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Effects of sonication condition on milk-soymilk yogurt properties

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Abstract

The present work was aimed to develop a new type of yogurt made from a mixture of milk and soymilk. Balanced Incomplete Block Ranking Test and 9-Point Hedonic Scale were employed in order to determine the best formulation. The effects of sonication condition (time and temperature) were evaluated on the developed milk-soymilk yogurt properties (viscosity, texture, and syneresis). Microscopic photographs were used to check the size distribution of fat globules. The sensory evaluations showed that milk to soymilk ratio of 3:1 was the best formulation. Moreover, proximate analysis was carried out in order to determine the nutritional values of the product. It was found that sonication had positive effects on the yogurt properties such as viscosity, firmness, cohesiveness, and consistency. However, the syneresis of the yogurt sample did increase when compared with the control.

Introduction

Milk, a widely consumed dairy product, is a complete food as it contains nearly all the nutrients such as carbohydrates, proteins, fats, minerals, as well as vitamins required by humans (Salau, 2012). Milk is also a rich source of calcium which is important in the maintenance of bones and teeth during childhood and adolescence, during which the bone mass is being built. Milk consumption is therefore important to reduce the risk of osteoporosis (NCCFN, 2005).

Soymilk is a popular traditional non-dairy product in China and other Asian countries such as Japan, Korea, Singapore, and Thailand (Jooyandeh, 2011). Basically, soymilk is the liquid extraction of soybean (*Glycine max*), a type of legume which is commonly available and grows well in wide range of soil (Salau, 2012). Most plant proteins are incomplete proteins which lack in one or more essential amino acids. However, legumes such as soybeans are an exception as they contain high amount of proteins (Jooyandeh, 2011). The quality of soybean proteins which contain a rich source of amino acids with good balance is comparable to milk. In addition, soymilk is also relatively cheaper than cow's milk (Hajirostamloo, 2009).

Yogurt is a type of coagulated milk product which is globally consumed. It is thought to be one of the oldest fermentation products which originated from the Middle East. The original production of fermented milk derived from the need to extend the shelf life of milk (Tamime and Robisons, 2007). Basically, yogurt is obtained through lactic acid fermentation by the addition of starter culture which converts lactose, the main carbohydrate in milk, to lactic acid. The conventional microorganisms which have been used as starter cultures are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Serra *et al*., 2009). Yogurt is popular for its health benefits and contains the digestible form of all the nutrients present in milk (Olugbuyiro and Oseh, 2011). It is tolerated by lactose intolerance population, who are lactase-deficient, due to the simpler form of lactose that can be easily digested by the body. Generally, the nutritional constituents of yogurt are derived mainly from three components which are the milk used, fermentation products due to lactic acid bacteria and the added ingredients by manufacturers

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(Vargas *et al*., 2008). Yogurt is an excellent source of protein, riboflavin (vitamin B_2), niacin (vitamin B_3), cobalamin (vitamin B_{12}), and minerals such as calcium, phosphorus, magnesium, and zinc. Regular consumption of yogurt can help in the maintenance of gastrointestinal health and improvement of the immune system (Hui and Özgül Evranuz, 2012).

In recent years, there has been an increasing demand for the development of new processing methods in the food industry. These new methods should have minimal impact on the nutritional contents and quality of the produced food. Sonication which is also known as ultra-sonication is one of these developed cost-effective food processing methods, and it utilises ultrasound to process food (Jeličić *et al*., 2012). Ultrasound can be defined as a form of energy generated by sound waves with frequencies higher than 20 kHz which is unable to be detected by human ear (Dolatowski *et al*., 2007). In sonication, longitudinal waves generated by the sound energy passes through the medium in a continuous wave-type motion, agitates and puts stresses on the medium particles, thereby producing high energy (Gordon and Pilosof, 2010). Different physical, chemical, and biochemical effects can be observed on the medium during the sonication process.

Although soymilk has many advantages, the undesirable beany flavour of soymilk has no market values because sensory properties such as aroma and taste will affect consumers' purchasing decision. Hence, there is a need to solve this problem, and one of the solutions would be mixing milk and soymilk. Thus far, milk-soymilk yogurt has not been developed, hence, not yet available in the market, and there is a lack of knowledge on improving the undesirable taste through mixing milk and soymilk.

The present work was conducted to introduce a new type of yogurt made from a mixture of milk and soymilk in order to improve its sensory attributes, and to evaluate the effects of sonication condition on the developed yogurt's properties such as viscosity, texture, and syneresis as well as the size distribution of fat globules.

Materials and methods

Materials

Reduced fat milk powder was provided by Fernleaf (Sabah, Malaysia). Soymilk powder was purchased from Melilea (Melilea Sdn. Bhd., Malaysia). The proximate compositions of reduced fat milk and soymilk are shown in Table 1. The starter culture (*S. thermophilus* and *L. bulgaricus*) was provided by Yógourment (Lyo-San Inc., Canada).

Table 1. Proximate composition of milk and soymilk.

