 5392)

Furthermore, this is also mentioned in the *Qur'an*:

> "Children of Adam! Take your adornment at every time of Prayer, and eat and drink without going to excesses. For Allah does not like those who go to excesses." (*Al-Qur'an* 7:31)

While it is cautioned against overeating, complete dietary restriction is also discouraged, as it may lead to issues such as starvation and deprivation of essential nutrients. This is based on Islamic teachings' general principles of preserving life and avoiding harm. Depriving the body of its nutritional needs is considered akin to ingratitude for the gift of the body from *Allah* (Anonymous, 2023). Islam indirectly prescribes a balanced diet to maintain overall wellbeing in this context.

As defined by Kaushik (2018), a balanced diet comprises various food items such as fats, carbohydrates, proteins, vitamins, and minerals in proper amounts and calories for maintaining health. The well-balanced diet may include meat, fish, fresh milk, cheese, fruits, vegetables, and whole grains. Consuming these nutritious foods provides essential elements that contribute to overall wellbeing and strengthen the immune system. This helps support the body's natural defences and restore health in illness. Interestingly, direct and indirect references have been made regarding these foods in the *Qur'an* and *hadith*. For example, the benefits of fruits as good nourishment are stated in this *Qur'anic* verse:

> "And from the fruits of date palms and grapes, you desire strong drink and a goodly provision" (16:67).

The Prophet Muhammad(ﷺ) also highlighted the significance of milk.

> "When one of you eats food, he should say: 'O God, bless it and give better nourishment.' When given milk to drink, he should say: 'O God! Bless it and grant us more, for no food or drink satisfies like milk'" (narrated by *Muslim*, 2052).

In addition to the food mentioned above and drinks, it is noteworthy that certain foods carry special significance in the *Qur'an*, with God emphasising the importance of olives and figs through solemn oaths.

> By the fig and the olive *(Al-Qur'an* 95:1)

The Prophet Muhammad(ﷺ) would sometimes praise certain foods, as exemplified by his statement on vinegar:

> "The best of condiments or condiment is vinegar." (*Sahih Muslim* 2051a).

The references to foods such as dates, olives, figs, milk, and vinegar underscore their value and importance within Islamic teachings on balanced nutrition. During Ramadan, Muslims observe fasting from dawn to sunset, demonstrating control over their energy intake and meal frequency. This practice aligns with the guidance of the Prophet Muhammad (ﷺ) to prioritise well-balanced meals during non-fasting hours, ensuring sufficient energy levels throughout the fasting day. Additionally, the tradition of breaking the fast with dates and water, as advocated by the Prophet Muhammad (ﷺ), reflects the incorporation of nutritious foods into daily practices. Dates, known for their rich nutritional content, serve as an excellent source of sugars, fibre, and essential nutrients, aiding in replenishing energy swiftly and providing various health benefits (Al-Farsi & Lee, 2008).

Consumption of halal food is crucial for various reasons, as it not only impacts personality and individuality development (al-Ghazali, 1989) but also influences the quality of mental development. Individuals are responsible for ensuring that what enters their stomach is obtained from a halal source and is clean, safe, and nutritious. Islam recognises the connection between food and mental performance and discourages the consumption of forbidden (haram) foods and drinks, such as alcohol. Binge drinking and a diet high in calories but low in nutritional value can adversely affect cognitive function, mood, and overall mental performance.

Conversely, a diet consisting of permissible (halal) foods, including a balanced intake of fruits, vegetables, and quality meat, can positively impact mental wellbeing, cognitive function, and behaviours. Verses in the *Qur'an* redirect humanity's focus towards self-reflection, urging individuals to carefully study their body and soul and understand the nature of their mutual relationship. Through self-reflection, individuals recognise that Allah has created humanity and all other beings with a purpose, as mentioned in the *Qur'an*.

> "Our Lord! You have not created (all) this without purpose." (*Al-Qur'an* 3:191)

### 1.3 Good etiquette an integral part of *Halalan toyiban* concept

Islamic food etiquette forms an integral component of *halalan toyyiban*. Following Islamic teachings, it guides Muslims in the manners and behaviours associated with eating and drinking. The source of manners and etiquettes associated with eating and drinking in Islam is derived primarily from the *Qur'an* and the authentic traditions of Prophet Muhammad (ﷺ), known as the Sunnah. The *Qur'an* does not provide explicit details on specific food etiquettes, but it emphasises the importance of gratitude. On the other hand, the *hadith* contains numerous narrations that provide specific guidance on food etiquette. The Prophet Muhammad's (ﷺ) practices and teachings on food are perfect guidance for Muslims. The Prophet Muhammad (ﷺ) emphasises the importance of starting with the name of *Allah,* expressing gratitude, observing proper etiquette, and seeking blessings in meals. Table 1 provides a concise overview of the practices and teachings of Prophet Muhammad (ﷺ) regarding food and eating, along with the corresponding *hadith* references.

Considering Islam's wealth of dietary teachings, integrating food interventions that align with Islamic principles can impact individuals' lifestyles and positively promote healthier diets. This article begins by delving into the *halalan toyyiban* concept to establish a contextual foundation for the review. The review then focuses on food interventions - discussing dietary changes and health improvement - emphasising youth, specifically university students, as the target demographic. The applicability of digital and faith-based interventions was then discussed, acknowledging the contemporary tools and methods available to influence behaviour.

## 2. Methodology

This article employs a narrative literature review, which leverages existing evidence to construct a coherent message. This approach allows various perspectives to be presented in a balanced manner, thereby fostering scholarly discourse. In contrast to systematic reviews, which adhere to detailed and explicit methods, narrative reviews lack established guidelines (Green *et al.,* 2006). While systematic reviews offer a structured and methodically rigorous evidence synthesis, narrative reviews provide flexibility to contextualise the topic. Narrative reviews also provide a general overview of a topic and set the stage for future research. They allow researchers to provide insights and new ways of thinking (Sukhera, 2022). In employing a narrative review, the article aims to reveal the potential application of faith-based interventions, specifically within the framework of *halalan toyyiban*, in promoting healthier dietary habits among youth.

### 2.1 Food intervention

Interventions are crucial in improving dietary habits and promoting healthier eating behaviours. Interventions related to food and dietary habits can take various forms, such as mobile-based programs, web-based programs, cooking classes and nutrition education (Brown *et al.,* 2014; Clifford *et al.,* 2009; Schnoll and Zimmerman, 2001). By providing individuals with information, resources, and support, food interventions empower them to make informed choices and adopt healthier eating patterns. They aim to increase the consumption of nutritious foods like fruits, vegetables, whole grains, and lean proteins while reducing the intake of unhealthy options. Successful food interventions have positively impacted dietary outcomes, such as increased fruit and vegetable consumption, improved nutrient intake, enhanced dietary self-efficacy, and better overall eating habits. Food interventions promote better health and wellbeing among individuals and communities by addressing the complex factors influencing food choices and behaviours. Common target groups for healthy diet interventions include the general population, children and adolescents, individuals with chronic conditions, low-income communities, pregnant women and new mothers, and older adults. Youth is also an important target group for healthy diet interventions.

#### 2.1.1 Youth as target for food intervention for healthier diet

The United Nations defines youth as persons between 15 and 24 (United Nations, n.d.). Healthy eating is essential among youth or adolescents to support their rapid physical growth and development. Insufficient intake of essential nutrients can negatively affect their growth, sexual maturation, and overall function. Excessive food consumption, on the other hand, may harm one's health by increasing susceptibility to non-communicable diseases. Furthermore, adolescents' eating habits are rapidly changing (Savige, Macfarlane and Ball, 2007). Takeaway meals, consumption of food outside typical meal hours, peer influence, a wider range of dietary choices, and higher levels of restrained eating are more common among adolescents (Mohammadi *et al.,*, 2020). The rising prevalence of obesity and unhealthy eating habits among youth is causing concern due to their link to chronic diseases such as cardiovascular disease.

In Malaysia, the Malaysian Dietary Guidelines for Children and Adolescents (2013) by the Ministry of Health (MOH) includes recommendations on healthy eating for children and adolescents. The guidelines recommend eating fruits and vegetables, consuming milk and milk products, and drinking plenty of water (Loh *et al.,* 2017). They are also encouraged to limit their daily fat, salt, and sugar intake. Despite guidelines, children and adolescents' food intake does not meet the recommended amounts (Moy, Ying and Kassim, 2006). Studies reveal that when adolescents reach puberty, the quality of their nutrition deteriorates (Soo, Shariff and Taib, 2008). The consumption of fruit, vegetables, and milk decreases through

Table 1: The practices and teachings of the Prophet Muhammad(ﷺ) regarding food etiquette

| Practice/Teaching | *Hadith* | References |
|---|---|---|
| **Saying "*Bismillah*" (in the Name of *Allah*) before eating** | "When any one of you eats, let him mention the name of *Allah*. If he forgets to mention the name of *Allah* at the beginning, let him say *Bismillaahi fi awwalihi wa aakhirihi*." | *Al-Tirmidhi* (1859), *Abu Dawood* (3767) |
| **Not criticising food** | "He never criticised food at all. If he liked it, he would eat it; if he did not, he would leave it and not say anything." | *Al-Bukhari* (3370), *Muslim* (2064) |
| **Expressing disinterest in certain food instead of criticising food** | "I do not feel like eating this." | *Al-Bukhari* (5076), *Muslim* (1946) |
| **Eat from what is in front of them in the dish*** | "Say *Bismillah* and eat from that in front of you in the dish." *At the time of the Prophet Muhammad(ﷺ), people used to eat together from one dish, and children would sometimes forget the etiquette | *Al-Bukhari* (5061), *Muslim* (2022) |
| **Urging guests to eat generously** | The Prophet Muhammad(ﷺ)repeatedly said to him (the guest), "Drink!" and he kept telling him to drink until he said, "By the One Who sent you with the truth, I have no more room for it!" | *Al-Bukhari* (6087) |
| **Making supplication (*dua*) for others before leaving the meal** | The Prophet Muhammad (ﷺ) made *du'a* in the house of 'Abdullah ibn Bisr and said: "O *Allah*, bless for them that which You have provided for them, forgive them and have mercy on them." | *Muslim* (2042) |
| **Eating with the right hand and forbidding eating with the left hand** | The Prophet Muhammad(ﷺ) said: "The *Satan* eats with his left hand and drinks with his left hand." | *Muslim* (2020) |
| **Encouraging eating together and mentioning *Allah's* name for blessings** | "I command you to eat together and mention the name of Allah over your food so that He might bless it for you." | *Abu Dawood* (3764), *Ibn Maajah* (3286) |
| **Avoiding reclining while eating** | "I do not eat reclining." | *Al-Bukhari* (5083) |

adolescence and early adulthood, while the consumption of sugar-sweetened beverages and confectionery grows. According to the National Health and Morbidity Survey (NHMS), the obesity prevalence among Malaysians aged 10–17 increased from 5.7 percent in 2011 to 11.9 percent in 2015 (Mohammadi S *et al.,* 2020). Obesity and overweight among adolescents continue to rise and persist into adulthood; promoting healthy eating among adolescents has become a top public health and research goal (Sharifah Intan Zainun *et al.,* 2019).

