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PROXIMATE ANALYSIS AND SHELF-LIFE STUDIES OF YOGHURT CHIA (*SALVIA HISPANICA L.*) PUDDING

Noranisa Zainal Abidin[1], Siti Jameelah Md Japilus[3], Ahmad Fikri Kassim[3], Syafiq Miswan[3], Hisham Mohd Nooh[1,2*]

[1]*Department of Food Science and Technology, Faculty of Applied Sciences,*
[2]*Alliance of Research and Innovation for Food (ARIF), Faculty of Applied Sciences,*
Universiti Teknologi MARA (UiTM), Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000 Kuala Pilah, Negeri Sembilan, Malaysia.
[3]*Faculty of Sports Science and Recreation,*
Universiti Teknologi MARA (UiTM), Cawangan Perlis, Kampus Arau, 02600 Arau, Perlis, Malaysia.

[*]*Corresponding author: hishamnooh@uitm.edu.my*

Abstract

Consuming *Salvia hispanica L.*, also known as chia seed, has been linked to studies that suggest positive impacts on individual health outcomes. This study was sought to examine the proximate composition and energy value, as well as the shelf life the formulated yoghurt chia pudding in different storage temperatures during 20 days of storage. The sample was evaluated for proximate analysis (moisture, protein, fat, ash, fibre, and carbohydrate) and the energy value using method of drying method, Kjeldahl, Soxhlet, dry ashing, acid and alkali digestion, and the arithmetic difference method. The shelf-life analysis was also evaluated for the effects of the storage temperature on the microbial analysis and pH across 20 days. The results obtained from this study indicated that the formulated yoghurt chia pudding has an energy value of 134.95 Kcal/100g and contained high fibre, carbohydrate, protein, and fat content with a value of 9.01%, 9.85%, 6.06%, and 7.92%, respectively, which were primarily imparted by the chia seeds' excellent nutritional composition. The lower count level of mould and yeast (CFU/mL) recorded for the yoghurt chia pudding sample in both chilled and frozen temperature throughout the storage period signifies the antimicrobial properties which shows a zone of inhibition of 8 mm diameter against the gram-positive bacteria (*S. aureus*) but less effects on the gram-negative bacteria (*E. coli*). For all storage temperatures, a gradual fall in the pH of yoghurt chia pudding over time can be observed. To sum up, the study demonstrated that chia seeds improve nutritional value, shelf life, and also possess antimicrobial properties. Thus, the consumption of yoghurt chia pudding as a healthy snack or meal alternative may support satiety or a sense of fullness, enhance digestive health, and offer sustained energy, making it a perfect choice for active people or a quick healthy snack for everyone.

Keywords: Yoghurt chia pudding, proximate analysis, shelf-life study, antimicrobial

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Introduction

The pursuit of novel food products is a prevalent activity and has risen in demand nowadays. In addition, due to increase of urbanisation and changes in lifestyle in today's fast-paced world, there is a growing desire for instant food, natural, and nutritious food products (Sharma et al., 2020; Singh et al., 2020). Known as one of nature's superfoods, chia seeds (CS) which helps to increase satiety level, low in calories, and may be customised in flavour (Many & Sarasvathi, 2016). Chia, also scientifically referred to as *Salvia hispanica*, is a flowering plant that belongs to the Lamiaceae family and is native to Central and Southern Mexico as well as Northern Guatemala (Sharma et al., 2020). Studies have proven that CS are loaded with natural antioxidants including tocopherols, carotenoids, sterols, and phenolic compounds. In addition, the seeds offer high level of protein (19-23%) than other traditional crops like wheat, rice, corn, and barley (Dumbrava et al., 2021; Romankiewicz et al., 2017). CS is also a favoured and captivating option for healthy food products due to the relatively low level of saturated fatty acids

contents namely palmitic acids and stearic acids, sufficient concentration of linoleic acid ω-6 (18-20%), and high proportion of alpha-linolenic acid ω-3 (55-60%) (Cardenas et al., 2018). Due to many health benefits that being offered, recent recognition of CS as a novel food by the European Parliament has led to a surge in its use in food manufacturing (Romankiewicz et al., 2017).