Proximate composition	Milk	Soymilk
Moisture (%)	86.11 ± 0.12	88.08 ± 0.10
Total solid (%)	13.90 ± 0.13	11.92 ± 0.07
Ash (%)	0.77 ± 0.02	0.35 ± 0.03
Protein (%)	2.82 ± 0.03	3.29 ± 0.06
Fat (%)	1.35 ± 0.04	0.66 ± 0.01
Carbohydrate (%)	8.97 ± 0.08	7.63 ± 0.01
Lactose (g)	1.42 ± 0.06	-
Dietary fibre (mg)	-	4.81 ± 0.07
Total energy vale (kcal)	59.27 ± 0.83	49.68 ± 0.08

Sample preparation

Milk and soymilk were prepared by adding 82.5 g milk powder or soymilk powder into 375 mL of water. Then, both types of milk were mixed based on different product formulations (Table 2). Next, 2.5 g of yogurt starter (Food and Drug Administration standard yogurt starter culture) was added to each formula by volume. After that, they were incubated at 43°C for 7 h. The milk-soymilk yogurts were cooled down and refrigerated at 4°C to stop the fermentation process. Fermentation stopped when the mixture achieved a pH in the range of 4.2 - 4.4 (Osman and Razig, 2010).

Sensory evaluation

Balanced incomplete block (BIB) design and 9-Point Hedonic Test were employed for sensory evaluation in order to choose the best formulation. Then, the best formulation was subjected to proximate analysis.

Balanced incomplete block design

A total number of nine samples including two control samples (yogurt and soy yogurt) and seven formulations of milk-soymilk yogurt were compared (Table 2). Generally, panellist cannot evaluate more than four to six samples at one time due to sensory fatigue. Hence, balanced incomplete block (BIB) design was used as this design allows sensory analysts to obtain consistent and reliable data from their panellists even when the total number of samples in the study is greater than the number that can be evaluated before sensory fatigue sets in. Typically, it is a method to be used when the number of samples to be compared is from 6 to 12 or at most 16. In BIB design, treatments are assigned randomly in a balanced way so that each treatment receives the same number of assessors and degree of replication. Instead of presenting all the samples in one large block, BIB design allows sensory analysts to present them in smaller blocks. The BIB design is based on blocks containing k samples, evaluated at r times and every pair of samples are evaluated together within a block at λ times. The entire BIB design was repeated three times in the present work in order to obtain a sufficiently large number of total replications, where the number of repetitions of the fundamental design is denoted by *p* (Meilgaard *et al*., 2006). According to Cochran and Cox (1957), a total of 36 randomly selected panellists were required to complete the BIB Ranking Test in order to choose the four most preferred formulations. They were asked to evaluate the samples by ranking them in a descending order from 1 to 6, with 1 indicates the sample they like the most and 6 indicates the sample they dislike the most (Lawless and Heymann, 2010). Table 1 shows the plan for BIB Ranking Test.

Table 2. Product formulations of milk-soymilk, the balanced incomplete block (BIB) design, and rank sum for each yogurt sample.

Formula	Milk:Soymilk Ratio	Sample	Rank Sum
1 (S3)	1:1	S3	61[a]
2 (S4)	1:2	S7	64[ac]
3 (S5)	1:3	S8	65[ab]
4 (S6)	2:1	S9	69[ac]
5 (S7)	2:3	S4	83[bc]
6 (S8)	3:1	S5	83[ab]
7 (S9)	3:2	S6	95[be]
		S1	116[de]
		S2	120[d]

Block	Sample Pairing					
1	1	2	4	5	7	8
2	2	3	5	6	8	9
3	1	3	4	6	7	9
4	1	2	5	6	7	9
5	1	3	4	5	8	9
6	2	3	4	6	7	8
7	1	3	5	6	7	8
8	1	2	4	6	8	9
9	2	3	4	5	7	9
10	4	5	6	7	8	9
11	1	2	3	4	5	6
12	1	2	3	7	8	9

Rank sum followed by same superscripts are not significantly different at 5% significance level. S1 = milk (100%), S2 = soymilk (100%). Note: t = 9, k = 6, r = 8, b = 12, λ = 5, E = 0.94, where t = total number of samples, k = number of samples evaluated by each panellist during a single session, r = number of times each sample is evaluated, b = number of blocks, and λ = number of times each pairs of samples are evaluated together. Source: Cochran and Cox (1957).

9-Point Hedonic Test

9-Point Hedonic Test is a type of sensory test which is used to quantify the degree of liking or disliking of a product. It is vital to carry out sensory analysis of a newly developed product because it has a great impact on consumers' choice, which determines their acceptance and largely influences their purchasing decisions (Andrés *et al*., 2015). The selected most preferred four formulations from BIB Ranking Test together with two control samples (yogurt and soy yogurt) were further tested in 9-Point Hedonic Test in order to determine the best formulation. Fifty untrained panellists were selected randomly in order to complete the 9-Point Hedonic Test and the sensory test was conducted in the sensory laboratory. Samples were drawn from the refrigerator immediately before serving to the panellists. Each panellist was served with six samples, labelled with three-digit random codes. The evaluated sensory attributes included colour, aroma, taste, texture, and overall acceptability. Sensory scorecard consisted of the following hedonic ratings: dislike extremely (1), dislike very much (2), dislike moderately (3), dislike slightly (4), neither like nor dislike (5), like slightly (6), like moderately (7), like very much (8) and like extremely (9). Panellists were instructed to rinse their mouth with drinking water in between each yogurt sample to cleanse their palate (Meilgaard *et al*., 2006; Stone *et al*., 2012).