While the rising incidence of obesity and overweight among adolescents is a growing concern, it is equally important to recognise the significance of promoting healthy eating among university students. The transition from adolescence to young adulthood, mostly spent at colleges or universities, is gaining recognition as an important time for health promotion and disease prevention (Nelson *et al.*, 2008). Targeting university students in healthy diet interventions allows for shaping their dietary behaviours, equipping them with tools for healthier choices, and providing knowledge that positively impacts their overall wellbeing, academic performance, and long-term health outcomes. The university phase marks a critical transition from adolescence to adulthood as individuals prepare for future responsibilities and the workforce. By focusing on interventions for university students, essential life skills, including healthy eating and self-care, can be developed as they navigate this pivotal stage.

### 2.2 Food interventions studies among university students

University students between 18 and 24 gain new experiences and personal freedom and develop a sense of identity as they ascend from adolescence to adulthood (Franko *et al.,* 2008). This is crucial during which young people establish independence and adopt lasting health behaviour patterns. Unfortunately, there is a potent tendency for university students to engage in unhealthy dieting, meal skipping (especially breakfast), and fast-food consumption. Lack of physical activity has also become a common practice.

Although university life is considered an age of optimal health and wellbeing, it is well documented that university students nowadays have poor dietary habits. The students often fail to meet recommended targets for fruits and vegetables, whole grains, milk, and dairy products compared to their adolescent years. According to Nelson *et al.,* 2008, poor eating habits and limited physical activity can likely increase the risk for osteoporosis, obesity, hyperlipidaemia, diabetes, and cancer in the long term. This unhealthy lifestyle is further linked to health-related quality of life (HRQoL). All of these associations suggest that it is important to establish good eating habits at an early age (White *et al.,* 2009). Therefore, early interventions are needed to improve health behaviours in this age group. Few nutritional education interventions (NEI) have targeted college or university students relative to interventions designed for children and the elderly.

Table 2: Food or dietary intervention studies among university students

| (Reference) | Design | Sample/ Country | Description of Intervention | Summary of Findings |
|---|---|---|---|---|
| Brown *et al.,* (2014) | Pre-post design | • 150 university students from the non-health department.<br>• USA | Mobile MyPlate text messages over 7 weeks vs. brochure (for controlled group). Pre-post survey on nutrition knowledge & and behaviour. | Text message intervention is effective in increasing nutrition knowledge and promoting good diet behaviour (breakfast, eating at restaurants, daily consumption of whole grain products, fruits, vegetables or potatoes, milk, yoghurt, or cheese) |
| Clifford *et al., (*2009) | Randomised controlled pre-post design | • 101 university students<br>• USA | Television program (15 minutes) based on the Social Cognitive Theory vs. sleep disorders program (5-minute internet-based). Impact on dietary behaviour and knowledge. | The result showed that television programs have little impact on dietary behaviour but still can influence changing people's knowledge. |
| Schnoll & Zimmerman, (2001) | Randomised controlled trials (RCT) | • 113 university students from nutrition class & and introductory health<br>• USA | Incorporating two self-regulatory strategies (goal setting and self-monitoring) into a nutrition education class to enhance dietary fibre self-efficacy and consumption. Four treatment conditions | Goal setting (GS) significantly impacted dietary fibre self-efficacy and consumption. No significant interaction with self-monitoring (SM). Supports Social Cognitive Theory. |
| Tas *et al.,* (2020) | Cross-sectional test and re-test | • 378 university students (no knowledge of food or nutrition knowledge before & and after study<br>• UK | Four weeks of intervention for key topics (i.e., eat more dietary fibre, eat less sodium, eat less sugar, and eat less saturated fats and trans-fats) was given in dietary guidelines. The intervention uses Healthy snacks and Informative leaflets. | Improvement in knowledge related to "less saturated fat and trans-fat." Limited impact on practical dietary outcomes (e.g. serving sizes or making informed food choices) |
| You *et al.,* (2009) | Pre and post test | • 22 university students from nutrition education class.<br>• Korea | Intervention: The 8-week body weight control program consists of an Introductory class (individualised low-calorie diet), Diet therapy, Exercise, Behavioural modification (online nutrition lecture; Supplement (sea tangle powder). | The results showed that the program could help decrease participants' body weight, body fat mass, per cent body fat, waist-hip ratio, and BMI. Additionally, it can improve dietary habits and enhance the quality of life. |
| Chiba T., et. al., (2020) | Randomised controlled trials (RCT) - Pre- and post-intervention | • 328 university students<br>• Japan | Intervention on dietary supplements: A single educational lecture on dietary supplements to different groups of students with different backgrounds. An assessment scale with 14 questions to evaluate the effects of the educational intervention | A one-hour lecture on dietary supplements improved university students' knowledge. |

| (Reference) | Design | Sample/ Country | Description of Intervention | Summary of Findings |
|---|---|---|---|---|
| Peterson *et al.,* (2010) | Pre and post surveys | • 104 university students (Both pre-survey & post-survey)<br>• USA | Healthy choice indicators, large signs, table tents, flyers, and colourful photographs with "benefit-based messages" promoted targeted foods. Pre-survey collected in the dining hall, followed by 3-week intervention. After the intervention period, post-survey data was collected via email. | Significant increase in consumption of certain foods. Short-term marketing strategies can enhance students' perceptions and choices of healthful foods. |
| Winzelberg *et al.,* (2000) | Randomised controlled trials (RCT) | • 60 female students from public university<br>• USA | 3-month internet-delivered health education program. Aims to enhance body satisfaction. Participants who completed the online CAHE program underwent body image and disordered eating attitude assessment at baseline, post-intervention and during a 3-month follow-up | Significant improvement in body image and reduction in desire to be thin. Demonstrates the effectiveness of using the internet for health education. |
| Poddar *et al,* (2010) | Randomised controlled trial (RCT) | • 294 undergraduate students from personal health<br>• USA | Five-week online course on dairy consumption. Topics self-efficacy, outcome expectation, and regulation regarding dairy consumption). Included email messages and behaviour checklists. | Successful in improving self-efficacy and self-regulation, but not outcome expectations or actual dairy consumption. |
| Shahril *et al.,* (2013) | Cluster randomised controlled – pre and post | • 417 university students<br>• Malaysia | Intervention: 10-week multimodal nutrition education (NEI) to enhance dietary intake among participants. Interventions include conventional lectures, brochures, and text messages. | Significant improvement in dietary intake of various nutrients (e.g. calcium, vitamin C, thiamine, fruits) and food items (eggs, milk, and dairy products). NEI is effective in improving participants' dietary intake |

Different interventions have been employed to promote healthy eating among university students, with mixed results (Table 2). One approach utilised mobile technology, as Brown *et al.,* (2014) demonstrated, who delivered behaviour-directed motivational dietary guideline messages to participants through Mobile MyPlate text messages. Another intervention focused on point-of-selection marketing, where Peterson *et al.,* (2010) implemented a multifaceted marketing strategy in university dining halls, using visual cues such as indicators, signs, tents, flyers, and photographs with "benefit-based messages" to promote healthier food choices. Web-based interventions were also applied, as seen in the study by Poddar *et al.,* (2010), who used an online course system to deliver tailored email messages, posted information, and behaviour checklists to improve milk intake. The study successfully improved self-efficacy and self-regulation but not outcome expectations or actual dairy consumption.

Additionally, a multimodal nutrition education intervention conducted by Shahril *et al.,* (2013) employed conventional lectures, brochures, and text messages over 10 weeks to enhance dietary intake among university students. The diversity of target behaviours and strategies employed in these studies highlights the complexity of interventions in this context. This underscores the importance of adopting multifaceted approaches that address various aspects of knowledge, attitude, and behaviour to promote healthy eating among university students effectively.

As indicated in Table 2, studies on promoting healthy eating among university students were conducted in various countries, highlighting the global focus on this important issue. Brown *et al.,* (2014) and Peterson *et al.,* (2010) implemented interventions to improve dietary behaviours in the United States. Shahril *et al.,* (2013) conducted their study in Malaysia, contributing to understanding healthy eating interventions in a Southeast Asian context. Similarly, Tas *et al.,* (2020) conducted their research in the United Kingdom, adding to the growing body of knowledge on promoting healthy eating among university students in European settings. Lastly, the study by You *et al.,* (2009) took place in Korea, providing insights into interventions tailored to Korean university students' specific needs and preferences. Table 2 overviews university students' food or dietary intervention studies.

### 2.3 Digital-based intervention

The rapid advancement and increasing complexity of digital technologies, including websites, mobile apps, wearable devices, and smartphone applications, have become popular and cost-effective tools for encouraging positive changes in diet and overall health. These digital interventions encompass a wide range of tools, such as internet programs, mobile apps, websites, emails, videos, CD-ROMs, games, tele services, SMS text messages, and social media, sometimes used in combination (Public Health Ontario, 2021).

The history of employing digital interventions for health and wellbeing among youth has an enduring trajectory. As evidenced by the interventions summarised in Table 3, there are diverse digital approaches implemented over the years, underscoring a sustained commitment to explore and refine digital strategies. The popularity of digital interventions reflects the increasing global accessibility of these digital technologies. With internet penetration reaching 95% in the most developed countries and reaching 60% worldwide, these interventions have witnessed a broadening reach. The advantages of employing digital strategies, particularly internet- and mobile-based approaches, are multifaceted. These interventions possess the capability to deliver services irrespective of space and time constraints, ensuring accessibility at the convenience of the users. Additionally, they offer potential anonymity, flexibility in conduct, and a strong appeal to the youth demographic. The advantages also include potential cost-effectiveness and scalability on a larger, consistent basis (Andersson *et al.,* 2019; Domhardt *et al.,* 2018; Domhardt and Baumeister, 2018; Ebert *et al*., 2018).

Various digital interventions have been utilised to enhance health and wellbeing outcomes for youth (Table 3). This includes web-based interventions that leverage online platforms or applications to deliver treatment, monitor health conditions, and provide valuable resources such as modules, psychoeducational, self-management strategies, and disease-specific information (Nijhof *et al.,* 2012; Stinson *et al,,* 2010). Similarly, mobile interventions that harness the power of mobile applications to track health information and monitor disease progression (Berndt *et al.,* 2014) were also applied. These mobile interventions offer flexibility and convenience, as they can be accessed anytime, anywhere through mobile devices.

As shown in Table 3, digital interventions were conducted in various countries, demonstrating the applicability, widespread implementation, and diverse impact. For example, Nijhof *et al.,* (2012) conducted their intervention in the Netherlands, shedding light on the effective use of digital strategies to enhance the wellbeing of adolescents in that specific region. In the study by Stinson *et al.,* (2010), the intervention took place in Canada, showcasing how digital platforms have been harnessed to improve adolescent health outcomes within the Canadian context. There are also few studies conducted in Malaysia (Shahril *et al*., 2013; Ahmad *et al.,* 2018; Nawi & Jamaludin, 2015).

Digital interventions can be implemented as stand-alone interventions (Andersson *et al*., 2019; Domhardt *et al.,* 2018; Domhardt and Baumeister, 2018; Ebert *et al*., 2018; Yonek *et al*., 2020) or in combination with human support (Therese and Holter, 2023). These interventions employ diverse delivery methods, some exclusively using a single mode, such as web-based applications, while others are evolving towards multimodal approaches. Multimodal strategies involve integrating different tools or platforms—from web-based applications to mobile devices—effectively delivering interventions. For instance, Ahmad *et al.,* (2018) conducted a study targeting overweight and obese primary school students aged 8 to 11 years old. The researchers used several digital platforms, including Facebook and WhatsApp, to incorporate face-to-face sessions. Despite the innovative approaches evident in these studies, an area less commonly explored is the integration of faith-based interventions within digital strategies.

