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FULL PAPER

Okara yoghurt – a high value-added fermented product from soybean curd residue

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Abstract

Okara is a by-product that consists of insoluble parts of soybean that remain after filtration of the pureed soybean during the manufacturing of soymilk or tofu, which is often underutilized or dumped into the landfills and caused environmental pollution. However, being rich in nutrients, it can, therefore, serve as a good growth medium for lactic acid bacteria (LAB) and developed as fermented products. The purpose of this study was to develop a yoghurt formulation using okara and determine the physicochemical properties and chemical composition of the okara based yoghurt. In this study, different formulations were assessed to develop the okara yoghurt. Higher numbers of colony forming units (CFU/mL) of the LAB starter cultures were observed in okara yoghurt (4.3×10^9CFU/mL) in comparison to plain milk yoghurt (1.43×10^9 CFU/mL). A lower pH was attained due to the higher amount of titratable acidity (1.421±0.53%) produced at a faster rate, especially for okara yoghurt (without skimmed) in comparison to milk yoghurt. The chemical composition of the okara yoghurt showed a substantially higher amount of protein (4.46±0.0424%), carbohydrate (16.15±0%), fibre (4.43±0%) and energy (101±0 kcal), in comparison to the commercial milk yoghurt. Furthermore, the addition of okara in the yoghurt formulation also showed an increase in microbial growth during the fermentation due to the presence of dietary fibre. In conclusion, the incorporation of okara with the fortification of skimmed milk not only can enhance probiotic growth but also assist in improving the texture and producing a better taste of okara yoghurt. Therefore, the development of new okara yoghurt from soy by-product is a valuable approach where the low value and low-cost by-products can be turned into a high value-added product with health benefits.

1. Introduction

Soybean curd residue (SCR), also known as okara is a soy by-product generated during the manufacturing of soymilk and soybean curd (tofu). It consists of insoluble parts of the soybean seeds that remain after the extraction of the aqueous fraction during the production of soymilk and soybean curd (tofu) (Swallah *et al*., 2021). About 1.1 to 1.2 kg of okara is produced from every kilogram of soybeans processed into tofu or soymilk production (Li *et al*., 2012). Therefore, an enormous amount of okara is generated with an estimation of more than a hundred million tonnes of okara has been produced annually, especially in the Asian countries where soybean consumption is high and has led to a significant disposal problem (Vong and Liu, 2016).

Okara is an excellent, low value but high nutritive raw material since it is rich in protein and fibre. It contains approximately 50% carbohydrates, 20 to 30% proteins, 10 to 20% lipids, and 40 to 60% dietary fibre, as well as mineral elements and phytochemicals such as isoflavones, saponins, phytosterols (O'Toole, 1999), which made it a promising nutritional substance with health benefits that has great potential to be applied as functional ingredients for human consumption (Jiménez-Escrig *et al*., 2008; Vong and Liu, 2016). However, the

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high moisture content (70-80°C) of okara has made it susceptible to spoilage and difficult to handle. Therefore, it is often used as animal feed resources or biofertilizer, being discarded or dumped in landfills which caused environmental and economic problems.