Incorporation of chia seeds in pudding recipes enhances its nutritional value and gives the thickening effect as a substitute of corn starch. CS immersed in water forming the slimy gel (chia mucilage) as shown in Figure 1. This chia mucilage can be developed even at a very low concentration has excellent water binding capacity which also enhancing viscosity and emulsion activity possessed an enormous potential for use in the development of food products as a thickening, emulsifier, and stabiliser (Kibui et al., 2018; Many & Sarasvathi, 2016). However, there are limited studies that utilizing CS as an ingredient in the production of pudding. Therefore, there is a need to conduct the proximate analysis of the formulated yoghurt chia pudding sample to provide insights to the consumers on the nutritional content of the yoghurt chia pudding sample that offers a diverse range of health benefits from the incorporation of the CS in the pudding production. Furthermore, analysing the shelf life of the yoghurt chia pudding sample through the microbial analysis and the pH changes during storage period aids to establish the recommended storage duration and conditions for maintain the product's safety and quality.

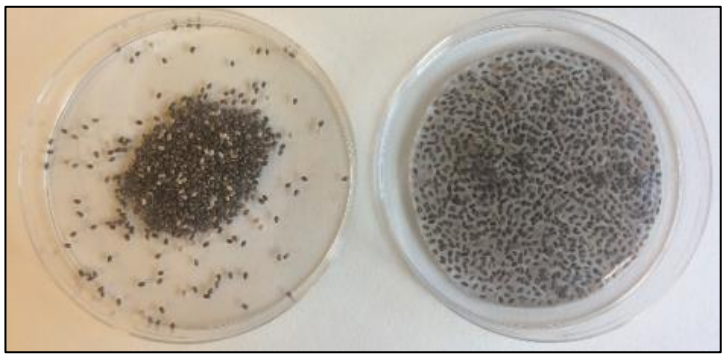

Figure 1. Picture of the formation of chia mucilage when water being absorbed (Castejón et al., 2017).

Methods

Preparation method of yoghurt chia pudding sample

Approximately 30 g of CS was soaked in a small quantity of hot water in a bowl and was then being let to sit for about 5 min to hydrate the CS more quickly. The other ingredients in the formulation of yoghurt chia pudding including vanilla essence (5 ml), honey (30 g) and sea salt (0.3 g) were added into the bowl containing hydrated CS. Then, about 180 g of Greek yoghurt and 180 ml full cream milk were also be added into the bowl and were thoroughly mixed. The mixture was divided into 13 small jars and were kept in the refrigerator at chilled (5 jars), frozen (5 jars) temperature and at ambient temperature (3 jars). The sample was taken for analysis in every 4 days intervals for 20 days (chilled and frozen). The yoghurt chia pudding will be spoiled at room temperature in the third day of analysis. Hence, no further data was collected after 3 consecutive days. The formulation of the yoghurt chia pudding was presented in Table 1. The amount of CS used in the formulation was 7.05% which was similar to the previous study by Ayaz et al. (2017) as to match the European Union decision which approved the recommended dosage intake up to 15 g daily (Ayaz et al., 2017). Figure 2 shows the experimental work of study which includes the nutritional composition and the shelf-life study of the yoghurt chia pudding

Table 1. Formulation of yoghurt chia pudding

Ingredient	Percentage (% w/v)
Chia seed	7.05
Greek yoghurt	42.32
Full cream milk	42.32
Honey	7.05
Vanilla essence	1.18
Sea salt	0.07