### 2.4 Faith-based intervention

Despite being less known, faith-based interventions are gaining significance within healthcare initiatives as they integrate spiritual and religious beliefs into their framework. Religion, a multifaceted concept encompassing beliefs, behaviours, rituals, and ceremonies, can be privately or publicly practised. These interventions have considerable potential to promote positive health outcomes among believers by incorporating spiritual and religious beliefs, practices, and teachings. Two distinct types of faith-based interventions exist faith-placed interventions, which are spiritually grounded initiatives.

Table 3: Digital intervention to improve health and wellbeing among youth

| Authors | Country | Sample | Age | Duration | Focus | Intervention | Outcome |
|---|---|---|---|---|---|---|---|
| Berndt *et al.,* (2014) | German | 68 | 8-18 years | 14 weeks | Diabetes management | Mobile/web-based disease monitoring applications (Mobil Diab). | Increased self-efficacy, improved quality of life, weight, and BMI values in diabetes management. |
| Carlsen *et al.,* (2017) | Denmark | 29 (intervention) and 21(control group) | 10-17 years | 30 months | Inflammatory bowel disease | Patient-managed eHealth (a web-based disease with symptom monitoring | Acceptable patient adherence, improved knowledge and understanding of inflammatory bowel disease. |
| Toole & Craighead, (2016) | USA | 80 | Age not specified (Undergraduate) | 6 - 8 day | Body image distress (BID) | Self-compassion meditation podcast Interactive; Self-directed; Audio-delivered podcasts | Minimal attrition, low compliance with meditation practice; impact on body image distress not specified. |
| Franklin *et al.,* (2006) | UK | 92 | 8-18 years | 12-month | Diabetes care | Mobile-based support system (Sweet Talk) - Automated, scheduled text messaging. | Improved self-efficacy and adherence in diabetes care, potential support for introducing intensive insulin therapy. |
| Newcombe *et al.,* (2012) | Australia | 42 | 10-17 years | 9-week time | Psychosocial wellbeing of a Chronic Respiratory Condition patient | Web-based problem-solving program (Breathe Easier Online) for psychosocial wellbeing. | No significant group differences post-intervention; preliminary evidence suggests program efficacy, improvement in attitudes, and reduction in depression symptoms. Decreased maladaptive social problem solving (impulsive/careless style) for participants |
| Nijhof *et al.,* (2012) | Nether-lands | 135 | 12-18 years | 6 months | Internet-based therapeutic program | Web-based (FITNET)-Guided and tailored iCBT with 21 modules and a comprehensive psychoeducation part | More effective than usual care in achieving full school attendance, absence of severe fatigue, and normal physical functioning at 6 months. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | (e.g., goals, sleep routine, cognition, fatigue-specific interventions, physical activities, and balance) | |
| Stinson *et al.,* (2010) | Canada | 43 | 12-18 years | 12 months | Juvenile Idiopathic Arthritis Self-Management | Web-based self-management programme (Teens Taking Charge) plus telephone support. | Effective in reducing pain intensity and interference and improving health-related quality of life in adolescents with juvenile idiopathic arthritis. |
| Shahril *et al.,* (2013) | Malaysia | 417 | 18-24 years | 10-week | Dietary Intake | Multimodal intervention using three modes. Conventional lectures, brochures, and text messages Daily servings of food intake. | Significant improvement in dietary intake compared to the control group. |
| Nawi & Jamaludin (2015) | Malaysia | 97 | 16 years old school students | 12 weeks | Weight management | Website intervention with topics related to weight management for school students. Websites include games and exercise videos. | Between baseline and end-line, BMI, waist circumference, and body fat percentage significantly decreased only in the intervention group. Comparing the two groups, however, there were no significant differences in the change in these measures over time. |
| Ahmad *et al.,* (2018) | Malaysia | 134 | 8 - 11 years old primary school children | 4 months | Weight management | Four-week training program using face-to-face sessions, Facebook, and WhatsApp for weight management in primary school children. | Effective in reducing childhood adiposity. |

conducted in organised religious settings, and faith-based interventions, which either have a spiritual foundation or involve a significant presence of a faith group but may not necessarily unfold within religious establishments. Faith-based dietary interventions offer several advantages compared to alternative methods. Ismail *et al.,* (2013) suggest that integrating faith-based objectives into the intervention is a tactic for achieving enduring behavioural changes. These interventions have the capacity to engage a substantial and consistent group, allocate space for programming, provide social support, and involve influential leaders who can encourage participation, potentially ensuring the sustainability of programs in the long term (Peterson *et al.,* 2002, Holt *et al.*, 2013, Wilcox *et al.,* 2013).

Numerous studies have explored faith-based interventions tailored for Muslims, addressing health and wellbeing concerns such as physical exercise, cardiovascular health, diabetes prevention, healthy lifestyle promotion, mental health, substance use, and cancer screening within their communities. In their comprehensive review, McLaren *et al.,* (2022) examined studies integrating the development and delivery of health interventions for Muslim minorities in Canada, Australia, the United States of America, and the United Kingdom. A recurring theme across these studies is the emphasis on religious tailoring, community consultations, and integrating Islamic principles. The cumulative evidence from these studies strongly suggests that integrating religious and cultural elements into interventions enhances their effectiveness and relevance among Muslim populations.

Faith-based intervention studies adopted diverse settings such as mosque-based programs, community centres, and collaborations with healthcare institutions (McLaren *et al.,* 2022). Collaboration is necessary for interventions to be effective, as they need to be co-designed and culturally and religiously sensitive, combining scientific guidelines on healthy living with the Islamic narrative (Sufyan *et al.,* 2021). This diverse and culturally attuned approach underscores the significance of faith-based initiatives in promoting health and wellbeing within Muslim communities (McLaren *et al.,* 2022).

Integrating food interventions rooted in Islamic principles, particularly the *halalan toyyiban* concept, has substantial potential to positively influence the dietary habits of tech-savvy youth, including university students. This could be done through digital platforms such as interactive modules, educational videos, mobile applications, and online support networks. This multi-approach strategy encourages healthier choices and aligns dietary practices with religious beliefs. This approach fosters a deeper intrinsic motivation to adopt healthier habits. A review indicates a limited exploration or incorporation of faith-based concepts within the context of digital interventions. This suggests a potential area for further investigation and development in this field. Moreover, by effectively combining the strengths of *halalan toyyiban* principles with digital technologies, interventions can guide and empower Muslim youth to make healthier dietary choices while adhering to their faith.

## 3. Conclusion

Healthy eating habits are crucial for maintaining physical and mental wellbeing, particularly among youth. Transitioning from adolescence to young adulthood is critical for establishing healthy eating habits, and interventions are needed to improve health behaviours in this age group. Different interventions have been employed to promote healthy eating among university students, with mixed results. The range of target outcomes and strategies used in these studies emphasises the complexity of influencing university students' dietary knowledge and habits, highlighting the need for comprehensive strategies to address these outcomes effectively. Recognising the significant role of religion, particularly for Muslims, faith-based interventions could serve as valuable tools in promoting a healthy diet. The *halalan toyyiban* concept, in particular, could be integrated into digital interventions to create a digital faith-based approach to foster healthy eating habits among Muslim youth.

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HALALSPHERE
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# Halal Laundry Detergents: Ingredients and Regulations in Malaysia

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**Abstract**

Cleaning products are necessary for our daily lives since they are highly effective in cleaning and washing. They assist in personal hygiene by loosening and removing soil and dirt from the surface, diminishing germs or bacteria that are the source of infectious diseases and making the surroundings comfortable. There are three most common detergents in various places: laundry, dishwashing, and household cleaning. Hence, this review paper focuses on laundry detergent to clean the dirt on fabrics and clothes. This paper aims to provide general knowledge for consumers, particularly Muslims, of the ingredients used in laundry detergents and the halal-related regulations in Malaysia. Ingredients used in detergent products, such as enzymes derived from animals, plants, fungi, and bacteria, have issues with where the sources were obtained, which subsequently become one of the reasons many productions have issues fulfilling the halal certification requirement. Other than enzymes, many other ingredients are added to detergent products to provide specific properties and characteristics, such as surfactants, builders, alkalis, bleaches, colourants, and fragrances, which consumers should know before purchasing. Manufacturers must inform Muslim consumers about the purity and impurity of the ingredients used in laundry detergents to make wise decisions in purchasing halal products.

## 1. Introduction

Globally, Muslims are the second-largest religious group, with 1.9 billion people in the world's population in 2020. The huge Muslim population has increased demand for halal products. Simultaneously, halal products have also started to gain worldwide recognition as a new standard for following the *Shari'ah* requirements and the concept of hygiene, sanitation and safety (Azmi *et al.*, 2018). Furthermore, it has also piqued the interest of non-Muslims in purchasing halal products (Chong *et al.*, 2021). The term halal means permissible, allowed, and lawful in Islam. Everything related to *najs* or animals not slaughtered according to *Shari'ah* is prohibited for Muslims. Thus, every halal product must follow the standard requirements and *Shari'ah* compliance. In particular, halal products include food and beverages, detergents, and other household products.

Detergents are often used as a cleaning agent at home and are also commonly found as good-performance products. They function effectively, cleaning the dirt or soil from the surface and making it easier to wash away (Kogawa *et al.*, 2017; Tan, 2019). The industry has introduced detergent products such as laundry detergent, household cleaners, and fabric softeners, pending the interest of many consumers(Cheng *et al.*, 2020). Every detergent production has various ingredients and mixtures to produce their specific brands, intended to appeal to consumers. Thousands of chemical compositions have been formulated to clean the surface. Detergents can be formulated with various organic and inorganic chemicals to produce a particular level of cleaning power or biodegradability at the same concentration (US Environmental Protection Agency, 1974). Furthermore, each ingredient in the detergent formulation and combination affects its cleaning capacity and ability.

However, detergent products may contain ingredients that can bring *syubhah* (doubtful) to Muslim consumers. Moreover, if there is no halal certification that verifies the status of halal and *ṭoyyibān* of the detergent, it will cause doubt among Muslim consumers. In modern production, animal fats are a basic component in the manufacture of detergents. For instance, animal fats can be found in enzymes like protease and lipase, commonly used in detergents (Naganthran *et al.*, 2017). It raises concerns among Muslim consumers since the ingredient used in the detergent may be derived from haram animals or animals that have not been slaughtered following *Shari'ah*, or the product was not manufactured under conditions that comply with halal production. It must be emphasised because Islam attaches great importance to personal hygiene and cleanliness (Armutcu, 2020). Islam requires Muslims to ritually clean themselves from any *najs* or dirt since it is required by Islamic rituals such as *wudu'*, cleansing before praying or reading the Qur'an, which is practised regularly and requires ritual cleanliness.

The awareness of using halal products is currently increasing among Malaysian communities. Muslim consumers must

ensure that the products' ingredients, processing, and distribution follow halal requirements (Hashim & Mat Hashim, 2013). In Malaysia, products must adhere to the Malaysian Standard and the Halal Certification Procedure Manual– Domestic 2020 (MPPHM) under the consumer goods scheme and related Malaysian standards to be certified as halal by the Department of Islamic Development Malaysia (JAKIM). The halal certification ensures that products are high-quality, safe, and *ṭoyibān* to use. Based on the discussions above, this review will cover the ingredients used and the halal-related regulations in Malaysia.