Currently, extensive studies have been carried out on okara's chemical composition, nutritional qualities, biological activities and its potential application (Li *et al*., 2012; Vong and Liu, 2016; Xia *et al.*, 2016; Swallah *et al*., 2021). Owing to its high content of dietary fibre and low production costs, okara could be used as a dietary supplement or functional ingredient to prevent diabetes, obesity, hyperlipidemia, as well as protect the gut environment due to the high antioxidant properties and prebiotic effects (Jiménez-Escrig *et al*., 2008; Swallah *et al*., 2021). Despite the vast health-benefiting effects exhibited in okara, there are several limiting factors such as the anti-nutrients and flatulence effect caused by the presence of certain oligosaccharides, besides the unpleasant 'fishy' and 'beany' flavour and unpalatable taste that seriously affects the consumers' acceptance towards okara-based products, thus, limit its application. Numerous studies have shown that the health benefits and nutritional quality of okara were proven enhanced through the fermentation process (Bedani *et al*., 2013; Filho *et al*., 2018). In recent years, there has been growing interest in the valorization of by-products or waste biomasses from the food industry as new sources of functional ingredients such as prebiotics (Xia *et al*., 2016; Voss *et al*., 2021). The functional biological activities of okara can be enhanced through fermentation, chemical or enzymatic hydrolysis by microbial proteases that metabolise proteins into amino acids and bioactive peptides and increase the soluble fibre content which consequently improves its nutritional quality and health benefiting effects. This fibre content of okara could be a potential substrate for probiotic growth and survival under simulated gastrointestinal conditions (Xia *et al*., 2016). The dietary fibre which comprises polysaccharides is not only able to escape from enzymatic digestion but also acts essentially as a substrate for the gut microbiota and could impact the host-microbial community and immunity (Shawallah *et al.*, 2021).

Yoghurt is a popular fermented milk product obtained through LAB fermentation on milk. It is gaining popularity and better acceptability owing to the increase of awareness on its health beneficial effects among the customers (Chen *et al*., 2017). According to the Codex Alimentarius International Food Standard CXS 243-2003, yoghurt is characterised by the presence of symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Codex Alimentarius Commission, 2018). There are three main biochemical processes involved in the formation of yoghurt which is the fermentation of lactose into lactic acid, hydrolysis of casein into peptides and free amino acids (proteolysis), and breakdown of milk fat into free fatty acids (lipolysis) (Steele *et al.*, 2013). These processes subsequently resulted in the production of various metabolites that led to the reduction of pH, formation of semi-solid texture (curd) and a distinctive yoghurt flavour (Irigoyen *et al*., 2012). The synergistic interactions between these two main starter cultures have provided mutual growth-stimulating effects. It was reported that *S. thermophilus* produces pyruvic acids, formic acid, ornithins, long-chain fatty acids and carbon dioxide which stimulate the growth of *L. delbruekii* subsp. *bulgaricus*. The pH of milk was also reduced to an optimum level for the growth of *L. delbruekii* subp. *bulgaricus* due to the lactic acid produced (Settachaimongkon *et al*., 2014). Conversely, *L. delbruekii* subsp. *bulgaricus* produces peptides, free amino acids and putrescine that stimulate the growth of *S. thermophilus* (Sieuwerts *et al*., 2008). Additionally, the lower pH caused by the lactic acid produced served as a preservation method as it inhibited the growth of pathogenic bacteria. It has been found that the association of these two microorganisms also affects the production of volatile and non-volatile molecules involved in flavour development (Chen *et al.,* 2017). In this research, an attempt has been made to incorporate okara as an ingredient for yoghurt production through lactic fermentation due to its high nutritional properties that could lead to health enhancement. Therefore, the objectives of this study were to evaluate the feasibility of incorporating okara in the formation of yoghurt and determine the physicochemical properties and proximate composition of the okara based yoghurt.

2. Materials and methods

2.1 Preparation of okara

The okara was obtained from a small manufacturing plant of soymilk and tofu production at Seri Kembangan, Selangor. The okara was collected and dried immediately in an oven at 40 to 50°C for 12 to 16 hrs, until it reached 4% moisture content. It was then ground using a grinder (Retch ZM 200, GmBH Germany), vacuum-packed (Fullwell DZ-400/2ES, Malaysia) and stored at 4°C for future usage.