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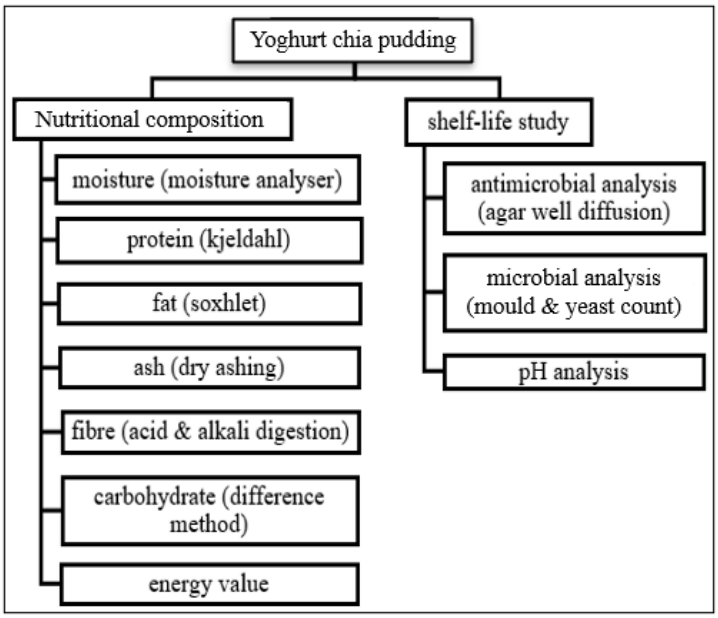

Figure 2. Experimental work of study

Moisture content determination by using moisture analyser

This analysis on moisture determination using the moisture analyser (Model PMB 53, Adam, UK) was based on the procedure obtained from (Bujang et al., 2017; Nielsen, 2010). Firstly, the empty sample pan was placed in the pan handler and the tare button was pressed to set reading to zero. Then, approximately 1 g of the yoghurt chia pudding sample was placed and being distributed evenly on an aluminium pan. The test sample was allowed to elevate to a constant temperature (150 °C) for 13 minutes. The instrument weighed and displayed the moisture percentage automatically as the moisture is driven from the sample at the end of the drying process.

Crude protein content determination by using Kjeldahl method

The procedure of the determination of the crude protein in yoghurt chia pudding sample was based on the method by Bujang et al. (2017) which includes digestion, distillation, and titration method. Approximately, 1 g of sample was weighed into the digestion tube. Two pills of the catalyst mixture (5 g potassium sulphate + 5 mg selenium) and 30 mL of concentrated sulphuric acid were added into the tube. The digestion unit (protein analyser Model Turbosog, Gerhardt) was then switched on and the yoghurt chia pudding sample was allowed to digest until the samples obtained clear with no charred material remained. Finally, the sample was taken off the digestion block and allowed to be cooled before proceeding with the distillation method. Then, the receiving flask was filled in with 60 mL of 2% boric acid and 3 drops of screened methyl red as an indicator. Approximately, 70 ml distilled water was added to the digestion tube following by the addition of 50 ml of 40% NaOH to the digestion tube by pressing the NaOH button until desired volume being achieved. The steam switch was turned on to start distillation process and about 200 ml of the distillate was collected at the end of the distillation process. For the titration part, the normality of the standardize solution was recorded prior to titration. Then, the

contents of the receiver flask were then being titrated with 0.1 M HCl. Lastly, the volume of HCl used for sample and blank were recorded. The following formulas (Equation 1) were used to calculate the crude protein content in the food sample.

$$\text{Total nitrogen (g)per 100 g food sample} = \frac{(\text{titre}-\text{blank})\times 1.4 \times 100}{1000 \times \text{ sample weight (g)}} \text{ x } 100 \quad \text{Equation 1}$$

Total protein (g) per 100 g food sample = total nitrogen x factor for foodstuff analysed

Fat content determination by using Soxhlet extraction method

The determination of crude fat was performed according to the method of (Bujang et al., 2017). Approximately, 2 g of pre-dried yoghurt chia pudding sample was weighed into a pre-dried extraction thimble and then was covered loosely with cotton. The thimble was then placed into a Soxhlet extractor. A dried 250 mL round bottom flask was weighed accurately, and 150 mL of petroleum ether was added. The boiling flask, Soxhlet apparatus, and the condenser were assembled, and water was then allowed to flow into the condenser. The extraction process was conducted for a minimum of 8 hours on an electrothermal extraction unit. After the extraction has completed, the flask containing the petroleum ether extract was removed. The petroleum ether was evaporated off on a boiling water bath (Model LAB-133D, Labtech). The flask was then being transferred into a drying oven (Model SFCN-302, Finetech) to dry the extract at 105 °C. After an hour, the flask was transferred immediately into a desiccator to cool prior to weighing. Equation 2 was used to estimate the fat content.