## 2. What is detergent?

The use of detergents is not an innovation for cleaning. Since ancient times, traditionally, people have been washing their clothes using soaps made from heated animal fat, oils and wood ashes mixed in a kettle. Then, there are innovations in soap that use the same process, substituting caustic soda for wood ashes as cleaning ingredients (Whitten & Whitten, 1990). After that, the first laundry detergent was introduced in Germany (Smulders *et al.*, 2001), and soap was made as one of the ingredients in the components of detergents for washing clothes. In these, soap has been combined with builders, or usually, it can be found as sodium carbonate, sodium perborate and sodium silicate (Smulders *et al.*, 2001). Known today simply as detergents, they can be produced using any soap or non-soap ingredient used as a cleaning agent. Chemically, they are compounds or preparations containing soaps or other ingredients intended for water-based laundry processes (Osadebe *et al.*, 2018). They mainly aim to remove visible stains and provide a hygienically clean surface, including removing the microbiological contamination and malodorous compounds as apparent stains (Bockmühl, 2017). Therefore, there was a need for an effective cleaning agent such as detergents, and unlike soap, it does not react with the mineral salts in water that form an insoluble compound, commonly known as soap curd.

A detergent can also be defined as any compound used as a cleaning agent, a mixture of active components, additives and mainly from water. It includes laundry detergents, bleach, fabric softeners, glass, and toilet cleaners (Zarogianni *et al.*, 2017). Detergents can be classified into natural soaps and synthetic detergents. However, nowadays, detergent is a term that is commonly used to refer to synthetic compounds of soaps and detergents (Kogawa *et al.*, 2017). Synthetic detergents are multicomponent compositions that can be obtained in various forms. They are composed of surface-active agents, or surfactants, organic and inorganic chemicals that enhance the efficiency of the surfactants (Info Mine Research Group, 2012). A large number of surfactants are based mainly on various crude petroleum products. Nonetheless, for a healthy and self-sufficient economy and green environment, they are commonly derived from synthetic organic chemicals as raw ingredients for products (Deshmukh *et al.*, 2015; Osadebe *et al.*, 2018). The surfactants in the detergent are usually alkylbenzene sulfonates, a group of compounds related to soap, but they are more soluble in hard water and have better washing performance. These compounds are widely used in synthetic laundry detergent products because of their superior washing performance (Abadi Kiswandono & Akmal, 2020; Akyüz & Roberts, 2002).

## 3. How detergent are made

Producing laundry detergent is similar to making soap saponification since they have the same function: they can clean soils, germs, and other contaminants. Both also have many similarities regarding their molecular structure and how they clean the surfaces (Nurul Ika Amira, 2015). Practically, after the saponification process is made, which involves heating fats and oils and reacting them with a liquid alkali, the detergent is produced since the saponification process is part of the detergent formulation (Ranji *et al.*, 2019; SDA, 1994; Tareila, 2004). Although people commonly refer to laundry detergent as soap, it differs from soap since there are certain major improvements in the ingredients of detergents. A carboxylic group (fatty acids) and a hydrocarbon chain performed two important functions in the soap-making process. The carboxylate end of the soap molecule, also known as hydrophilic (water-loving), is attracted to water. On the other hand, the hydrocarbon chain, known as hydrophobic (water-hating), is attracted to the oil and grease and repelled by water (SDA, 1994). When the oil and grease chemically attach to the carboxylate end of the soap molecule, they are drawn away from the cleansed clothes.

However, to effectively remove any hydrophobic or germs, it is necessary to use something much more capable of emulsifying so that the water can carry it away. Using animal or vegetable oils with a strong water-based solution, such as sodium or potassium hydroxide, lye or NaOH, can be more effective. These oils are mainly triglycerides containing three fatty acids, react with the alkali and produce glycerine and metal soap (Nurul Ika Amira, 2015). Thus, almost any triglyceride can be found in soap, but some salts of triglycerides are harsher than others. If any surplus base remains after the salt is formed, it must be neutralised, or it will be caustic and cause material damage (Tareila, 2004). Furthermore, adding alkali will not bond with the minerals in hard water because it will form scum, affecting the cleaning action's effectiveness. Therefore, soap sensitive to water hardness was eventually replaced with synthetic surfactants with more desirable characteristics.

A synthetic detergent is distinguished from soap by the absence of fatty acids, which are substituted by an acid formed when sulfuric acid reacts with hydrocarbons derived from petroleum. The resulting surfactant displays a reduced tendency to bond with minerals in water to form curds than soap and is more effective in hard than soft water (Whitten & Whitten, 1990). Surfactants, as main components in detergents, are made from petrochemicals derived from petroleum or oleochemicals derived from fats and oils. Both contain hydrocarbon chains repelled by water but attracted to oil and grease in soils, similar to the fatty acids used in soap-making. They are utilised to build the water-hating end of the surfactant molecule, composed of hydrocarbon chain sources (SDA, 1994). There are more ingredients in detergent besides surfactants, such as builders, which fulfil various functions. Since the new millennia, detergent production has been developing green and eco-friendly products, which are described as made using natural or oleochemical surfactants and do not affect the environment. These ingredients benefit the environment and consumers (Siwayanan *et al.*, 2015).

## 4. Types of laundry detergent

The use of laundry detergents becomes essential since it tends to make the process of cleaning easier. In ancient times, when it was time to wash the laundry, it would be taken to the river and rubbed against the rocks to help remove dirt and soil from the clothes (Tareila, 2004). However, various laundry detergents are available in the market, such as detergent bars, powders, and liquids. A detergent or soap bar made from saponification is one of the oldest cleaning and washing

methods. Detergent bars or soap bars are commonly made from fatty acids extracted from animal tallow or a combination of fatty acids extracted from animal tallow and vegetable oils (Bajpai & Tyagi, 2007). Other components, including colourants, fragrances, pigments, and other additives, are added to certain detergent bars. In most developed countries, detergent bars have been phased out in favour of detergent powder.

Detergent powders are used for both manual and machine washing, depending on the market that has been distributed. Depending on the manufacturer, surfactants, builders, bleaching agents, enzymes, and fillers are commonly found in detergent powders in varying amounts. Surfactants are among the most important of these components, and their cleaning activity has been a driving force in developing new detergents for many years (Siwayanan *et al.*, 2015). This type of detergent is particularly effective at removing dirt and soil from the surface (Bajpai & Tyagi, 2007; Zoller, 2008). However, the main limitation of detergent powders is that they do not dissolve well in the liquid and might leave marks of chalky residue on clothes after washing. As a result, it will be necessary to run another cycle in order to remove them completely.

Liquid detergents are gaining popularity worldwide due to their ease of use, dispersion, and dissolution in the wash water. Liquid detergents are often more profitable than bars or powders, as liquids are filled with water, whereas bars and powders are typically filled with additional fillers (Zoller, 2008). They developed rapidly due to their superior performance, laundering delicate fabrics such as silk, wool, and synthetic. This product has developed into extremely complex formulations incorporating builders and auxiliary speciality chemical ingredients (Dixit, 2003). Detergent production is competitive in the industry, where numerous laundry detergents have been produced globally. Powdered laundry detergent has dominated the Malaysian market, accounting for approximately 62% of the total value, followed by detergent liquid at 36% and detergent bars at 2% (Hee, 2017). Among the biggest global industries in laundry detergent are Procter & Gamble, Unilever, Henkel, Lion Corporation and Kao Corporation (Siwayanan *et al.*, 2015).

## 5. Ingredients of laundry detergent

Numerous detergent products are available on the market, from which consumers can choose and purchase based on their preferences. Therefore, most laundry detergent production will follow consumer trends currently prevalent in the market. For instance, producing green innovation or eco-friendly products free of harsh chemicals harmful to the environment and humans. They will also produce detergents that appeal to consumers' emotions through touch, feel and smell. Some manufacturers also produce detergents based on the value of detergent that benefits consumers and enhances the washing efficacy of laundry, such as hygienic cleaning, whitening, colour protection, anti-bacterial, etc. (Hee, 2017). Nowadays, the need for detergents capable of killing germs and bacteria is high due to the outbreak of COVID-19 that has afflicted the entire country. Many customers will choose detergents that effectively kill germs and bacteria to avoid getting viruses or other diseases. The following are the basic ingredients used and their function in the manufacture of detergents.

### 5.1 Surfactants

Surfactants or surface-active agents are the most common ingredients in laundry detergent formulations. A surfactant molecule consists of two components. One component of the molecule is hydrophobic, whereas the other is hydrophilic. These molecules are highly active at the interfaces between oil and water (Tai & Nardello-Rataj, 2001). Their major function is to improve the wetting ability of water and reduce the surface pressure between soil and water. The soil and dirt are removed from the surface to be cleansed and disseminated in the aqueous phase (Bajpai & Tyagi, 2007; Showell, 2016). Surfactants are commonly found in laundry detergents in a variety of forms. Surfactants are classified into four major categories: anionic, nonionic, cationic, and amphoteric. These surfactants differ in their ability to remove different types of dirt, their effectiveness on various fabrics, and their reaction to changes in water hardness (Bajpai & Tyagi, 2007).

Surfactants are organic chemicals that serve a specific function in a process or product (SDA, 1994). However, there have been reports of surfactants derived from chemicals harming the environment at times. Thus, there are concerns regarding their impact on the environment, specifically their biodegradability and toxicity to organisms and humans. The term "chemical" has also become synonymous with "toxic chemical," which has raised concerns among human beings. According to the studies by (Rosen & Kunjappu, 2012), cationic surfactants are more harmful than anionic surfactants, and anionic surfactants have a higher level of toxicity than nonionic surfactants. As a result, every manufacturer is responsible for ensuring that the appropriate materials and quantities are used to ensure the safety of the finished products for human consumption.

### 5.2 Builders

Builders are often combined with surfactants to lower the content of surfactants in the detergent formulations (Gürkök, 2019). Builders are one significant ingredient generally added to control the water's hardness. Detergent manufacturers have recognised the significance of controlling water hardness to achieve an optimum level of cleaning by using builders. Divalent ions were found in most water, specifically calcium and magnesium, which negatively impacted the laundry process (Mole, 1990). Therefore, a wide variety of suitable detergent builders are needed. Sodium tripolyphosphate (STPP), sodium carbonate, sodium citrate, and zeolites are builders in laundry detergents (Camerson & Cameron, 2011).

STPP is one of the most well-known and commonly used detergent builders, providing excellent calcium control and dispersion, suspension, and anti-encrustation benefits (Showell, 2016). However, most phosphates are not biodegradable, and it will cause health problems and major environmental hazards. Phosphate residues on the surface may cause nausea, diarrhoea and skin irritations. Therefore, many countries, including Japan, Korea and China, have substituted zeolite for phosphates in their laundry detergent formulations (Siwayanan *et al.*, 2015). Zeolites were more effective in cleaning than STPP at low temperatures, but zeolites take time to diffuse into the wash. In order to compensate for the inadequacies of the detergent builder, an alkali ingredient such as soda ash or sodium silicate should be added (Yunusa *et al.*, 2018).

### 5.3 Alkalis

Alkalis are soluble salts that can neutralise or adjust the acidity of other ingredients in a mixture. Alkalis effectively clean the fabrics and remove dirt or soil without heavy rubbing. During the washing process, soluble salt of alkalis, such as sodium carbonate and sodium silicates, give negative charges to the soil

and substrate (Bajpai & Tyagi, 2007). The other purpose of alkalis is to slow the corrosion of metal components in washing machines, boost the anti-resorption capacity of detergents, and lower the hygroscopic qualities of detergents (Info Mine Research Group, 2012). However, using strong alkalis in the washing process may damage fabrics and cause the clothes to feel rough to the touch.