Proximate analysis of developed milk-soymilk yogurt

The moisture, total solid, ash, crude fat, and crude protein contents were determined by oven method, direct heating method, Soxhlet solvent extraction method, and Kjeldahl method, respectively, as described by AOAC (2000). The total solid non-fat content was determined by subtracting the percentage of fat content from total solid content (Ehirim and Onyeneke, 2013). The total carbohydrate content was determined by subtracting contents of moisture, ash, protein, and fat from 100 (Obadina *et al*., 2013).

Ultrasound sample preparation

The developed milk-soymilk yogurt (best formulation) was prepared by mixing of milk and soymilk in the ratio of 3:1 (S8) based on the preference of the panellists. 20.62 g of soymilk powder and 61.87 g of milk powder were measured and added into 375 mL of water. The milk-soymilk was sonicated using a sonicator bath (Bransonic 8510E-DTH, USA) at different conditions (temperature: 25, 30, 35 and 40°C; time: 2, 4, 6 and 8 min). In contrast, the control milk-soymilk was not sonicated and was continued to the next step. Then, 2.5 g of yogurt starter culture was added to the mix. It was then incubated at 43°C for 7 h in an incubator (Friocell 55, Germany). The yogurt produced by sonicated milk-soymilk and control yogurt were then refrigerated at 4°C in a cold room (Thermal-Matic, Malaysia) to stop the fermentation process (Osman and Razig, 2010).

Morphology structure

Microstructure of the yogurts was examined by using a scanning electron microscope (SEM) (Hitachi S-3400N, Japan). The yogurts were examined in the microscope chamber at a temperature of -30.0°C and voltage of 15 kV (Duan *et al*., 2018). Reproducibility of the SEM images were assured by taking at least three pictures of each sample at final magnifications (×10).

Texture

The texture of each yogurt sample was measured using a texture profile analyser (Stable Micro System, TA.XT plus, UK) following the method of Patel and Roy (2016). Back extrusion test was performed on each sample. The sample was placed in a standard back extrusion container with 50 mm diameter approximately 75% full. It was then placed centrally under the back extrusion cell with 35 mm disc diameter. The settings of the texture analyser were a 5 kg load cell, pre-test speed and a test speed of 1.0 mm/s, the post-test speed of 10.0 mm/s and distance of 30 mm. Texture attributes including firmness, consistency, cohesiveness, and index of the viscosity of each sample were measured in triplicate.

Viscosity

Viscosity of each yogurt sample was measured following the method of Akalin *et al*. (2012) with slight modification. The viscosity of each sample was measured after stirring the product for 60 s, using a Brookfield viscometer (Brookfield DV-E, USA) with spindle No.4 at a spindle rotation speed of 20 rpm. Each experiment for viscosity measurement was done in triplicate.

Syneresis

Syneresis of each yogurt sample was measured following the method of Riener *et al*. (2010). Briefly, 30 g of each sample was spread on a Whatman No.1 filter paper in a funnel, which was placed on top of a 50 mL measuring cylinder. The measuring cylinder was then kept at 4°C for 5 h and the volume of liquid collected was recorded. Each experiment for syneresis was done in triplicate. The syneresis percentage was calculated as (liquid weight / initial sample weight) ×100.

Statistical analysis

All the statistical analysis was performed using IBM SPSS Statistical software version 22.0 and significance differences were determined at $p < 0.05$. The results of BIB Ranking Test and 9-Point Hedonic test were subjected to Friedman test and Kruskal-Wallis analysis, respectively. Two-way analysis of variance (ANOVA) and Tukey's post hoc test was performed on the data obtained to determine the effects of sonication temperature and time on the properties of control and milk-soymilk yogurt samples.

Results and discussion

Sensory evaluation

Balanced Incomplete Block (BIB) Ranking Test

A summary of rank sum for each yogurt sample in ascending order after performing BIB Ranking Test is presented in Table 1. As can been seen from Table 1, S3, S7, S8, and S9 were the four most preferred samples by the panellists, whereas S2 was the least preferred. This finding indicates that all the milk-soymilk yogurts have better rank sums than both yogurt (S1) and soy yogurt (S2), which means panellists like the milk-soymilk yogurts more than the yogurt and soy yogurt. However, there was no significant difference among the top four most preferred samples (S3, S7, S8, and S9) with rank sums of 61, 64, 65, and 69, respectively (Table 2). Therefore, S3, S7, S8, and S9 were selected as the top four most preferred formulations together with two control yogurts (S1 and S2) to be further evaluated using 9-Point Hedonic Test in order to get the best formulation and to examine whether there were any significant differences in terms of sensory attributes among these six yogurt samples.

9-Point Hedonic Test

Table 3 shows the sample mean scores for the samples S1, S2, S3, S7, S8, and S9 in terms of colour, aroma, taste, mouth feel, and overall acceptability. Regarding the colour, S3 obtained the highest mean score and S2 (soy yogurt) the lowest (Table 3). Results indicated that mixing of milk and soymilk did not significantly ($p > 0.05$) affect the colour of yogurt produced.

Besides colour, aroma also plays an important role in increasing consumers' demand towards a product. A comparison was done between all the milk-soymilk formulations, denoted by S3, S7, S8, and S9. However, the results obtained did not illustrate a clear trend on panellists' preference. Based on Table

Table 3. Developed milk-soymilk yogurt's mean scores for colour, aroma, taste, mouth feel, and overall acceptability.