$$\% \text{ Fat in sample} = \frac{\text{weight of fat in sample (g)}}{\text{weight of sample taken (g)}} \text{ x } 100 \quad \text{Equation 2}$$

Weight of fat in sample = (weight of flask + fat) – weight of flask

Ash content determination by using dry ashing method

The determination of ash content in the sample was conducted according to Bujang et al. (2017) method with slight modification. A shallow porcelain dish was dried in the oven at 105 °C for 3 hours and was then being cooled in a desiccator. Once the porcelain dish has attained room temperature, the crucible was weighed. Since yoghurt chia pudding sample contain high moisture, the sample was dried in the drying cabinet (Model DC 1000, Genlab) at 60 °C for 2 days prior to analysis. Approximately 4 g of the homogenized yoghurt chia pudding sample was weighed into a porcelain dish. The dried sample was charred gently over a Bunsen burner until ceased smoking has been obtained. The crucible was then being placed in a muffle furnace (Model SEF-301, Finetech) and heated at 550 °C for 3 hours. The sample was ashed until a whitish or greyish ash has obtained. Then, the dish was removed and cooled in desiccator immediately before weighing. The dish was replaced in the muffle furnace and heating process was continued twice to obtain a constant weight. Finally, the total ash content of the yoghurt chia pudding sample was calculated. The following Equation 3 was used to estimate the ash content.

$$\text{ash (g) per 100 g of the total sample} = \frac{\text{weight of ash (g)}}{\text{weight of sample (g)}} \; x\; 100 \quad \text{Equation 3}$$

Fibre content determination by using acid and alkali digestion method

The determination of crude fibre using fibre apparatus (Model FOSS, Fibertech E) was performed according to the method of Bujang et al. (2017). Approximately, 1 g of dried ground sample was weighed into a crucible. Then, 200 ml of 1.25 N H_2SO_4 was added and boiled for 30 minutes. The content of the crucible was then filtered, and the residue was washed with hot water until free from acid. Next, 200 ml of 1.25 N warm NaOH was added into the beaker which contained the insoluble material and was then being boiled for 30 minutes. The content was then filtered, and the residue was washed with hot water for about four times, then followed by washing with 15 ml of 1% hydrochloric acid for two times. The residue was washed again with hot water until it is neutral or became free from acid.

Next, the crucible with the insoluble material was transferred into the oven at 105 °C to achieve a constant weight. Then, the weight of the residue and crucible was recorded. The residue was then being charred gently over the Bunsen burner until it produced ceased smoking. The residue was ignited in a muffle furnace at 550 °C for 3 hours. The crucible was then being cooled in dessicator prior to weighing. The weight of ash and crucible was recorded and finally, the loss in weight on ignition represent the weight of the crude fibre. The following Equation 4 was used to estimate the fibre content in the chia pudding sample.

$$\text{crude fibre (g) per 100 g sample} = \frac{(\text{weight of crucible+dried residue})\ (\text{g}) - (\text{weight of crucible+ash})(\text{g})}{\text{weight of sample taken (g)}} \quad \text{Equation 4}$$

Carbohydrate content determination by using difference method

The carbohydrate content in the yoghurt chia pudding sample was attained by applying the arithmetic difference method. This can be obtained by deducting the sum of the percentages of moisture, crude protein, crude fat, and ash from one hundred (Marina & Azizah, 2014).

Energy value content determination

The amount of energy was being calculated by multiplying the amounts of carbohydrate, protein, and lipid by factors of 4, 4, and 9 correspondingly, and then adding the results (Ganogpichayagrai & Suksaard, 2020).