### 5.4 Enzymes

Many laundries detergent products contain at least one enzyme or a combination of enzymes, such as proteases, amylases, cellulases and lipases, to increase their efficiency in the laundry process (Hasan *et al.*, 2010). Enzymes are frequently used as stain removers in detergent formulations and are extremely effective. If the enzymes were not in the detergent formulations, some soils and dirt would be challenging to remove and require repeated attempts (Gürkök, 2019). Therefore, detergent enzymes are needed to break and dissolve certain compounds into their basic components (US SDA, 2005). For instance, proteases, which have a wide range of applications and have been used in detergent products (Park *et al.*, 2018), are needed in detergent formulations to break down stains (Singh, 2021). Amylases are generally used with proteases and increase detergent cleaning (Gürkök, 2019). Besides that, lipases have been used in detergent products to create biodetergents. Most can tolerate harsh circumstances, such as oxidising agents and surfactants (Devi *et al.*, 2020). Cellulose is the most abundant renewable biological resource and a relatively inexpensive energy source. It smooths the fabrics, removes dirt, and helps prevent stains and dust from redepositioning on the fabric's surface (Gürkök, 2019).

Enzymes are proteins that all living creatures produce. Many different species of enzymes have been derived from plants, animals, bacteria and fungi (Gürkök, 2019; Hasan *et al.*, 2010; Singh, 2021). However, sufficient enzyme exposure can result in allergy symptoms, including asthma (Basketter *et al.*, 2012). Even though enzymes can cause allergic reactions, this has generally been seen as a minor issue in detergent products (Vanhanen *et al.*, 2000). Besides, certain enzymes may leave an effect and irritate the skin or eyes and cause irritation of the upper respiratory system due to their proteolytic action (US SDA, 2005). Therefore, the industry is currently focused on manufacturing enzymes derived from natural sources that are environmentally benign (Hasan *et al.*, 2010).

### 5.5 Bleaches

Bleaches are agents to whiten, brighten, and remove stubborn stains from clothing. Bleaches have proven to be quite powerful and useful in removing and carrying away the soil and dirt that has accumulated in the wash water due to the presence of detergents. Two types of bleach can be categorised in detergents: chlorine bleach and oxygen bleach. Chlorine bleach is sodium hypochlorite that reacts swiftly with various inorganic and organic compounds. As a result, it is an effective stain remover, cleanser, sanitiser and disinfectant of fungi, viruses and bacteria (Girotti, 2015; SDA, 1997). At the same time, oxygen bleach acts as an all-fabric bleaching agent for soil and dirt removal in detergents. Additionally, sodium percarbonate and sodium perborate containing hydrogen peroxide are used for this purpose (Bajpai & Tyagi, 2007). Bleaching agents have several downsides, including an unpleasant odour and the ability to change the colour of coloured fabrics.

### 5.6 Colourants

Colourants are used in detergent products to bring the consumer's attention to a unique characteristic contributing to the product's performance. Detergent products normally add a transparent blue colour, which may provide bluing derived from ultramarine and organic dyes (Info Mine Research Group, 2012), impacting white fabrics. Sometimes, the product becomes transparent emerald with time. Discolouration of laundry detergents has been observed and investigated by many researchers. For example, oxidising phenolic antioxidant ingredients leads to yellowing and other ingredients that can lead to different colours (Missler *et al.*, 2014). The other significance of colourants in detergent products is that they provide a special identity to the product.

### 5.7 Fragrances

Another ingredient that brings to the unique identity of detergent products is fragrance. These ingredients are not added to detergent formulations to enhance their cleaning qualities but to provide a pleasant odour to the consumer's clothes. Fragrances are occasionally used to cover the odour of other chemical ingredients contained in a product, which might be unpleasant (Rastogi, 2002). For instance, lipases leave residual odours on clothes that need fragrances in detergent products to prevent bad odours (Hasan *et al.*, 2010). Nowadays, there is a concern that certain ingredients may be harmful to human health and the environment. Some of the chemicals used in detergent formulation can cause irritants or allergens, and when released into the indoor air, they can be detrimental and contribute to air pollution (Zarogianni *et al.*, 2017). Thus, it is essential to have effective detergents to prevent microbes that are too small to be seen by the naked eye but can cause disease and illness in human beings (Abney *et al.*, 2021).

In a nutshell, detergents used in daily life may contain doubtful sources due to a lack of awareness about the ingredients used in the detergent formulations. Moreover, only certain ingredients are listed on the product label, meaning most consumers are ill-informed about it. Furthermore, if a product does not have a halal label, it is not easy to know whether or not the ingredients used are halal. For example, there are issues with enzymes added to detergent products that are not labelled as being derived from animal or plant sources. Therefore, halal and haram aspects of detergent products should be considered, especially the sources derived. Detergent products can be derived from many sources, such as plants, animals, bacteria and fungi. It is not an issue for Muslim consumers if it is taken from plant sources. However, if it is taken from animal sources, it is a dubious source. In Islam, something derived from haram animals or halal animals that are unslaughtered according to *Shari'ah* are both prohibited.

Besides, it can be harmful and dangerous to humans when it comes from a chemical that does not follow the proper process and manufacture of products. Although most chemicals are neutralised and made harmless, some remain and cause environmental pollution. These concerns are growing in the public mind and have become a serious issue among Muslim consumers. In Islam, something that can harm human beings is not allowed to be used that includes the method of *fiqh* is *al-taḥrīm yatbaʿ al-khubthu wa al-ḍarar*, which means the prohibition of things due to their impurity and harmfulness. If something brings more harm than benefit, it is also categorised as haram, and what brings more benefit than haram is considered halal(Al-Qaradawi, 2013). Therefore, Muslims need

to know more about the ingredients used in laundry detergents. They must be careful in using a product, including detergents, by ensuring the products used are halal and *toyyibān* and safe to use daily.

## 6. Halal related regulations in Malaysia

### 6.1 General requirements for laundry detergents

The production of laundry detergents is expanding in demand and supply. They are important since they can reduce the number of germs and bacteria that have been contaminated with clothes. Therefore, regulations and guidelines have been enforced to ensure that products are considered safe and effective before reaching the consumer. There is a need for manufacturing requirements from Good Manufacturing Practice (GMP), which ensures that products are consistently produced and regulated according to quality standards. Other than that, the International Standards Organization (ISO), which establishes common standards for different countries, is important in ensuring the safety, reliability, and quality of products and services.

Every manufacturer is responsible for analysing applicable regulations and ensuring the products' safety, quality, and performance before releasing them to consumers. According to SGS Group Management (2013), there are standards and regulations for detergent products in Malaysia. It includes the Consumer Protection Act 1999, which protects consumers and fills gaps in other important laws that may be insufficient to protect customers. This act stated that no person or any company should supply, propose to supply or advertise for supplying any products or services that do not conform to the safety requirements under section 19. Thus, before the products are brought to the market, every manufacturer must ensure that the product complies with Malaysian safety standards. Apart from that, a need to meet an act of product safety established in Malaysia, the Environmental Quality (Prohibition on the Use of Controlled Substances in Soap, Synthetic Detergent, and Other Cleaning Agents) Order 1995. Every product that has been manufactured, including detergents, must adhere to the guidelines established by the government to comply with government standards.

### 6.2 Halal requirements for laundry detergents

Producing halal detergents adheres to the applicable laws, standards and regulations and the halal standards. Although Malaysia does not have a halal act, the manufacturer must adhere to the requirements of the Manual Procedure for Malaysia Halal Certification-Domestic 2020 (MPPHM, 2020), which contains guidelines from the Department of Islamic Development Malaysia (JAKIM) and the States Department of Religious Affairs (JAIN), to obtain a halal certificate. The purpose of these guidelines is to clarify the requirements that must be followed in acquiring and maintaining Malaysia Halal Certification. Besides, there is a need to follow the Malaysian Standard that has described the general guidelines and basic requirements for halal products in Malaysia. The halal certification aims to provide quality indicators, assurance, and indicators of religious compliance that can assist Muslim consumers in reducing discomfort and instilling confidence in products (Rizkitysha & Hananto, 2022). Therefore, halal certification for detergents is important since they are used to clean clothes and other things that come into contact with the human body, which is intrinsically related to man's daily worship.

According to MPPHM, the standards for halal detergents were stated in Section 5 - Consumer Goods, which must ensure that products manufactured and submitted for Malaysia Halal Certification Certificates (SPHM) benefit consumers other than food and beverage products, cosmetics, pharmaceuticals and medical or products that are not have a specific certification. Among the criteria that do not have a specific certification because there are doubts about the source of manufacturing, whether the materials or ingredients used are obtained from halal or non-halal sources, or it was used as processing aids in manufacturing or manufacturing products under the Malaysian Halal Certification scheme, such as bleaching earth, alum and gas. Besides, there is a requirement to follow the MS 2200-2: 2013 Islamic Consumer Goods-Part 2: Usage of Animal Bone, Skin and Hair-General Guidelines (Malaysian Standard, 2013), MS 2565: 2014 Halal Packaging-General Guidelines (Malaysian Standards, 2014), as well as other current legislation and regulations enforced by the relevant authorities.

### 6.3 Guidelines for obtaining halal certification

The requirement for a halal certificate for a product is to certify that a product complies with the requirements and teachings of Islam for Muslims (Siala, 2013). Therefore, it is essential to check the ingredients used in a product and everything involved in making the product to obtain a halal certificate. It includes the ingredients used, processing, packaging, labelling, manufacturing, distribution, and anything related to the product. It must be free from all things that *Shari'ah* prohibits, which can cause human beings to doubt it. Thus, any product, including detergent submitted for approval for SPHM, must comply with the MPPHM 2020 (MPPHM, 2020), MS 2200-2:2013 Islamic Consumer Goods-Part 2: Usage of Animal Bone, Skin and Hair-General Guidelines (Malaysian Standard, 2013) and MS 2565:2014 Halal Packaging-General Guidelines (Malaysian Standards, 2014).

The formulation of halal detergent must begin with undoubted ingredients of the sources obtained. The raw materials or ingredients used in detergent products must be halal and safe for human consumption. Each ingredient must be able to identify the original manufacturer of ingredients. Critical ingredients such as enzymes and other chemicals are permitted if only the ingredient is certified halal. It is not recommended to use other critical ingredients with unverified halal certification status in the production of halal detergent (Sugibayashi *et al.*, 2019). Therefore, the ingredients used must adhere to the laws and regulations enacted by the Malaysian government. It must not include any ingredients that could prohibit it from obtaining a halal certificate and raise concerns for Muslim consumers. Besides that, all operations related to the preparation and processing of the materials used in the manufacture of detergents must adhere to the requirements of *Shari'ah* law, act and regulations. The processing area must be free from any *najs* or contamination, and no mixing between raw materials or products with non-halal materials or those with uncertain halal status. During the preparation, processing, handling, or storage of products, they must always remain clean and comply with Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP) and other standards.

Generally, there are no issues with the packaging of detergent products since most of the sources used to create bottles, pouches, and poly bags are halal. However, manufacturers of halal detergents must ensure that packaging materials are sourced from halal suppliers and adhere to all requirement standards. The packaging material must be halal, not

contaminating the products, and safe for human consumption. It must be carried out under hygienic and sanitary conditions. Besides, the primary packaging, including secondary and tertiary packaging, should be clearly defined with evidence of compliance with the *Shari'ah* requirement law and requirements stated in the guidelines and should not contain any other materials classified as *najs*. Then, halal detergent products must be labelled according to labelling requirements stated by the regulatory organisation of each country. In Malaysia, a halal Malaysia logo, which is a symbol to indicate the product is certified halal by JAKIM, must be labelled on the product's packaging. Labelling material that comes into direct contact with the product must be non-hazardous and from halal sourced. Labelling should not contradict the principles of *Shari'ah* law and should not highlight indecency, which contradicts *Shari'ah* law. The packaging label should contain the information required by applicable laws, regulations, and standards. On the product label, no declarations, symbols, logos, names, or images containing religious or divine elements should be used to contravene the principle of *Shari'ah* law, such as the name of God.