Attributes	S1	S2	S3	S7	S8	S9
Colour	6.02 ± 1.57^{a}	6.00 ± 1.51^{a}	6.62 ± 1.21^{a}	6.60 ± 1.11^{a}	6.02 ± 1.68^{a}	6.38 ± 1.31^{a}
Aroma	5.96 ± 1.77^{bcd}	5.10 ± 1.31^{f}	5.62 ± 1.44^{cdef}	5.30 ± 1.69^{def}	6.40 ± 1.64^{ab}	5.18 ± 1.56^{ef}
Taste	5.78 ± 1.57^{cd}	4.46 ± 1.72^{f}	5.80 ± 1.61^{bcd}	5.12 ± 1.42^{e}	6.50 ± 1.64^{a}	5.42 ± 2.16^{de}
Mouth feel	5.66 ± 1.93^{def}	5.56 ± 1.43^{ef}	5.90 ± 1.15^{bcdef}	5.70 ± 1.27^{cdef}	6.52 ± 1.37^{a}	5.42 ± 1.74^{f}
Overall Acceptability	5.66 ± 1.53^{cde}	4.74 ± 1.56^{f}	5.76 ± 1.27^{bcde}	5.36 ± 1.50^{ef}	6.52 ± 1.37^{a}	5.54 ± 1.94^{de}

Means with same superscripts in the same row are not significantly different at 5% significant level. S1 = milk (100%), S2 = soymilk (100%), S3 = milk:soymilk (1:1), S4 = milk:soymilk (1:2), S5 = milk:soymilk (1:3), S6 = milk:soymilk (2:1), S7 = milk:soymilk (2:3), S8 = milk:soymilk (3:1), and S9 = milk:soymilk (3:2).

3, S8 (milk to soymilk = 3:1) had the highest mean score in terms of aroma followed by S1, S3, S7, S9, and S2. This result showed that, although the beany aroma of soymilk may not be completely masked by the milk, the combination of milk and soymilk for S8 was more favourable than milky aroma of yogurt.

As can be seen from Table 3, milk yogurt (S1) scored better than soy yogurt (S2) in terms of taste and there was a significant difference ($p < 0.05$) between these two samples. Furthermore, there were significant differences between S8 and the other yogurt samples, including yogurt and soy yogurt.

The most noticeable finding (Table 3) is that S8 had the highest mean score among all milk-soymilk yogurts, yogurt, and soy yogurt in terms of mouth feel. There was no significant difference ($p > 0.05$) between other milk-soymilk yogurts with yogurt and soy yogurt.

Based on Table 3, S8 was the most preferred yogurt by the panellists in terms of overall acceptability followed by S3, S1, S9, and S7. The lowest score was received by S2 (soy yogurt). Therefore, S8 (milk: soymilk = 3:1) could be a new type of yogurt made from mixture of milk and soymilk as it was well accepted by the panellists.

Proximate analysis

Table 4 presents the proximate compositions for the developed milk-soymilk yogurt (control). Water is a major constituent of most food products. The moisture content of food varies greatly according to different type of products. It is important to determine the moisture content of food because it will affect the growth of microorganisms, thus reducing the shelf life of food. On top of that, determination of moisture content is also necessary to calculate the content of other food composition. The mean moisture content of developed milk-soymilk yogurt was 86.78%. Total solid refers to the dry matter that remains after moisture removal (Nielson, 2010). The total solid content of a product is inversely proportional to the moisture content. The higher the moisture content, the lower the total solid content. It is important to study on the total solid content of yogurt because it is associated with the texture of yogurt produced. Increased yogurt viscosity is observed when the total solid content of milk is increased, which means higher total solid content contributes to desirable yogurt texture (Lee and Lucey, 2010). As illustrated in Table 4, the total solid content of developed milk-soymilk yogurt was 13.23%.

Total solid non-fat (SNF) components of milk mainly consist of lactose, protein, and minerals. Based on a review done by Sfakianakis and Tzia (2014), SNF content of milk varies from 11% to 14%, whereas SNF of the yogurt ranges from 9% to 16%. The higher the SNF level, the higher the resulting yogurt's viscosity and firmness. As can be seen from Table 4, the total solid non-fat content of developed milk-soymilk yogurt was 11.87%.

Ash value is an index of mineral content obtained through the ashing process. Basically, ash refers to the inorganic whitish residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. The ash content of foods is different with their nature properties. As shown in Table 4, the ash content of milk-soymilk yogurt was 0.62%.

Table 4. Proximate compositions of developed milk-soymilk yogurt.

Nutrients	Contents (%)
Moisture	86.78 ± 0.02
Total Solid	13.23 ± 0.02
Total Solid Non-Fat	11.87 ± 0.01
Ash	0.62 ± 0.01
Fat	1.36 ± 0.01
Protein	3.52 ± 0.03
Carbohydrate	7.73 ± 0.06

Crude fat refers to the crude mixture of fat-soluble material present in the sample. It is also known as the ether extract or free lipid content. It is vital to determine the fat content of yogurt because fat has a nutritional role which serves as a main source of energy. Besides, fat also plays an important sensory role in improving the consistency of yogurt (Mourad *et al*., 2014). Increasing the fat content of milk results in an increase in consistency and viscosity of the yogurt (Sfakianakis and Tzia, 2014). The amount of fat content of developed milk-soymilk yogurt was 1.36% (Table 4).