2.2 Preparation and fermentation of plain milk and okara yoghurt

The ingredients used for the preparation of plain milk and okara yoghurt are listed in Table 1. The formulation of okara yoghurt was based on the cow milk

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yoghurt formulation with some modifications. The UHT cow milk was substituted with soy milk and the feasibility of adding okara as an ingredient in the preparation of okara yoghurt was assessed (Table 1). Initially, the okara yoghurt was consisted of soymilk, sugar and okara, without the addition of skimmed milk, with the hypothesis that skimmed milk could be substituted with okara . The UHT cow milk used was the Commercial Full Cream Milk (Dutch Lady, Malaysia), while the soymilk used was the Commercial Original Soymilk (Homesoy, Malaysia). The yoghurt preparation was mixed and homogenized using a homogenizer (Silverson LSM-A, England) at 7500 rpm for 10 mins to allow a homogenous mixture of all ingredients prior to pasteurization at 90°C for 20 mins. Upon pasteurization, the mixtures were immediately mixed thoroughly using a stirrer to avoid agglutination and were cooled down to about 40°C prior to the inoculation process. The fermentation of both plain milk and okara yoghurt was performed by adding 3% of inoculum preparation into each yoghurt mixtures and incubated at 30°C and 37°C for okara yoghurt and plain milk yoghurt, respectively, for 16 hrs or until they reached pH 4.2 to 4.5, which indicated sufficient amount of lactic acid produced. The endpoint of yoghurt fermentation is measured through the determination of pH which is directly related to the total acidity of the mixture. During fermentation, lactose was converted into lactic acid and caused the pH to drop to pH 4.5 to 4.7. At this pH endpoint, the fermentation was stopped by cooling the yoghurt to 4°C.

Table 1. Formulation and ingredients employed in the production of plain milk and okara yogurt

Ingredients (g/L) of milk	Milk yogurt	Okara yogurt (without skimmed	Okara yogurt (with skimmed
Cow UHT milk (L)	1	-	-
Soy milk (L)	-	1	1
Skim milk (g/L)	60	-	60
Sugar (g/L)	120	120	120
Okara (g/L)	-	30	30

2.3 Inoculum preparation

The commercial Yogourmet® starter culture was used as inoculum in the preparation of yoghurt. The Yogourmet® starter culture consists of three probiotic strains namely; *Bifidobacterium longum, Lactobacillus casei* and *Lactobacillus acidophilus,* besides the two main yoghurt starter cultures, i.e., *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus.* The inoculum was prepared by adding 0.2 g of Yogourmet® starter culture powder into 40 mL UHT milk in sterile tubes. The mixture was mixed well and incubated at 37°C for 16 hrs or until pH 4.5 was achieved.

2.4 Physicochemical and microbiological analysis

All yoghurt samples were subjected to physicochemical and microbiological analysis such as determination of pH, Brix, titratable acid and enumeration of total cell count before and after fermentation. The analysis performed was recorded and the readings of before and after 16 hrs fermentation, where pH 4.5 was attained and were compared.

2.4.1 pH analysis

The pH of yoghurt samples was determined after a certain period of fermentation. The pH meter was calibrated using a buffer solution of pH 4.0 and 7.0. An aliquot of 2 mL of yoghurt samples was used and the pH reading was taken using a digital pH meter (Mettler Toledo, Switzerland) (Shori and Baba, 2011). The yoghurt fermentation was stopped when pH 4.5 was attained.

2.4.2 Brix analysis

Brix refractometer (Atago Pal-1 Digital) was used to measure the total soluble solid/sugar in each yoghurt mixture. A volume of 200 µL of each solution was pipetted onto the sample plate and the reading was recorded as indicated in the manufacturer's instruction.

2.4.3 Determination of total titratable acid

Ten times dilutions of yoghurt samples were used in the determination of total titratable acid (TTA). Approximately three drops of 0.1% phenolphthalein solution (0.1 g phenolphthalein in 100 mL of 95% ethanol) were added into the samples. The yoghurt suspension was titrated with 0.1 N NaOH while continuously stirring until the colour changed to pink, which lasted for 30 s. The volume of NaOH required was recorded, and amount of acid produced was calculated using the following formula (Shori, 2013):

% TTA = Dilution factor (10) × Volume NaOH × 0.1N × 0.009 × 100%

Where 10 = Dilution Factor, 0.1N = Normality of NaOH, 0.009 = conversion factor; whereby 1 mL of 0.1N NaOH neutralizes 0.009g of lactic acid.