Besides that, the manufacturing environment must also be designed and located in an area with no risk of contamination by non-halal materials or products to avoid cross-contamination. If the manufacturing has been contaminated by *najs Al-mughallazah*, such as dogs or pigs entering the manufacturing, it must undergo mandatory ritual cleansing (sertu). All the facilities and resources must be in good condition, clean, and follow GMP requirements and other quality standards. The purpose of these standards is to ensure the quality and safety of products. Manufacturers must ensure that halal-certified and non-halal products are physically separated or located in a separate location to ensure no ambiguity about the halal status of the finished product. In addition, manufacturers engaged in Original Equipment Manufacturing (OEM) must apply and obtain Halal Malaysia Certificates under the OEM scheme before being eligible to offer product manufacturing services to other companies. Apart from the manufacturing standards, manufacturers must also have a Halal Assurance System (HAS). HAS is a procedure used by a company to maintain a comprehensive halal assurance, and it also meets the specific requirements of Malaysian Halal Certification. The Malaysian Halal Management System (MHMS) should also be developed, implemented and maintained by a company to manage products and services to maintain halal assurance through HAS.

The distribution system must ensure that halal detergent products reach the market in a halal state without being contaminated by haram materials or *najs*. It is recommended that halal products be handled and distributed separately from non-halal products to prevent them from being mixed or contaminated with non-halal things. It is because mixing haram and halal materials or products in the same place leads to *syubhah*. For example, there are leaks in detergents that can cause a mixture of halal and non-halal products. There is a *fiqh* method related to this issue, which is *itqāʾ al-syubhah khasyīah al-wuquʿ fī al-ḥaram*, meaning *syubhah* should be avoided at all costs for fear of being involved with haram things. It is because the products that have been mixed cannot be ascertained whether they are halal or haram. Thus, there is a necessity in Islam for Muslims to segregate at every stage to avoid any *syubhah* things in order to stay clear of something that is haram (Al-Qaradawi, 2013). Lastly, transportation should also be dedicated and appropriate to halal packaging and satisfy hygiene and sanitation conditions.

## 7. Conclusion

As discussed above, there are numerous detergent products available on the market. However, how many of these detergent products are genuinely halal and *ṭoyyibān* remains to be determined. Muslim consumers sincerely concerned with halal and *ṭoyyibān* products will scrutinise everything they use, including detergent products. Nowadays, many consumers are unaware of the ingredients used in detergents; compared to food, people are more aware of the ingredients used in food, whether they are good for them or not. This reality should not continue since an individual's attitude and behaviour reflect the individual's religious beliefs. This paper provides general knowledge for consumers, particularly Muslims, of the ingredients used in laundry detergents and halal-related regulations in Malaysia, which must comply with the standards and regulations established by the authorities.

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HALALSPHERE
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# Harmonising Food Safety and Friendly Service through Halal and Toyyib Principles

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**Abstract**

The widespread problem of food poisoning poses a serious threat to public health, with potentially severe consequences, including death. Food vendors need to prioritize offering halal and safe, flavorful food, ensuring excellent taste and service quality for consumers. This paper explores implementing food safety and friendly services aligned with halal and *toyyib* principles to address food safety and quality issues effectively. These principles include the physical characteristics of the product in accordance with *Shari'ah* law (P1); products that are sourced ethically and responsibly (P2); products that meet high standards of quality and safety (P3); functioning as servant and caliph of *Allah* in managing according to *Allah's* commands and prohibitions (P4); not excessively wasteful or extravagant (P5); positive moral and spiritual implications associated with the product (P6); and aimed at achieving prosperity in both this life and the hereafter (P7). This review article delves into how widespread and consequential it is to establish a structure for ensuring food safety and quality in the halal industry. By closely examining the halal and *toyyib* principles in the food sector, it becomes clear that grasping the importance of each principle is crucial for upholding the utmost standards in the halal and *toyyib* food sectors.

## 1. Introduction

In the world of food services, where tastes and aromas come together to captivate our senses, a profound connection exists between the safety of our food and the warmth of the service we receive (Desmarchelier *et al.*, 2008 & Knudsen, 2010). This harmonious interplay between food safety and the art of friendly service is the foundation of a thriving culinary experience. Food safety is a paramount concern globally, as foodborne illnesses can have severe consequences, ranging from temporary discomfort to permanent health problems, disability, and even fatalities (Gizaw, 2019). However, within this delicate equilibrium, the stakes are far from insignificant. A single miscalculation can lead to a minor stomachache and severe health consequences, casting a shadow over lives and well-being. Food poisoning poses a significant challenge to public health. Its repercussions can range from momentary discomfort to dire complications like paralysis, kidney dysfunction, chronic joint pain, and, tragically, even loss of life, as reported in 2018, with over 60 cases and two deaths (Whitworth, 2018). In a world where meals are intended to nourish and delight, the potential for such harmful outcomes demands our unwavering attention and immediate action (Roger & Oyelakin, 2020). In the pursuit of ensuring the well-being of consumers, it is crucial that food vendors not only provide safe products but are also aligned with ethical, cultural, and spiritual values. In the context of Islamic dietary practices, this involves adhering to halal and *toyyib* principles.

In Arabic, the word "halal" signifies "permissible" or "lawful" in terms of Islamic law. Muslims adhere to a set of rules known as halal daily. In contrast, *toyyib* denotes something that is not only halal but also wholesome, pure, and good for one's health. The concepts of halal and *toyyib* are addressed in numerous verses of the Qur'an, and they are not limited to just food physically but also apply to other aspects of life, including services. For example, Surah al-Baqarah, verse 168, states, "O people, eat from whatever is on earth [that is] lawful and good and do not follow *Satan's* path. He is, in fact, a blatant enemy to you". This verse conveys the message that people should consume what is both lawful and good while avoiding the path of Satan. The verse encourages adhering to what is not only permissible but also morally upright and nourishing. These concepts are central to Islamic ethics and guide Muslims in making choices that align with legal and ethical standards. The halal and *toyyib* concepts emphasise the importance of adhering to religious laws and seeking what is beneficial and virtuous in their daily lives, fostering a holistic approach to living according to Islamic principles, as Tsani *et al.* (2021) mentioned.

This paper explores the implementation of food safety and friendly services, the concept introduced in the next section through halal and *toyyib* principles, that create a harmonious

balance between meeting health standards and adhering to cultural and spiritual values. It seeks to address the multifaceted challenges posed by food safety issues by combining these principles' physical, spiritual, material, and supernatural dimensions. Doing so enhances the safety and quality of food products and fosters a deep sense of responsibility towards consumers and society.

### 1.1 The concept of food safety

According to the Department of Food Safety and Quality Division, Ministry of Health, Malaysia, "safe food" must be free from harmful bacteria, poison, toxins, faecal and other waste matter. A food safety assurance program is defined as a planned and documented system of practices which assure that any particular type of food will not cause harm to a consumer when it is consumed, as stated in the Food Hygiene Regulations 2009 (Ministry of Health, 2015). Consumers' demand for and preferences regarding food safety has gradually changed due to rising incomes and urbanisation, and consumers are now paying more attention to it (Joya *et al.,* 2022). It is further stated that consumers are now looking for food that offers several aspects, including safety, quality and healthy features, and not only serving basic dietary needs.

The limitation of knowledge on food safety is linked with poor food-handling practices, which are more likely to occur in mass catering and collective dining, especially food prepared by food handlers (Sani & Siow, 2014). Foodborne illness, hospitalisation, fatality, and financial losses are consequent outcomes of consuming foods contaminated with foodborne pathogenic bacteria, all of which have a negative impact on public health (Subramaniam *et al*., 2023). To address these challenges, Malaysia introduced the Hazard Analysis and Critical Control Point (HACCP) certification scheme in April 2001, followed by implementing the Food Hygiene Regulations in 2009. The Malaysian Ministry of Health took these measures to protect consumers from foodborne illnesses and ensure they access safe, high-quality food. Consequently, individuals who own or operate food premises, as well as those who handle and cater food, are now legally obligated to adhere to stringent hygiene standards to guarantee the safety of the food they provide to consumers (Petruzzelli *et al*., 2018). Furthermore, food operators must establish procedures to assess the effectiveness of their food management systems, including proper storage and handling of raw materials and the production process (McFarland *et al*., 2019).

All individuals, particularly food service providers, are responsible for providing clean and hygienic food. The halal and *toyyib* aspects also offer a holistic idea, emphasising goodness in every aspect, especially regarding food preparation (Shafiee *et al*., 2017). However, the halal concept goes beyond the certification process, which encompasses the ideas of cleanliness, hygiene, and all-encompassing safety, or halal and *toyyib*, as emphasised by the *Shari'ah* principles. Thus, consuming halal food is becoming more and more important because it is associated with quality, cleanliness, and safety. Halal is almost always associated with how Muslims slaughter farm animals for food (Aliff *et al*., 2015). For instance, when a chicken has been cut into pieces, it is impossible to trace that the poultry has not been properly slaughtered, and consequently, eating that chicken is haram (prohibited) for Muslims. Aside from establishing procedures to ensure that food management systems are effective, food operators must also consider how raw materials and finished goods are handled (Petruzzelli *et al*., 2018). Food is prohibited from being eaten by Muslims due to three main aspects: what it is made of, how it is obtained, and how it is processed (Dewi & Agustina, 2021).

Aside from establishing procedures to ensure that food management systems are effective, food operators must also consider how raw materials and finished goods are stored (Petruzzelli *et al*., 2018). Food must be stored at the proper storage temperature, which is categorised into three different temperature types which are room temperature storage (25 to 35ºC), frozen storage (-18ºC) and Chill Storage (0 to 4ºC) (Ministry of Health, 2015). According to the Food Handling Training Module by the Malaysia Ministry of Health (2015), all perishable foods such as fish, chicken, and meat need to be stored in Frozen Storage; semi-perishable food such as vegetables and fruit can be stored in the Chill Storage, and non-perishable food together with dry food can be stored at Room Temperature. For the cook food category, different types of storage will affect the duration of the food's shelf life. Cook food stored at room temperature can only be consumed within 4 hours; in the chiller, it can be stored up to a maximum of 3 days; in the freezer, it can be stored up to one week to 3 months, depending on the type of cooked food. To prevent cross-contamination, raw meat should also be stored in separate bags from things ready to eat because it is a crucial step for meat storage (Ehuwa *et al*., 2021). It is further stated that when handling raw meat, hands should be washed both before and after. Before consumption, all types of meat must be cooked to the right temperature to kill any pathogens and cooking a whole chicken should take place at 180°C for 20 minutes. Improper manufacturing procedure control, such as temperature regulation and storage conditions, were found to be important outbreak-causing factors (Food Safety News, 2022).

In conclusion, food safety encompasses two fundamental aspects: the safety and quality of sources, which include raw materials and ingredients, and the safety of the processing methods employed in the food management systems. This multifaceted approach to food safety ensures that the end product meets basic dietary needs and aligns with evolving consumer demands for safety, quality, and health-conscious features. The changing consumer preferences, driven by rising incomes and urbanisation, have highlighted the importance of providing food that is nourishing and free from potential hazards.