Crude protein is the approximate amount of total protein in foods. Basically, a crude protein contains nitrogen from not only protein but also non-protein sources. As shown in Table 4, the protein content of milk-soymilk yogurt was 3.52%.

It is essential to determine the carbohydrate content in food because carbohydrate is important as a major source of energy and it is also required for nutrition labelling of a food product (Nielson, 2010). Based on Table 4, the total carbohydrate content of developed milk-soymilk yogurt was 7.73%.

Fat-globule size

Fat globules in milk are usually large and their particle sizes can be easily affected by external stress such as sonication. Its average size usually ranges from 3.4 µm to 4.5 µm (Chandan, 2006). The size of fat-globule particles correlates linearly with the oral smoothness of yogurt, and yogurt with smaller fat-globule size is desired by consumers. For particle size distribution, the median diameter, known as D50 or Dv0.5, is used to characterise the size of the yogurts' fat-globule particles which indicates the value of particle diameter at 50% in the cumulative distribution (Cayot *et al*., 2008). The results obtained for D50 of the yogurt produced by sonicated milk-soymilk at different sonication times and temperatures are given in Table 5. There were no significant main effects ($p > 0.05$) of time and temperature on particle size distribution of the yogurt produced by sonicated milk-soymilk. Additionally, interaction of temperature and time showed no significant ($p > 0.05$) effects on particle size distribution of the yogurt produced by sonicated milk-soymilk.

Microscopic observations were primarily used to check for fat globule clusters or other abnormalities. Figure 1 demonstrates microstructure images of yogurt samples. From these images, it is apparent that control yogurt appeared to have less branching as compared to yogurt produced by sonicated milk-soymilk. According to Ciron *et al*. (2010), fat globules in yogurt produced by sonicated milk-soymilk were small in size thus allowing them to be incorporated within the protein matrix and formed highly cross-linked networks easily.

Viscosity

Viscosity of yogurt is an important aspect as it affects the mouth feel sensation and acceptability of consumers. Yogurt usually has high viscosity which indicates that it has a thick and concentrated consistency. It is important for yogurt to flow in the mouth; but it should resist flow linearly with time when stress is applied. Viscosity of yogurt can be influenced by factors such as particle size (Vijayakumar *et al*., 2015).

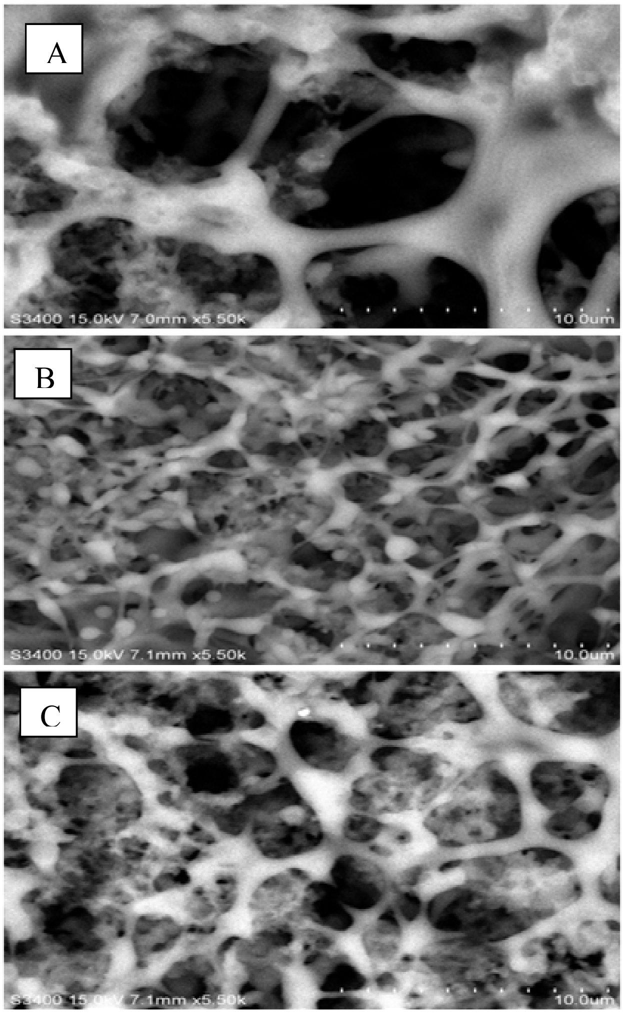

Figure 1. SEM microstructure images of (A) control milk-soymilk yogurt, (B) milk-soymilk yogurt sonicated at 30°C for 6 min, and (C) milk-soymilk yogurt sonicated at 30°C for 8 min.

Table 5 shows that there was a significant difference between the viscosities of the yogurt produced by sonicated milk-soymilk at different temperatures ($p < 0.05$). The viscosity of the yogurt increased with temperature from 0°C until 25°C and then decreased at 30°C but increased again until 40°C. Additionally, the viscosity of the yogurt produced by sonicated milk-soymilk at 30°C was the lowest and it was significantly lower than the control. As temperature reached 30°C, it might have favoured the penetration of the ultrasonic waves, decreased the implosion force of the formed bubbles during cavitation, and reduced the viscosity of the liquid (Villamiel and de Jong, 2000). Other than that, the decrease in viscosity could be due to the contraction of gel with the rearrangement of casein micelles. From the results, the yogurt produced by sonicated milk-soymilk at 40°C had the highest viscosity value. Other than the yogurt produced by sonicated milk-soymilk at 30°C which had the lowest viscosity, it was also indicated that the viscosity of the yogurt produced by sonicated milk-soymilk was significantly higher than control ($p < 0.05$). The viscosity of the yogurt increased significantly as the temperature increased. This could be due to the formation of fat aggregates during heat exposure. Vijayakumar *et al*. (2015) found that as temperature increased, whey proteins unfolded and underwent sulfhydryl-disulphide interchange reactions. This increased the bonding between the denatured whey proteins and casein micelles, forming yogurt matrix with more ease, thus causing an increase in viscosity.