2.4.4 Enumeration of total viable cell count

The number of total viable cells was estimated using the spread plate method (Hasanzadeh-Rostami *et al*., 2014). A serial dilution of the yoghurt samples was prepared and spread plating was performed using appropriate dilutions. An amount of 100 µL samples from selected dilutions were spread plated on the MRS agar plate and was left dried prior to incubation at 37°C

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for 24 - 48 hrs for observation of colonies. The colonies formed for each dilution was then enumerated after incubation.

$$\text{CFU/mL} = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{\text{0.1 mL of sample}}$$

2.4.5 Proximate analysis of okara yoghurt

The moisture, ash, crude protein, fat content and dietary fibre were determined according to the method recommended by the Association of Official Analytical Chemists (AOAC) standard methods (AOAC, 1998). Moisture content was determined using the oven drying method (AOAC Method 950.46) and ash content was also obtained using the drying method (AOAC Method 923.03). Crude protein was determined using Kjeldahl's method (AOAC Method 981.10). Crude fat was obtained using the Soxhlet method (AOAC Method 905.02 and 989.05). The dietary fibre was determined using the enzymatic gravimetric method (AOAC Method 991.43). The total carbohydrate content was estimated based on the formula as follows: [Carbohydrate (%) = 100% - % (moisture + protein + fat + ash)]. Whereas, total energy value was determined based on the calculation formula: Energy value (Kcal) = (% Crude Protein × 4) + (% Crude Fat × 9) + (% Carbohydrate × 4) and measured in kilo-calories/100 g (kcal/100 g).

2.4.6 Statistical analysis

All analyses were performed in triplicates and the results were expressed as mean ± SD (standard deviation). The statistical analysis was performed using one-way analysis of variance (ANOVA, SPSS 27.0) and the Tukey test was used for comparison of means at a 5% significant level.

3. Results and discussion

3.1 Fermentation of milk yoghurt and okara yoghurt

Soymilk as milk-based incorporated with okara was used in the initial trial of okara yoghurt product development (Table 1), resulted in the phenomenon of syneresis to occur, too sour taste and unacceptable beany flavour of okara yoghurt. Syneresis or whey separation is a textural defect in yoghurt which occur due to the rearrangement of the casein micelles network which caused water to expel due to shrinkage of gel, which leads to syneresis (Rani *et al*., 2012). However, when skimmed milk was incorporated into the okara yoghurt at the same amount as in the milk yoghurt preparation (Table 1), an improved texture and taste of the okara yoghurt was obtained. There was no whey separation in okara yoghurt with better taste (less sour) and firmness compared to the okara yoghurt without skimmed milk. These results showed that different compositions of the milk base and ingredients used in the yoghurt formulation might affect the taste, texture and appearance of the yoghurt produced, which probably contributed by the different physicochemical properties of the yoghurt. According to Rani *et al*. (2012), the texture of yoghurt is strongly influenced by the composition of the milk base used and the fermentation method employed in the production of yoghurt, with appropriate firmness without syneresis which is critical for excellent quality yoghurt.

3.2 Physicochemical properties of yoghurt

The physicochemical properties of yoghurt samples such as the pH, °Brix and titratable acidity (TTA) of all yoghurt samples are shown in Table 2. It was observed that the pH of both okara yoghurt reduced faster than the milk yoghurt (data not shown). Both okara yoghurt (with and without skimmed milk) attained a final pH of around pH 4.5 after 16 hrs (overnight) of incubation at 30°C (P<0.05), in comparison to milk yoghurt which required a prolonged incubation time of up to 48 hrs to attain a final pH of 4.5. Similarly, a faster rate of pH reduction which resulted from the faster acidification rate in soymilk than in milk could be explained due to the lower

Table 2. Physicochemical properties of the milk and okara yogurt before and after fermentation