Antimicrobial properties analysis using well diffusion assay method

The analysis on the antimicrobial properties was conducted by using the agar well diffusion method according to Luo et al. (2019) with slightly modification. The bacterial strains used in this analysis was *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) which were activated in nutrient broth at 37 °C until the exponential growth phase was reached which is approximately about 12 hours. The concentration of bacterial suspension was recorded at optical density at 630 nm at the end of the incubation period using a UV/Vis spectrophotometer (Model T 801, PG instrument). Then, 100 µL of each bacteria suspension was injected using pipette into the nutrient agar plate. The agar was punctured (6 mm diameter) on each of the nutrient agar where 6 wells were made (the positive control was streptomycin, the negative control was distilled water, while the other 2 wells were for the 50% 100% sample each). The agar plates were then incubated at 35 °C for 24 hours. Then, the diameter of the inhibition zone formed for each agar plate containing *S. aureus* and *E. coli* were measured.

Microbial analysis (mould and yeast counts)

The microbial analysis was conducted according to Upasen et al. (2018). Spread plate technique was used to measure the mould and yeast counts. For agar preparation, approximately, 39 g of potato dextrose agar (PDA) was dissolved in 1 L of distilled water. The solution was then being autoclaved (Model FVG 2, Fedegari) at 121 °C under 15 psi for about 15 minutes. Next, about 15 ml to 20 ml of the solution was poured into each petri dish and was then being kept in a laminar air flow or the clean bench to allow it to solidify. Next, 0.1% peptone water was prepared and autoclave to be used as a diluent for the serial dilution. Serial decimal dilution was performed using the 0.1% peptone water solution. After the desired concentration was achieved, 0.1 ml of the solution was spread on the surface of the prepared culture medium and was then being incubated at 35 ± 2 °C for 48 hours before colony counts were performed. The results obtained was expressed as colony forming unit per mL (CFU/mL) calculated using Equation 5.

$$N = \frac{\Sigma C}{(n1+0{,}1\ n2)d} \quad \text{Equation 5}$$

Where,

ΣC = the sum of the colonies counted on all the plates from two successive dilutions

$n1$ = the number of plates counted in the first dilution

n2 = the number of plates counted in the second dilution
d = the dilution from which the first counts were obtained

Determination of pH

The method of pH determination in yoghurt chia pudding sample was based on the method by (Mcglynn, 2003). The pH meter (Model (765 Calimatic, Knick, Berlin, Germany) was calibrated properly before analysis. Then, the probe was first cleaned with distilled water, then dried carefully. The sensing tip of the probe was immersed in the sample and the pH reading were recorded. The meter was allowed to be stabilized for at least 1 minute. The probe was then being rinsed, blotted dried and the analysis was repeated by using a fresh portion of yoghurt chia pudding sample.

Statistical analysis

Simple statistical analysis of descriptive statistics using Microsoft Excel was done to determine the standard deviation of the proximate analysis and pH analysis. The difference between the mean values were evaluated using the Duncan's multiple range test.

Result and Discussion

Nutritional composition analysis and energy values of yoghurt chia pudding

The results of the nutritional composition of the yoghurt chia pudding evaluated in this study was presented in Table 2. The yoghurt chia pudding formulation values obtained was compared with different previous studies.

Table 2. Nutritional composition (%) of yoghurt chia pudding and energy value (Kcal/100g)

Nutrients	Contents
Moisture (%)	75.43 ± 0.2254
Crude protein (%)	6.06 ± 0.7832
Crude fat (%)	7.92 ± 0.5672
Crude ash (%)	0.74 ± 0.01099
Crude fibre (%)	9.01 ± 0.5662
Carbohydrate (%)	9.85 ± 0.2136
Energy value (Kcal/100g)	134.95 ± 0.4734

Notes: Data are expressed as means values ± SD (n = 3)

Based on the results obtained, the average moisture content for the sample was 75.43%. According to Shember et al. (2014), the yoghurt samples being evaluated exhibited a moisture content in the range of 78.62 to 82.41% which is similar to the studies conducted by Matela et al. (2019) on various commercialised yoghurt product which ranging from 76.08% to 80.07%. Study conducted by Muñoz et al. (2012) on the hydration of chia mucilage, a 100 mg sample of the substance has the capacity to soak up 2.7 mL of water, or 27 times its weight. The moisture content of the yoghurt chia pudding sample was within the limit as the maximum moisture level of yoghurt should be 84% since too much water reduces viscosity and affects texture and mouth feel (Shember et al., 2014).