Besides that, there was a significant difference between the viscosities of the yogurt produced by sonicated milk-soymilk for different times ($p < 0.05$) as shown in Table 5. Viscosity of the yogurt produced by sonicated milk-soymilk was significantly higher than control and they increased with increased sonication time. Control had the lowest viscosity whereas the yogurt produced by sonicated milk-soymilk for 8 min had the highest. This was due to the increased in gel strength of yogurt with increasing sonication time (Ashokkumar *et al*., 2010). As sonication time increased, it could increase the loss of tertiary structure of globular proteins due to cavitation which would lead to an increase in volume of protein. The rearrangement of protein and protein-protein contacts would then increase the formation of protein aggregates over time leading to increased viscosity (Sahan *et al*., 2008). Moreover, there was a significant interaction ($p < 0.05$) between the effects of temperature and time on viscosity of yogurt.

Syneresis

Syneresis is one of the crucial parameters of yogurt quality. It is the shrinkage of gel which occurs simultaneously with the expulsion of liquid or whey separation (Vareltzis *et al*., 2016). Syneresis is related to the instability of the gel network and the lack of its ability to entrap all the serum phase. The unstable gel network can be due to the increase in rearrangement of gel matrix or damage to the weak gel network (Lee and Lucey, 2010).

The time and temperature showed a significant interaction ($p < 0.05$) on syneresis of yogurts. As shown in Table 5, results indicated that there was a significant difference in the syneresis of control and yogurt produced by sonicated milk-soymilk at different temperatures ($p < 0.05$). The syneresis of the yogurt produced by sonicated milk-soymilk increased from 0°C until 30°C and then decreased until 40°C. Based on Table 5, the treated yogurt has significantly higher syneresis than control.

In the aspect of sonication time, there was significant difference in the syneresis of control and yogurt produced by sonicated milk-soymilk ($p < 0.05$) as shown in Table 5. From the results, there was no significant difference in the syneresis of yogurt produced by sonicated milk-soymilk for 2, 4, 6 and 8 min ($p > 0.05$). The syneresis of the yogurt produced by sonicated milk-soymilk was significantly higher than control and it increased with increased sonication time. In contrast, Riener *et al*. (2010) reported that yogurt produced from milk sonicated for 10 min at 45°C had lower syneresis level than unsonicated milk. Wu *et al*. (2000) also reported that yogurt produced from sonicated milk had reduced syneresis due to the change in water holding capacity of the milk proteins.

Texture

Textural characteristics of yogurt are affected by the structural arrangement of its network. Four texture parameters of yogurt samples, namely firmness, consistency, cohesiveness, and index of viscosity were analysed in the present work. These parameters are viscoelasticity measurements and can be obtained with the back extrusion method.

Firmness is the force necessary to attain a given deformation (Walia *et al*., 2013). One of the most important textural characteristics of yogurt is firmness or curd texture. Based on Table 5, there was significant difference ($p < 0.05$) between the firmness of yogurt produced by sonicated milk-soymilk at different sonication temperatures. The firmness of yogurt produced by sonicated milk-soymilk was significantly higher than control. The yogurt produced by sonicated milk-soymilk at 40°C had the highest firmness value. This might be due to the increasing thermal denaturation of whey proteins, especially β-lactoglobulin, as the heat increased. Partially denatured whey proteins bind with casein micelles in the milk by disulphide bridging to form strong networks and increase the gel strength of yogurt (Ozcan, 2013). Additionally, they could associate with large number of small fat globules present in the yogurt produced by sonicated milk-soymilk to form a good gel structure and attain increased firmness (Riener *et al*., 2010).

Furthermore, Table 5 also indicates that the firmness of yogurt produced by sonicated milk-

Table 5. D50, viscosity, syneresis, and texture of yogurt produced by sonicated milk-soymilk at different sonication times and temperatures.