Before fermentation			
Physicochemical properties	Milk yogurt	Okara yogurt (without skimmed milk)	Okara yogurt (with skimmed milk)
Brix (°)	26.36±0.07^{c}	20.60±0.14^{a}	24.00±0.57^{b}
pH	6.36±0.01^{a}	6.42±0.00^{a}	6.64±0.03^{b}
TTA (%)	0.27±0.00^{a}	0.27±0.13^{a}	0.32±0.06^{a}
After fermentation			
Physicochemical properties	Milk yogurt	Okara yogurt (without skimmed milk)	Okara yogurt (with skimmed milk)
Brix (°)	20.45±0.07^{b}	17.95±0.07^{a}	23.90±0.07^{c}
pH	4.54±0.05^{b}	4.33±0.01^{a}	4.41±0.04^{ab}
TTA (%)	1.06±0.15^{a}	1.42±0.53^{a}	0.90±0.07^{a}

Values are presented as mean ± standard deviation of three replicates. Values with different superscripts within the same row are significantly different analysed using the Tukey Method with 95% confidence level (P<0.05).

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buffering capacity of soymilk as reported by Champagne *et al*. (2009) when inoculated with co-cultures of yoghurt starter cultures and *B. longum* (Battistini *et al*., 2018). However, due to technical restrictions that needed an overnight incubation, the milk yoghurt was incubated at a higher temperature of 37°C and accomplished a final pH of pH 4.5 after the overnight incubation period (16 hrs) of the milk yoghurt preparation. It is generally accepted that incubation temperature plays a major role in determining the quality of yoghurt produced. Rani *et al*. (2012) reported that a longer incubation time is needed to attain certain pH and firmness when yoghurt preparation is incubated at a lower temperature. Therefore, milk yoghurt preparation was incubated at a higher temperature (37°C) in order to achieve pH 4.5 at the same incubation period with okara yoghurt. A study by Oladimeji *et al*. (2016) showed that the rate of reaction was strongly dependent on the incubation temperature, in which low- or poor-quality yoghurt will be produced if the incubation temperature is not properly maintained.

Yoghurt fermentation is mainly performed by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* as starter cultures at 40 to 45°C for 2.5 to 3 hrs or at ambient temperature for 18 hrs or more until it reaches a certain level of acidity (Wardani *et al*., 2010). However, at a lower temperature (e.g., 30°C), the incubation would require a longer fermentation time for improved yoghurt quality. Previous studies have demonstrated that lactic fermentation caused a further reduction of pH after prolonged incubation time (Oladimeji *et al*., 2016; Krisnaningsih *et al*., 2019). During the making of yoghurt, lactose is broken down into galactose and lactic acid (Krisnaningsih *et al*., 2019; Ityotagher and Julius, 2020). A high amount of lactic acid is produced and accumulated due to the LAB metabolic activity that produces lactic acid from lactose. The metabolic reaction of lactic acid bacteria not only produces lactic acid but also acetaldehyde that causes the lowering of pH to between pH 3.6-4.5, depending on the technological operating condition. The lowered pH subsequently affects the casein (milk protein) to coagulate and precipitate, forming a solid, thick curd that makes up the yoghurt (Oladimeji *et al.,* 2016).

The time taken to achieve a final pH near pH 4.5 also varied depending on the types of substrates and starter culture used. Furthermore, the use of suitable microorganisms is important for soymilk fermentation. A perfect match of substrate and the starter culture is a prerequisite to produce soy yoghurt with desired quality, because of different compositions of soymilk, thus, not all the yoghurt starter cultures are able to make full use of the components in soymilk (Guo and Yang, 2015). Soymilk might be a good medium for *Bifidobacterium* spp. growth, due to the presence of raffinose and stachyose, which could be fermented by strains belonging to this genus. Besides, *L. acidophilus* was reported able to metabolize oligosaccharides during the fermentation of soymilk (Donkor *et al*., 2007) and other LAB strains that possess α-galactosidase are also reported able to grow and utilize soymilk (Fung and Liong, 2010; Bedani *et al*., 2013). The Yogourmet® starter culture used containing *B. longum, S. thermophilus* and *L. acidophilus* which known could grow in soymilk, consume the non-digestible oligosaccharides such as raffinose, stachyose and other sugars such as sucrose and convert them into lactic acid and acetic acid that present in the okara yoghurt (with or without skimmed milk) and subsequently, caused the pH of okara yoghurt to reduce faster.