Matela et al. (2019) reported that yoghurt product should have a protein composition of not less than 2.70% in accordance with Codex guidelines. According to the nutritional content of the utilised Greek yoghurt namely Lactel Greek style yoghurt strawberry sold by the Lactalis Training Malaysia Sdn. Bhd., the protein content is 4%. This shows that yoghurt chia pudding being evaluated has a high protein content of 6.06% (Table 2). This was derivable from the existence of protein in CS which contained 16.54% (Kulczyński et al. 2019), 19.78% (Cardenas et al. 2018), and 20.90% (Kibui et al. 2018).

The fat content of the evaluated yoghurt chia pudding was 7.92% which was above the minimum standard for low fat yoghurt (< 3.5%) (Ndife, 2014). According to the nutritional content of the utilised Greek yoghurt, the fat content is 4.4%. Greater fat contents were reported by Kibui et al. (2018) where the evaluated chia enriched yoghurt of variety of formulation were from 14% to 15.13% which was due to the utilisation of normal yoghurt of higher lipid content. Previous studies have proven that fat content in yoghurt products plays a crucial role which impart positive effects on its physical and sensory

characteristics, and negative effects on its shelf life (Farinde et al., 2009; Saint-Eve et al., 2008).

The crude ash content of chia yoghurt (0.74%), as shown in Table 2, was found to be slightly lower than those reported by studies as 0.89% on average by Kibui et al. (2018). This high ash content was derived by high ash content of the CS (4.8%) (Cardenas et al., 2018; Cassiday, 2017).

According to Tremblay & Panahi (2017), yoghurt has a low fibre content which were between 2.6% to 2.9%. The high value of crude fibre content (9.01%) of the chia yoghurt was expected as CS has higher fibre content as reported in various studies (Cardenas et al., 2018; Cassiday, 2017; Kulczyński et al., 2019). Similar results on the fibre content were obtained where 2.20% and 4.40% when being enriched with 7 g and 14 g CS, respectively. Meanwhile, there is no fibre content obtained (0%) through the sample for yoghurt without the enrichment of CS (Ayaz et al., 2017). The consumption of the chia pudding may aid in slowing down the digestion process and the release of glucose since it exerts higher fibre content (Ayaz et al., 2017).

According to the nutritional composition of the utilised Greek yoghurt, the carbohydrate content is 16.6%. Lower carbohydrate content value was expected of the yoghurt chia pudding (9.85%) because most of the available lactose present in the yoghurt had been changed to lactic acid, making it a perfect diet for those who are lactose intolerant (Kibui et al., 2018).

The amount of energy calculated was 134.95 kcal/100g when the total sums of amounts of carbohydrate, protein, and lipid were multiplied by factors of 4, 4, and 9, correspondingly and added (Ganogpichayagrai & Suksaard, 2020). Similar caloric value was also observed through a study from Kibui et al. (2018), where yoghurts enriched with CS has an energy value from 132.71 to 157.87 kcal/100g.

Antimicrobial analysis on yoghurt chia pudding

Figure 3 shows the inhibition zone of the formulated yoghurt chia pudding sample against the gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*) and the gram-negative bacteria, *Escherichia coli* (*E. coli*).