Temp. (°C)	Time (min)	D50 (μm)	Viscosity (mPa/s)	Syneresis (%)	Texture			
					Firmness (g)	Consistency (g/s)	Cohesiveness (g)	Index of viscosity (g/s)
0	0	2.10 ± 1.13^{aA}	3735.00 ± 7.07^{eD}	34.73 ± 0.11^{Cd}	26.58 ± 0.08^{cC}	717.33 ± 1.06^{dE}	-15.55 ± 0.93^{cD}	-36.57 ± 0.63^{dD}
25	2	0.99 ± 0.44^{aA}	3935.00 ± 7.07^{cC}	39.38 ± 0.21^{bB}	36.62 ± 1.17^{bB}	1015.09 ± 0.92^{cB}	-23.11 ± 0.87^{bAB}	-62.56 ± 0.77^{bB}
	4	1.11 ± 0.64^{aA}	3890.00 ± 14.14^{dC}	40.89 ± 0.36^{bB}	36.87 ± 0.45^{aB}	1018.57 ± 0.84^{bB}	-22.73 ± 0.57^{aAB}	-61.90 ± 0.49^{aB}
	6	1.06 ± 0.57^{aA}	3930.00 ± 14.14^{bC}	42.35 ± 0.08^{abB}	34.08 ± 1.04^{aB}	964.76 ± 0.57^{bB}	-21.40 ± 0.85^{aAB}	-54.33 ± 0.97^{cB}
	8	1.18 ± 0.64^{aA}	3935.00 ± 7.07^{aC}	42.75 ± 0.13^{aB}	33.92 ± 0.45^{aB}	900.58 ± 1.08^{aB}	-21.41 ± 0.91^{Aab}	-49.67 ± 0.96^{aB}
30	2	2.93 ± 1.75^{aA}	3490.00 ± 14.14^{cE}	43.12 ± 0.18^{bA}	32.66 ± 1.20^{bB}	827.62 ± 1.37^{cC}	-20.18 ± 0.93^{bB}	-46.54 ± 1.04^{bC}
	4	2.04 ± 1.93^{aA}	3430.00 ± 14.14^{dE}	44.21 ± 0.74^{bA}	36.75 ± 0.92^{aB}	996.04 ± 0.59^{bC}	-22.46 ± 0.82^{aB}	-57.92 ± 0.91^{aC}
	6	1.64 ± 1.26^{aA}	3610.00 ± 14.14^{bE}	43.03 ± 0.68^{abA}	33.29 ± 0.45^{aB}	890.39 ± 1.47^{bC}	-20.57 ± 1.24^{aB}	-46.91 ± 0.25^{cC}
	8	0.66 ± 0.00^{aA}	3780.00 ± 7.00^{aE}	44.24 ± 0.83^{Aa}	35.75 ± 0.58^{aB}	1010.86 ± 0.76^{aC}	-21.91 ± 0.23^{aB}	-57.52 ± 1.23^{aC}
35	2	2.26 ± 2.25^{aA}	4495.00 ± 7.07^{cB}	41.52 ± 0.33^{bB}	30.92 ± 1.06^{bB}	759.5 ± 1.63^{cD}	-12.53 ± 0.93^{bC}	-75.05 ± 0.98^{bA}
	4	2.81 ± 0.59^{aA}	4385.00 ± 7.07^{dB}	40.55 ± 0.31^{bB}	35.86 ± 0.46^{aB}	813.59 ± 1.46^{bD}	-18.08 ± 1.02^{aC}	-78.82 ± 0.66^{aA}
	6	2.32 ± 0.91^{aA}	4680.00 ± 0.00^{bB}	41.33 ± 0.83^{abB}	37.37 ± 0.81^{aB}	871.89 ± 0.60^{bD}	-21.38 ± 1.02^{aC}	-52.43 ± 1.14^{cA}
	8	0.64 ± 0.01^{aA}	4635.00 ± 7.07^{aB}	42.27 ± 0.59^{Ab}	37.13 ± 1.26^{aB}	948.71 ± 1.22^{aD}	-17.78 ± 0.94^{aC}	-78.61 ± 0.97^{aA}
40	2	1.47 ± 0.37^{aA}	4770.00 ± 14.14^{cA}	40.14 ± 0.85^{bC}	36.94 ± 0.67^{bA}	902.55 ± 1.54^{cA}	-19.68 ± 1.03^{bA}	-46.65 ± 0.97^{bB}
	4	1.13 ± 0.62^{aA}	4765.00 ± 7.07^{dA}	38.65 ± 0.08^{bC}	37.81 ± 0.51^{aA}	957.95 ± 1.51^{bA}	-26.19 ± 1.16^{aA}	-55.63 ± 0.62^{aB}
	6	1.16 ± 0.14^{aA}	5170.00 ± 14.14^{bA}	40.08 ± 0.36^{abC}	41.45 ± 0.89^{aA}	1067.92 ± 1.37^{bA}	-22.10 ± 0.29^{aA}	-52.79 ± 0.35^{cB}
	8	1.06 ± 0.04^{aA}	5710.00 ± 14.14^{aA}	41.14 ± 0.01^{aC}	45.39 ± 0.90^{aA}	1165.79 ± 1.47^{aA}	-25.73 ± 0.73^{aA}	-70.48 ± 0.75^{aB}

Means with different lower-case letters (a-e) in a column are significantly ($p < 0.05$) different between different times. Means with different upper-case letters (A-E) in a column are significantly ($p < 0.05$) different between different temperatures.

soymilk for 4, 6, and 8 min was not significantly different ($p > 0.05$). The control had the lowest firmness value whereas the yogurt produced by sonicated milk-soymilk for 8 min has the highest. Increased sonication time could increase the denaturation of whey protein structure and decrease the size of fat globules in the milk. These unfolded protein structures could then bind with casein micelles or the small fat globules with more ease, thus increasing the gel strength and firmness of the yogurt samples (Ashokkumar *et al*., 2010).