The value of titratable acidity (TTA) which indicate the amount of lactic acid produced was shown to increase after 16 hrs of incubation of yoghurt preparation. The titratable acidity was increased due to the activities of LAB, which break down lactose and other sugars into lactic acid. Okara yoghurt without skimmed milk showed the highest amount of titratable acidity (1.42±0.53%), followed by milk yoghurt (1.05±0.15%) and okara yoghurt (with skimmed milk) (0.9±0.07%) (Table 2), although there was no statistically significant difference in the quantity of titratable acidity among the yoghurt samples. The high TTA value obtained in okara yoghurts (without skimmed milk), when inoculated with Yogourmet® starter culture, was probably due to the low buffering capacity of soymilk compared to that in milk (Champagne *et al*., 2009; Battistini *et al*., 2018).

The total soluble solid or sugar content in a food product such as yoghurt is measured as °Brix. Different Brix values were observed in different yoghurt formulations (P<0.05) depending on the difference in concentration and ingredients used. It was observed that the sugar content is reduced with the increase in incubation time as indicated by the reduction of Brix value in the yoghurt samples, suggesting that the sugars present has been converted by LAB during the lactic fermentation. Unlike the okara yoghurt (without the addition of skimmed milk), higher total soluble solid was observed in milk and okara yoghurt (fortified with skimmed milk) at the initial and end of fermentation. Fortification of skimmed milk into milk and okara yoghurt has increased the total soluble solid in both, milk and okara yoghurt. A similar finding of increased total solids when the increased concentration of milk powder has been reported by Ityotagher *et al*. (2020), whereas, Roslan *et al*. (2021) found that the addition of okara has

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contributed to an increase in total soluble solids in all yoghurt samples and was proportional with the amount of okara added in the yoghurt formulation.

Many researchers reported that the growth of starter cultures and the quality of yoghurt produced are greatly affected by several factors such as chemical milk composition, the incubation time, temperature and concentration of inoculum (starter cultures) used (Trejo, 2014; Oladimeji *et al*., 2016). These factors were represented by the final yoghurt's pH, titratable acidity, Brix, viscosity and nutritional properties (Oladimeji *et al*., 2016). In addition to that, the starter culture will also affect the textural properties of milk yoghurt during and after the fermentation process. The viability of the starter cultures depends on several factors including types of strains used, the interaction between the starter cultures, incubation temperature, fermentation time and nutrient availability (Guo and Yang, 2015; Krisnanningsih *et al*., 2019). Loveday *et al.* (2013) also found that milk composition, thermal treatment and incubation temperature influenced the acidification process and the final yoghurt characteristics. It has also been reported that the types of milk can influence microbial metabolism, meaning that the same microorganism applied to different milk sources can generate a final product with distinct chemical composition and different volatile compounds (Madeiros *et al*., 2015). Overall, Oladimeji *et al.* (2016) obtained similar findings of an increase in viscosity of the yoghurt, reduction in pH and Brix (percentage of sugar) and increase in titratable acidity as the incubation temperature increased and subsequently, resulted in a thicker gel, more firmness and adhesiveness of the yoghurt produced.