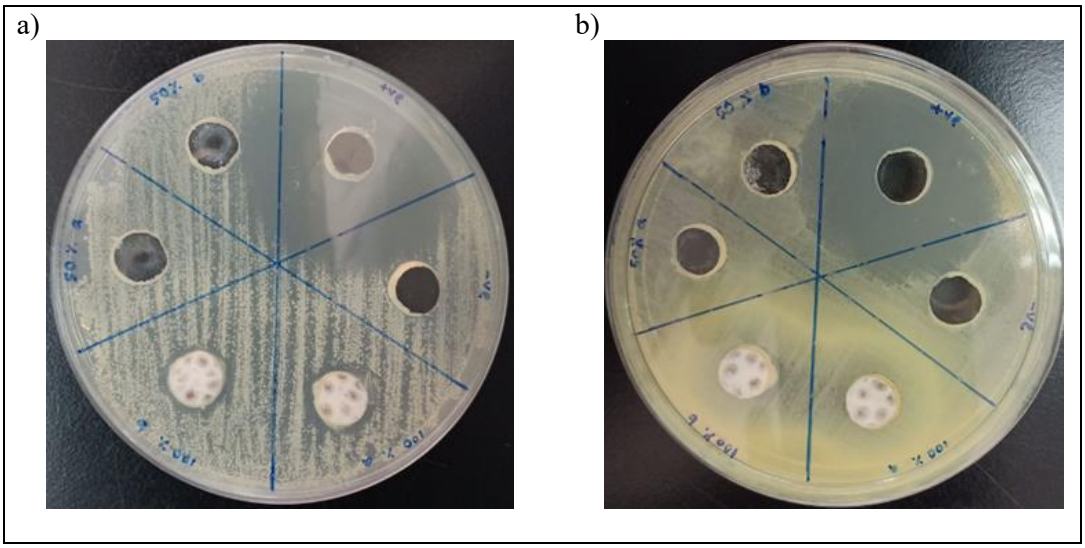

Figure 3. Zone of inhibition against a) gram-positive bacteria (*S. aureus*), and b) the gram-negative bacteria (*E. coli*)

The yoghurt chia pudding sample in Figure 3 shows an inhibition zone of 8 mm in diameter against the gram-positive bacteria (*S. aureus*), whereas no inhibition zone was observed for the gram-negative bacteria (*E. coli*). Luo et al. (2019) stated that, S. *aureus* was more susceptible to the antibacterial effects than *E. coli* generally. This is probably because gram-negative bacteria had an outer membrane covering

their cell walls known as lipopolysaccharide at the external surface and surrounded by a thin layer of peptidoglycan, which protected them from the effects of antibacterial agents (Aguilar et al., 2020; Luo et al., 2019). Studies by Aguilar et al. (2020) proved that CS possess good antimicrobial properties against *S. aureus* and *E. coli* with the inhibition zones of diameter > 14 mm and < 10 mm, respectively. Also, based on previous study by Shah (2000), live beneficial bacteria found in yoghurt including *Lactobacillus* and *Bifidobacterium* species, wherein during fermentation process producing organic acids such as lactic acid which helps to reduce the yoghurt's pH, resulting in an acidic condition that prevents the growth of numerous pathogenic bacteria and microorganisms causing deterioration. Yoghurt's probiotic bacteria have been proven to have antimicrobial effects on several pathogens, including *Salmonella sp., E. coli, and S. aureus* (Shah, 2000). The positive results on antimicrobial properties shown by the yoghurt chia pudding signifies that it can contribute to extension in its shelf life as it is useful in reducing or preventing the risk of microbial contamination caused by foodborne pathogens (Aguilar et al., 2020).

Shelf-life assessment on yoghurt chia pudding

Data shown in Table 3 indicated the mould and yeast count in CFU/mL and the pH in different storage temperatures during 20 days of storage period. The yoghurt chia pudding was taken for mould and yeast count analysis for 3 consecutive days in the room temperature storage whereas, the sample was taken to analysis every 4 days interval for both chilled and frozen storage temperature for 20 days.