### 1.2 The concept of friendly service

The food industry is not only limited to the entities that are doing business, institutions or food and beverages (F&B) companies but also includes all the food operators in restaurants, schools, hospital cafeterias, catering operations and many other places that prepare food production and food service including the catering industry which is one of the food industry categories. The halal food industry is crucial to Muslims worldwide as it gives them a sense of security that whatever they consume, use, and purchase is *Shari'ah* compliant and lawful for consumption. Simultaneously, the halal industry contributes to societal development and national economic growth (Bohari *et al*., 2013).

Food service is also an important element embedded in friendly service, where it refers to the various components or aspects involved in providing food and beverage-related services to customers that are crucial for ensuring a seamless and satisfying dining experience. Food service encompasses menu planning, food preparation, service provided by staff, ambience and atmosphere of the restaurant, table setting, hygiene and food safety, customer service, timing and efficiency (DiPietro,

2017). People today prefer innovative food and beverage service that can be enjoyable, attractive, and presentable; they also want the physical environment, which includes good customer service, ambience, table setting, and lighting, to appeal to guests (Yashwant Singh Rawal, 2019). A key aspect of the food service industry is providing excellent customer service, which includes interacting with customers in a friendly and attentive way, accurately taking orders, addressing any issues or special requests, and guaranteeing overall customer satisfaction (Kanyan *et al.*, 2016).

Delivering exceptional customer service is a core aspect of the food service industry, involving friendly interactions, precise order handling, issue resolution, and ensuring overall contentment. Key components encompass timely greetings, knowledgeable staff, efficiency, personalisation, resolution of concerns, hygiene, and a welcoming ambience. These practices and showing appreciation and follow-up gestures contribute to a lasting positive impact. Such customer-centric approaches are essential for garnering satisfaction and fostering loyalty (Stranjančević, 2015). Factors like amiable staff, professionalism, service speed, food quality, and atmosphere are crucial in elevating customer service, which is achievable through warm greetings and addressing concerns (Schechter, 1994). Service quality dimensions, such as tangibility, reliability, empathy, assurance, and responsiveness, significantly influence customer satisfaction in fast-food establishments (Aftab, 2016). Customer preferences centre on taste, server friendliness, hygiene, and prompt food service, underscoring the significance of these factors in crafting a positive dining experience (Molitor, 1995).

In conclusion, the term "friendly services" within the food industry encompasses various practices focused on customer satisfaction, such as creating a welcoming environment, handling orders efficiently, and resolving issues promptly. This customer-focused approach is essential for building loyalty and ensuring positive dining experiences. Critical elements like friendly staff, professionalism, swift service, food quality, and a pleasant ambience are vital in enhancing customer service. Going beyond basic needs, friendly services strive to make customers feel valued and attended to, contributing to positive recommendations and customer loyalty. The flexibility of these services in catering to diverse customer preferences is notable, and in the context of the halal food industry, it takes on heightened importance. The halal aspect holds significance for Muslims globally, assuring them that the food aligns with Islamic dietary laws. Businesses emphasising halal options can access a substantial market while contributing to the overall prosperity of food-related enterprises.

## 2. Harmonising halal and *toyyib* principles for food safety and friendly service in the food service sector

The halal food service industry is booming in Malaysia due to the public's acceptance of halal food, which is good for business development (Yusuf & Oyelakin, 2020). The halal food service industry in Malaysia is expanding because the demand is high based on the Muslim population in the country (Kamarulzaman *et al.*, 2017), the working schedule of her workforce, and her contemporary lifestyle. Ab Hamid *et al.* (2017) stated that the fast growth demonstrated that the Malaysian halal business was well appreciated both domestically and internationally and given the rise in population around the world and the industry's acceptance by the non-Muslim community, thus presenting a range of opportunities for companies to produce goods and services, especially in the halal sector (Ab Hamid *et al.*,2017; Razak, 2023).

Moreover, non-Muslim customers are also seen to slowly accept halal certified eateries, including restaurants, cafes and other food outlets, because a halal-certified eatery provides an image of good hygiene practices and a well-managed food premise. Even in Malaysia, policymakers put several certifications, standards, and restrictions on the food industry to ensure food quality and safety (Ali *et al.*, 2022). Hence, the halal-certified status is also seen as a competitive advantage in the food service business. The trend of halal applications submitted by food manufacturers and operators nationwide is increasing due to the demand and popularity of halal food products. According to Yunos *et al.* (2017), in the context of the food sector, halal certification is related to an inspection of many elements, which includes the preparation, slaughtering, ingredients used, cleaning, handling, processing, and storage of food, as well as the transportation and distribution of it. The food can only be permitted to have the halal certification after it has been deemed nutritious and cooked using just permitted ingredients in a clean, hygienic manner. Halal certification is an example of an industrial standard practice and an instrument for coordination and quality standards.

In food service, halal refers to what is permissible to be served and consumed according to Islamic dietary laws, while *toyyib* refers to what is wholesome, nutritious, and good for the body and soul. Halal and *toyyib* principles apply to the entire process of food service, from preparation to presentation (Arifin *et al.*, 2021; Ibrahim & Othman, 2014). For example, restaurants and food providers must adhere to halal and *toyyib* practices when sourcing and preparing food. This includes using permissible ingredients, utensils, and cooking methods in accordance with Islamic guidelines. In the Qur'an, such as Surah Al-Baqarah, verse 168, the directive is clear: "O humanity! Eat from what is lawful and good on the earth, and do not follow *Satan's* footsteps. He is truly your sworn enemy." This verse emphasises the importance of cleanliness and purity, ensuring that the food served is halal and *toyyib*, meaning it should be of high quality, free from contamination, and prepared hygienically.

In addition, the principles of halal and *toyyib* extend to the treatment of customers and employees in the food service industry (Perdana, 2020). Providing excellent customer service and creating a welcoming and respectful environment for all patrons is essential. This aligns with the broader concept of halal and *toyyib*, emphasising ethical and wholesome practices in all aspects of life, including food service. By following these principles in the food service context, businesses can cater to Muslim customers' dietary needs and preferences, ensuring that the food is halal and of the highest quality, promoting physical well-being and spiritual fulfilment.

One of the studies on the application of the halal and *toyyib* principles in the food industry was conducted by Norazilawati Md Dahlal (2017 & 2021). The study established an ideal framework for food quality management based on the halal and *toyyib* principles, which consisted of seven basic principles. These principles were formulated by systematically researching selected Qur'anic verses related to the concepts of halal and *toyyib*. The selected Qur'anic verses were analysed and categorised according to a specific theme and supported by prominent *tafseers* (*Tafsir al-Azhar, Tafsir Ibn Kathir,* and *Tafsir al-Maraghiy*). The established seven principles consider all aspects of the physical and spiritual, as well as material and supernatural elements. These principles can be applied across

all industries, including the food service industry, to ensure their implementation aligns with halal and *toyyib*.

This paper uses the seven established principles of halal and *toyyib* as guidelines in food service. These seven halal and *toyyib* principles help to navigate the tangible aspects of the food service sector (referring to the physical and material) as a tool to safeguard the intangible aspects - referring to the *habl min Allah* (man's relationship with his Creator) while strengthening and enhancing the *habl min al-Naas* (man's relationship with other creatures including other human beings and nature), especially in the food safety and friendly service aspects. These tangible and intangible aspects can be incorporated into food service practices when the seven halal and *toyyib* principles are incorporated into food safety and friendly service. When these principles are embedded into the food service industry, there will be a significant impact on customers' trust and the quality of services offered, not just in tangible factors such as food preparation methods and quality control procedures but also in intangible factors such as ethics and morality. Figure 2 illustrates the seven principles of halal and *toyyib* that are posited to be used in the food service industry to ensure high-quality and spiritually wholesome food.

### 2.1 Principle 1 - Physical (tangible) characteristics of a product to be in accordance with Shari'ah law

The first principle of halal and *toyyib* focuses on the physical (tangible) aspects. For the food service industry, aligning food safety principles and friendly service to comply with *Shari'ah* or Islamic law is very important. *Shari'ah* law imposes specific requirements on how food is sourced, handled, and prepared to meet Islamic dietary guidelines. Regarding food safety, this entails diligently sourcing all raw materials and ingredients from halal-certified suppliers, ensuring no haram elements are present in the products and adopting halal-compliant processing methods (Kohilavani *et al.* 2013). Transparency is key, with clear labelling of halal menu items and rigorous periodic audits to maintain compliance. Concurrently, the concept of friendly service encompasses aspects like diverse menu planning, rigorous staff training in halal principles and halal food preparations, a welcoming ambience reflecting Islamic values, the use of clean and halal-certified tableware, a strong emphasis on hygiene, and the provisions for excellent customer service adhering to Islamic principles of respect and courtesy (Putit *et al.* 2016). Transparency is key, with clear labelling of halal menu items and rigorous periodic audits to maintain compliance. Concurrently, the concept of friendly service encompasses aspects like diverse menu planning, rigorous staff training in halal principles and halal food preparations, a welcoming ambience reflecting Islamic values, the use of clean and halal-certified tableware, a strong emphasis on hygiene, and the provisions for excellent customer service adhering to Islamic principles of respect and courtesy (Putit *et al.* 2016).

### 2.2 Principle 2 - Products that are sourced ethically and responsibly

The second principle of halal and *toyyib* is focused on how food sources are obtained. This principle is fixated on ethical and responsible sourcing practices for the food safety and friendly service model, which is essential for creating a positive dining experience. The halal and *toyyib* principle ensures that the production process of the item meets high ethical standards (Arifin *et al.* 2021). Food safety involves ensuring that raw materials and ingredients come from suppliers, adhering to fair labour practices, sustainable farming, and animal welfare. Responsible farming and harvesting practices enhance food safety while aligning with environmental and ethical concerns. Transparency and traceability in sourcing highlight