3.3 Total viable cell count

A high total viable cell count was obtained in all yoghurt samples after fermentation with over the recommended levels for probiotic action (10^6 CFU/mL) (Table 3). The amount of total viable cell count was comparable in both okara yoghurt (with and without skimmed milk) and was significantly higher than in milk yoghurt (P<0.05). The highest number of total viable cell count (P<0.05) was attained in okara yoghurt (incorporated with skimmed milk) with (4.3±0.19)×10^9 CFU/mL, followed by okara yoghurt (without skimmed milk) with (1.83±0.21)×10^9 CFU/mL and cow milk yoghurt with (1.43±0.22)×10^9 CFU/mL (Table 3). In this study, the total viable cell count was higher in okara yoghurt compared to that in plain milk yoghurt especially in okara yoghurt fortified with skimmed milk. Several studies have shown that soy products, particularly soy yoghurt might be a good medium for probiotic strains (Donkor *et al*., 2007; Wang *et al.*, 2009; Bedani *et al*., 2013) Furthermore, the presence of dietary fibre in okara also enhanced the probiotic growth and viability. Several researchers have reported that the presence of dietary fibre in okara could improve the growth and survival of lactic acid bacteria during incubation and refrigerated storage (Sendra *et al*., 2008; Ityotagher and Julius, 2020). Additionally, they also reported that dietary fibre in the whole soybean might have a positive impact on LAB growth and activity in the soybean enriched yoghurts, resulting in higher lactic acid production in the whole soybean enriched yoghurt compared to the plain milk yoghurt (Ityotagher and Julius, 2020).

Several factors that affect LAB viability are including the incubation time and temperature, pH, LAB strain, milk composition and supplementation. Krisnanningsih *et al*. (2019) reported that there is a gradual significant increase in acidity of yoghurt during the storage with the amount of lactic acid produced. The strategy of incorporating okara and skimmed milk in okara yoghurt would provide additional nutrients that improve probiotic growth and subsequently result in higher lactic acid production. The survival of LAB was also indicated by the low pH condition of the okara yoghurt. Moreover, the presence of dietary fibre in okara as a prebiotics source in the fermented milk had stimulated the metabolic activities of starter bacteria that resulted in improved acidity development. According to Swallah *et al*. (2021), okara could act as a carrier that provides a surface for bacteria cell adhesion, thereby enabling substrate uptake as well as cell growth. It was also observed that the insoluble dietary fibres in okara were converted into soluble fibres when inoculated with both strains, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Tu *et al*., 2014) and subsequently became the source for soluble dietary fibre or prebiotics that would enhance the growth of probiotic strains in the okara yoghurt.

Table 3. The total viable count of lactic acid bacteria

	Before fermentation (×10^7 CFU/mL)±SD	After fermentation (×10^9 CFU/mL)±SD
Milk yogurt	2.06±0.19^a	1.43±0.22^a
Okara yogurt (without skimmed milk)	2.06±0.25^a	1.83±0.21^a
Okara yogurt (with skimmed milk)	2.36±0.10^a	4.3±0.19^b

Values are presented as mean ± standard deviation of three replicates. Values with different superscripts within the same column are significantly different (P<0.05).

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3.4 Proximate analysis of okara yoghurt

The proximate analysis was performed on the best okara yoghurt sample (fortified with skimmed milk) and was compared with a standard chemical composition in milk yoghurt (Chandan, 2017) as presented in Table 4. Results obtained showed that overall, the okara yoghurt contained higher content of protein and carbohydrate compared to that in the cow milk yoghurt. Besides, the okara yoghurt also contains lower fat content but higher energy and dietary fibre in comparison to cow milk yoghurt. The protein content of okara yoghurt was slightly higher than cow milk yoghurt with 4.46±0.042%. The higher protein content in okara yoghurt was probably due to the incorporation of okara which contains 25% protein, as well as the incorporation of skimmed milk that contributed to the additional protein content. The protein content obtained in the okara yoghurt has met the requirement of the Codex Alimentarius International Food Standard CXS 243-2003 that the yoghurt sample should contain not less than 2.70% protein content (Matela *et al.,* 2019).