Table 3. Effect of different storage temperature on pH and mould and yeast count in 20 days storage period

Storage temperature	Time (day)	MYC (CFU/mL)	pH
RT	1	81^{a}	4.74 ± 0.00^{a}
	2	109^{a}	4.55 ± 0.00^{a}
	3	$2.2 \times 10^{3\,a}$	4.13 ± 0.05^{a}
CT	4	0^{a}	4.34 ± 0.00^{a}
	8	4^{a}	4.25 ± 0.00^{b}
	12	45^{a}	4.15 ± 0.01^{b}
	16	268^{b}	4.11 ± 0.00^{a}
	20	NE	4.05 ± 0.00^{b}
FT	4	0^{a}	4.29 ± 0.00^{a}
	8	13^{a}	4.30 ± 0.00^{a}
	12	18^{b}	4.20 ± 0.00^{b}
	16	13^{b}	4.18 ± 0.02^{b}
	20	18^{b}	4.14 ± 0.01^{a}

Notes:
Means with the same letter are significantly different ($P < 0.05$) by Duncan's multiple range test
Data of pH values are expressed as means values ± SD (n = 3)
RT- Room Temperature (27 °C), CT- Chilled Temperature (3 °C), FT- Frozen Temperature (-20 °C)
NE- Not evaluated

The declined in pH of the yoghurt chia pudding across the period of time can be observed for all the storage temperature as tabulated in Table 3. The pH falls gradually from 4.34 to 4.05 for sample stored in chilled temperature, whereas pH dropped from 4.29 to 4.14 for sample being kept at frozen temperature. Also, pH for sample stored at ambient temperature dropped drastically from pH 4.74 to 4.13. Similar results trends on decreasing of pH was observed by studies by Joung et al. (2016), across 28 days refrigerated which might be due to the accumulation of acetaldehyde, acetic acid, lactic and formic acid in the yoghurt samples evaluated. Similar results also presented by Alli et al. (2010) where mould and yeast proliferation in the yoghurt product use some of the acids resulting in a corresponding drop in the acidity, thus lowering the pH. The pH of samples might drop as a result from the remaining lactose which is the milk sugar was being further metabolized by the lactic acid even after storage at lower temperatures (Viljoen et al., 2003).

Based on Table 3, acceptable level of CFU/mL was observed during storage temperature from CT the 4^{th} day until 12^{th} day. The number of colonies obtained was less than 10 in 10^{-1} dilution. Hence, it

indicates that the count is less than 100 CFU/mL (Yamagata, 1992). No mould and yeast growth observed initially in the yoghurt chia pudding samples stored at low temperatures (CT: 3 °C, FT: -20 °C) when incubated after four days after the production. The low level of yeast and mould in the sample may attributed to antimicrobial effect of the CS in the production of chia pudding (Mukhekar et al., 2018). The count of mould and yeast in RT increased drastically to an unacceptable level until the 3rd day of evaluation signifies that this yoghurt chia pudding sample was unsuitable to be kept in this type of storage temperature. Besides, according to Zubairi et al. (2021), the presence of a mould and yeast colony could indicate poor processing hygiene and inadequate sanitation. Yoghurt that has levels of yeast and mould that are higher than 10^2 to 10^3 CFU/mL should not be consumed since they may pose health risks or indicates the probable spoilage of the product (Zubairi et al., 2021).

Conclusion

In conclusion, the results obtained from this study indicated that the formulated yoghurt chia pudding has an energy value of 134.95 Kcal/100g and contained rich fibre, carbohydrate, protein, and fat content with a value of 9.01%, 9.85%, 6.06% and 7.92%, respectively. The low level count (CFU/mL) indicated that the formulated yoghurt chia pudding exhibit the antimicrobial properties which shows zone of inhibition of 8 mm diameter against the gram positive bacteria (*S. aureus*), however no inhibition zone was observed against the gram negative bacteria being tested (*E. coli*). The pH falls gradually for sample stored at CT (4.34 - 4.05), and at FT (4.29 - 4.14) in 20 days storage. The shelf-life study reveals that yoghurt chia pudding remains stable and of acceptable quality until day 12 for CT and longer shelf life when stored in FT (> 20 days). The research recommends that the relationship between water activity and the mould and yeast growth should be done for shelf life studies. Additionally, sensory evaluation to assess preference and acceptance of consumers can also be done for further study.

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Author Contribution

N Zainal Abidin – writing original draft, formal analysis; SJ Md Japilus – review and editing; AF Kassim – review and editing; S Miswan– review and editing; H Mohd Nooh – supervision, review and editing.

Conflict of Interest

Authors declare no conflict of interest.

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