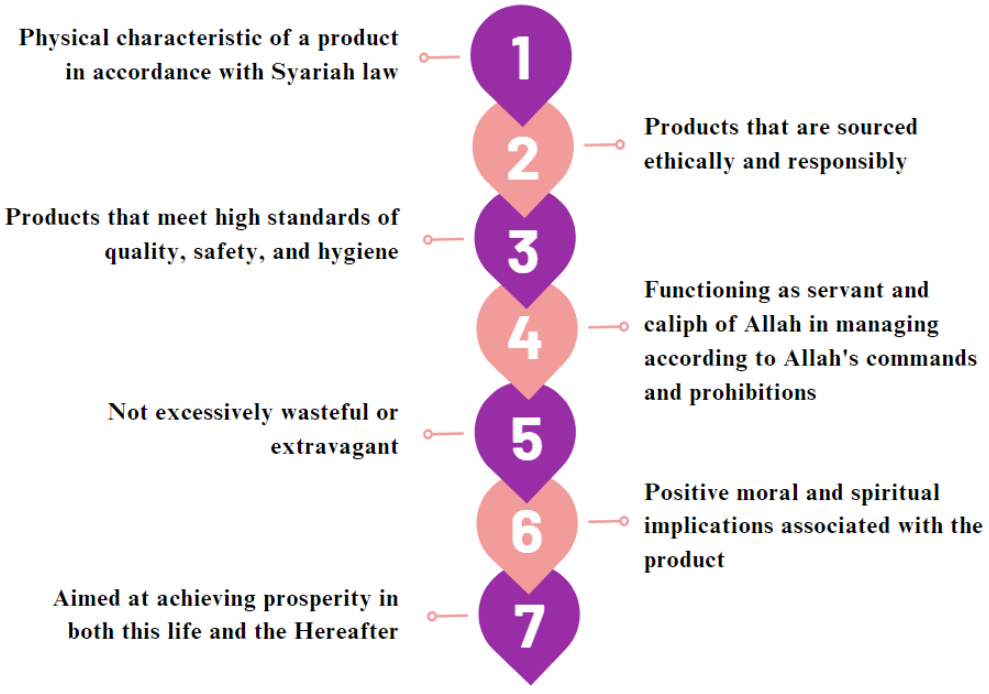


Figure 2: The seven principles of halal and *toyyib*.
Source: Norazilawati Md Dahlal (2017 & 2021)

accountability to customers (Ospital *et al.*, 2022). The process from the beginning to the end of the food products created is in facilities and business models centred on moral duty prior to profit. It is concerned with the facilities, how the product is produced, and even the employees' salaries, which reflect an ethical and responsible standard of production (Scheik, 2021). In the realm of friendly service, integrating halal and *toyyib* principles is vital for addressing both tangible and intangible aspects. Through ethical and responsible sourcing and prioritising local, organic, and ethically produced ingredients, businesses can enhance their commitment to fairness and sustainability in alignment with Islamic principles. This aligns with findings from various studies, such as Sharma (2021), which emphasises the food service system's autonomy, justice, and well-being, whereas Lesser (2013) emphasises the significance of wholesome selections. Guachalla (2022) argues that plant-based diets should be promoted to address ethical and environmental issues. Robson (2011) highlights that personal space should be considered while arranging a table, particularly in various dining situations. According to this principle, the comprehensive approach to providing friendly service should consider ethical, health, and comfort considerations. The staff adeptly communicates ethical choices and fosters customer loyalty rooted in shared values, contributing to the sustained success of businesses in the halal and *toyyib* food sectors.

### 2.3 Principle 3 - Products that meet high quality, safety, and hygiene standards

The food service industry's third principle of halal and *toyyib* emphasises ensuring that products meet high quality, safety, and hygiene standards. Incorporating this principle into the food safety and friendly service model is indispensable for an outstanding dining experience. When it comes to food safety, the process starts with carefully selecting raw materials and ingredients from reliable vendors known for their commitment to quality and safety. Rigorous inspection procedures upon receipt are imperative to verify the products' adherence to quality criteria and ensure freedom from defects or contaminants (Alli, 1990). Equally crucial is the proper handling and storage of ingredients to prevent contamination and preserve freshness. It is crucial to uphold hygienic processing practices throughout the food management system, including strictly cleaning kitchen tools and utensils (Ebert, 2018). Routine quality checks maintain consistency and safety throughout the food preparation process.

Regarding friendly service, excellence begins with crafting a menu emphasising top-notch ingredients, safety, and hygiene. The menu should prominently feature dishes that meet these standards and showcase the freshness and excellence of the products used, with clear communication of this commitment to customers (Filimonau *et al.* 2017). Food preparation by a well-trained culinary team should prioritise precision and care, emphasising the use of superior ingredients to highlight their flavours and textures. The overall dining experience is elevated through a thoughtfully designed ambience and impeccable presentation of clean and hygienic tableware (Nazri *et al.* 2022). This commitment extends to fostering a hygiene culture throughout the establishment, including dining areas and common spaces. Finally, staff should be trained to provide attentive and informed customer service that includes explaining the measures taken to ensure quality, safety, and hygiene in food preparation (West, 1992). This comprehensive approach not only assures customer safety and satisfaction but also establishes a reputation for excellence, fostering customer loyalty and positive recommendations, all essential for the long-term success of any food-related business, especially halal and *toyyib*.

### 2.4 Principle 4 - Functioning as servant and caliph of *Allah* in managing according to *Allah's* commands and prohibitions

The fourth principle of halal and *toyyib* emphasises the operational mechanism of the food service industry, paying close attention to the actors in this industry. The actors here refer to the policymakers, implementers, practitioners, and consumers directly or indirectly involved in the food service industry. In terms of the operational mechanisms, how these actors play their role in the food industry is crucial. Based on this fourth principle, the actors are not to merely provide the service in accordance with the halal and *toyyib* standards but, more importantly, to be able to deliver their purpose in life as servants of their Creator and fulfil his duty as the caliph of *Allah* throughout his existence on this earth (Fatimah, Ahmad EQ & Suhartini, 2020). This encompasses a comprehensive approach to operating a food service premise that sets the importance of managing food safety and friendly service according to Allah's commands and prohibitions whilst firmly grasping Islamic values. It posits food service as a tool for humans, whether in the capacity as consumers or food producers or regulators as the actors in the industry, to enhance their purpose as a servant of *Allah SWT*, simultaneously fulfilling their role as caliph of *Allah SWT* (Muhammad Syukri Salleh, 2003 & Fadzila Azni Ahmad, 2016, Norazilawati Md Dahlal, 2017). Food safety entails strict adherence to halal requirements, including sourcing and preparing ingredients in compliance with Islamic dietary guidelines and using certified halal processes and equipment. It is crucial to stay away from prohibited substances like alcohol and pork. There is a deep conviction that *Allah* sees and knows all outward and inward actions, even when only the self is making preparations without all others. It is a tool that enhances man's *habl min Allah* (vertical relationship) with his Creator.

Regarding friendly service, this principle encompasses not only the provisions of halal options but also the creation of a welcoming ambience that reflects Islamic modesty and respect. Maintaining impeccable cleanliness and hygiene, as emphasised in Islamic practices, is crucial. Additionally, staff are trained to deliver exceptional customer service that aligns with Islamic principles of respect and courtesy, treating patrons as honoured guests. Incorporating charitable practices, such as donating to causes in accordance with *zakat*, further demonstrates a commitment to community and ethical stewardship. It also aims to enhance and strengthen the *habl min al-Naas* (horizontal relationship). This holistic approach ensures compliance with Islamic dietary guidelines. It fosters an environment that resonates with patrons who share these values, ultimately enhancing customer loyalty and promoting the success and reputation of the food-related business within the Muslim community and beyond.

### 2.5 Principle 5 - Not excessively wasteful or extravagant

Integrating the principle of avoiding excess waste and extravagance into the food safety and friendly service model embodies responsible and ethical practices within the food service industry. Food safety involves minimising food waste through meticulous inventory management, portion control, and innovative repurposing of surplus ingredients to curb unnecessary disposal. It extends to sourcing from suppliers

products that prioritise sustainability and eco-friendly packaging, thus reducing environmental impact (Bouhlel *et al.*, 2023). The principle also encompasses optimising energy usage in food preparation and storage, lowering operational costs, and reducing environmental footprints.

Within friendly service, the commitment to avoid waste and extravagance is evident in menu planning strategies that harness ingredients across multiple dishes, reducing plate waste and offering smaller portions or shareable options. Kitchen staff are trained to execute efficient food preparation techniques, preventing overproduction and ensuring precise portioning to curtail leftovers (Hennchen, 2021). This fifth principle provides reminders that those who indulge in wastefulness are the brethren of man's common enemy, *Satan*. Thus, eco-conscious choices are made in decor and table settings, favouring reusable or biodegradable materials that minimise waste and environmental harm as efforts to steer away from the common enemy. Hygiene and cleaning practices incorporate eco-friendly products to maintain stringent standards while reducing harmful chemicals (Lehman, 2023). Customer education initiatives inform patrons of the dedication to waste reduction and encourage responsible ordering, potentially offering take-home containers to minimise food waste further. The principle also advocates resource conservation, including water-saving measures, recycling programs, and reducing single-use plastics.

Adhering to the principle of avoiding excess waste and extravagance diminishes the environmental impact of food service operations and underscores the commitment to ethical and sustainable practices. This resonates with environmentally conscious customers, elevating their reputation and attracting those who value responsible resource management. Furthermore, reducing waste translates into cost savings, presenting a mutually beneficial outcome for the halal and *toyyib* food service business and the environment.

### 2.6 Principle 6 - Positive moral and spiritual implications associated with the product

The sixth principle of halal and *toyyib* underscores the significance of positive moral and spiritual implications associated with safe, high-quality food products and friendly services. Choosing ethically produced and safe food ensures physical well-being and moral satisfaction, elevating the taste experience and influencing purchasing decisions (Bratanova, 2015). As observed by Kasingku (2023), nutritious food positively affects both physical health and spiritual development. The link between adherence to Islamic dietary principles and human development, encompassing intellect, morals, and psychology, is emphasised, with halal and *toyyib* eating contributing to a pure and clean heart (Metusin, 2020; Sawari, 2015). Moreover, halal and *toyyib* food consumption influences emotional and spiritual intelligence, fostering a healthy lifestyle and positive human growth (Farid, 2020).

Regarding friendly services, embracing halal *toyyib*-friendly practices, such as clear menu labelling, cultural sensitivity, and community engagement, enhances the dining experience, promoting customer satisfaction, loyalty, and positive word-of-mouth (Shafieizadeh & Tao, 2020). These practices benefit the halal sector and nurture a harmonious relationship between food operators and consumers, emphasising good morals and health. Additionally, halal *toyyib*-friendly services have been shown to evoke positive emotions and spiritual experiences, influencing satisfaction, trust, and loyalty among customers (Tama, 2014; Al-Ansi, 2019). The comprehensive impact of halal and *toyyib* principles on physical and spiritual dimensions highlights the significance of ethical food practices and customer-oriented services in the food service industry.

### 2.7 Principle 7 aims to achieve prosperity in this life and the hereafter

The seventh principle within the halal food service sector is to attain prosperity in this life and the hereafter, embodying a comprehensive and spiritually mindful approach. Aligned with the Islamic notion of success in worldly and hereafter endeavours (Al-Haq, 2023), the industry aligns with believers' aspirations by providing outstanding, high-quality food and friendly services. As a guide to behaviour, Islam instructs believers to lead successful lives through balanced and moderate social principles (Nasir, 2020). Emphasising meaningfulness and continuous improvement for a good quality of life (Qadir & Ghauri, 2021; Al-Haq, 2023), Islam encourages believers to seek well-being in both physical and spiritual dimensions, paralleling the approach to crafting quality food and services. This entails conducting business with integrity, ethical standards, and kindness towards customers and colleagues (Jelaini, 2014). Integrating faith into professional goals nurtures fulfilment and purpose for food operators and consumers, fostering a harmonious personal and spiritual growth journey. Essentially, this comprehensive approach within the food service industry embodies a life well-lived, aligning with the values of *Allah* and positively contributing to both temporal and eternal aspects of existence.

## 3. Conclusion

The halal and *toyyib* principles underscore the significance of ethical, moral, and sustainable practices, particularly in the food service industry. Embracing these ecologically sustainable concepts offers numerous benefits for food service providers, society, and the environment, fostering overall well-being. These principles enhance well-being across various dimensions such as economics, environment, society, health, and culture, with their effectiveness hinging on industry stakeholders' adept implementation of the seven halal and *toyyib* principles. The concept of *toyyib*, extending beyond religious contexts, highlights the importance of quality, wholesomeness, and ethical considerations in food production and service. In today's conscious consumer landscape, incorporating halal and *toyyib* principles into customer-friendly services is essential and a strategic move for long-term success and expansion in the food industry. This signifies a dedication to meeting the diverse needs of customers while upholding ethical and cultural values, thereby contributing to both societal advancement and economic growth.

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