Table 4. Chemical composition of okara yogurt and comparison with typical chemical composition of milk yogurt

Chemical Composition (per 100g)	Milk Yogurt*	Okara Yogurt (with skimmed milk)
Protein (g)	3.47	4.46±0.0424
Total Fat (g)	3.25	2.02±0.0282
Total Carbohydrate (g)	4.66	16.15±0
Ash (g)	-	0.7±0.141
Moisture Content (%)	87.90	76.67±0.134
Energy (kcal)	61	101 (424 kJ)±0
Dietary Fibre (g)	0	4.43±0

*Typical chemical composition of milk yogurt (adapted from Chandan, 2017).

The fat content of okara yoghurt was found slightly lower than that in cow milk yoghurt, as it is known that plant milk (soymilk) generally contains lower fat than that in cow milk. It has been reported that the percentage of fat content plays an important role in yoghurts since it improves the texture, appearance, flavour and taste of yoghurts (Oladipo *et al.,* 2014). According to United States Department of Agriculture (USDA) (2001), yoghurt samples with more than 3.25% of fat content should be labelled as yoghurt; yoghurt with fat content in the range of 0.5 to 2.0% should be labelled as Low-Fat yoghurt; whereas, yoghurt with less than 0.5% fat content is labelled as Non-Fat yoghurt. Additionally, based on the Codex Alimentarius International Food Standard CXS 243-2003, yoghurt should have less than 15% of fat content (Codex Alimentarius Commission, 2018). The fat content of okara yoghurt obtained from our study was in compliance with the standard fat content of yoghurt and can be categorized as low-fat yoghurt.

In addition, the carbohydrate content of okara yoghurt was more than 3 times higher than in the milk yoghurt with 16.15±0%. The higher carbohydrate content of okara yoghurt compared to milk yoghurt could be attributed to the high content of dietary fibre in the okara. According to Matela *et al*. (2019), based on Dairy Council (2013), yoghurt should contain a carbohydrate content of 13.7 - 17.7%. It also showed that the carbohydrate content of okara yoghurt was in good agreement with this standard. The disaccharides and lactose found in yoghurts have been hydrolysed by the enzyme lactase (α-galactosidase) from the starter cultures and converted into simple sugars, glucose and galactose. These simple sugars are easily absorbed by the body and thus, yoghurt is ideal for people with lactose maldigestion (Mckinley, 2005).

It was also observed that the value of moisture content of the okara yoghurt in this study (76.67±0.134%) is in agreement with other reported values of 76.08-80.07% (Matela *et al.*, 2019). According to Matela *et al*. (2019), the moisture content of yoghurt should be less than 84% as the presence of higher moisture content would affect the texture and mouthfeel. Besides, okara yoghurt also provides higher energy content compared to milk yoghurt. Based on the calculation for energy, which calculated the sum of total carbohydrate, protein and fat content and as okara contains more carbohydrate and protein, thus, okara yoghurt offers higher energy content than milk yoghurt. In addition, a substantial amount of dietary fibre was also detected in okara yoghurt (4.43±0 %) as the okara itself consists of insoluble fibres whereas milk yoghurt had no fibre content.

4. Conclusion

This study showed that in the development of okara yoghurt using the Yogourmet® starter culture, it was best with the fortification of skimmed milk. The incorporation of skimmed milk has improved the body, texture and taste of okara yoghurt. Furthermore, okara yoghurt also showed a higher total viable cell count, equal and acceptable physicochemical properties and chemical composition that met the Codex requirement for yoghurt. The application of okara as an ingredient in okara yoghurt had also increased the yoghurt nutritional compositions such as protein, carbohydrate and dietary fibre. Protein and dietary fibres can be converted into more valuable bioactive metabolites such as peptides, amino acids, soluble dietary fibres through microbial fermentation, which could enhance probiotic growth (due to the presence of prebiotic and the okara's cell adhesion effect) with extra health benefits, therefore, are

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potential for the development of functional ingredient for human consumption.

Conflict of interest

The authors declare no conflict of interest.

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