Consistency is a viscosity guide of yogurt. The back extrusion rig is often used to measure consistency of yogurts. Consistency relates to the firmness, thickness or viscosity of a liquid or semi-solid fluid. The intermolecular attraction which holds the elements of the yogurt is the main factor affecting its consistency. Yogurt which is more viscous or thicker has a higher consistency value (Yilmaz-Ersan *et al*., 2017). In the present work, the consistency was significantly ($p < 0.05$) affected by time and temperature. Besides that, there was a significant interaction effect between temperature and time on consistency of yogurt ($p < 0.05$). The consistency of the yogurt produced by sonicated milk-soymilk increased from 0°C until 25°C and then decreased at 30°C until 35°C but increased again at 40°C. Control had the lowest consistency value whereas the yogurt produced by sonicated milk-soymilk at 40°C had the highest. The increased denaturation of whey protein in relation to higher sonication temperature was more susceptible to associate with casein and casein micelles. In addition, the casein micelles tend to aggregate due to reduction of repulsive charge (Morand *et al*., 2011). Thus, denatured whey protein could act as bridging material between casein micelles and formed bonds with more ease, resulting in a stronger yogurt structure. Furthermore, the consistency of yogurt produced by sonicated milk-soymilk significantly increased with increasing sonication time ($p < 0.05$) as shown in Table 5. There was no significant difference in the consistency of yogurt produced by sonicated milk-soymilk for 4 and 6 min ($p > 0.05$). Control had the lowest consistency value whereas the yogurt samples treated for 8 min had the highest.

Cohesiveness is the tendency of yogurt to cohere or stick together. It indicates how well the semi-solid product such as yogurt withstands a second deformation relative to how it behaved under the first deformation (Walia *et al*., 2013). Cohesiveness also relates to the strength of the internal bond in the yogurt structure. Cohesive value of a yogurt is provided in the negative area of the graph in back extrusion method and therefore the value is denoted with a negative sign. Higher negative value indicates more cohesiveness in the product (Patel and Roy, 2016). It was found in the present work that the treated yogurt was more cohesive than control. The reduction in fat particle size due to longer sonication time allowed more incorporation of small fat particles into the interspace of the protein matrix (Ciron *et al*., 2010). However, there was no significant difference ($p > 0.05$) in the cohesive value of yogurt produced by sonicated milk-soymilk at 25°C and 40°C. There was also no significant difference in the cohesive value of yogurt produced by sonicated milk-soymilk at 25°C and 30°C ($p > 0.05$). From the results, the cohesive value of the yogurt produced by sonicated milk-soymilk increased from 0°C until 25°C and then decreased at 30°C until 35°C but increased again at 40°C. This could be due to higher whey protein denaturation at higher temperatures, and higher rate of bonding between denatured whey protein and casein which led to stronger gel network and smoother yogurt structure. Besides that, results shown in Table 5 also indicate that the cohesive value of yogurt produced by sonicated milk-soymilk were significantly higher than control ($p < 0.05$). The cohesive value of yogurt produced by sonicated milk-soymilk for 4, 6, and 8 min was not significantly different ($p > 0.05$). The control had the lowest cohesive value whereas the yogurt produced by sonicated milk-soymilk for 4 min had the highest. This might be due to the stronger gel network formed by higher rate of bonding of protein matrix and small fat globules of which size were reduced in the exposure to longer sonication time.

Index of viscosity indicates how the viscosity of fluid changes with stress. High values of index of viscosity indicate that the yogurt is more resistant to gradual deformation of shear stress (Yilmaz-Ersan *et al*., 2017). It is provided in the negative area of the graph in back extrusion method and therefore the value is denoted with a negative sign. In the present work, the index of viscosity was significantly ($p < 0.05$) influenced by time and temperature. Furthermore, there was a significant interaction effects between temperature and time on index of viscosity of yogurt ($p < 0.05$). Based on Table 5, there was a significant difference ($p < 0.05$) between the index of viscosity of yogurt produced by sonicated milk-soymilk at different sonication temperatures. However, the index of viscosity of yogurt produced by sonicated milk-soymilk at 25°C and 40°C was not significantly different ($p > 0.05$). There was fluctuation in the index of viscosity of the yogurt produced by sonicated milk-soymilk with increasing temperature. The control had the lowest index of viscosity whereas the yogurt produced by sonicated milk-soymilk at 35°C had the highest. The significantly higher index of viscosity of yogurt produced by sonicated milk-soymilk indicated that they could be more resistant to gradual deformation of shear stress than control. This could be due to the higher gel strength as a result of increasing denatured whey protein and casein bonding at higher temperature. However, there was no significant difference in the index of viscosity of treated yogurt for 4 and 8 min ($p > 0.05$). The significantly higher index of viscosity of treated yogurt samples demonstrated that they could be more resistant to gradual deformation of shear stress than control. Longer sonication time could reduce the size of fat globules, thus allowing them to incorporate better into the protein network, thereby forming a tighter gel network which is more resistant to stress.

Conclusion

The present work was able to produce a new type of yogurt made from mixture of milk and soymilk. BIB Ranking and 9-Point Hedonic tests were employed for sensory evaluation in order to choose the best formulation. The result revealed that milk-soymilk yogurt with a milk to soymilk ratio of 3:1 was the most preferred based on the evaluated attributes and it successfully secured the best mean score for overall acceptability by the panellists. Based on the sensory evaluation, milk-soymilk yogurt produced in the present work was yellowish-white in colour and possessed a combination of strong milky and weak beany aroma. On top of that, it had a combination of sourness with slightly sweet taste. Furthermore, the texture of this product could be described as soft and smooth. In addition, the present work was carried out to evaluate the effect of sonication condition (time and temperature) on milk-soymilk yogurt quality. The results obtained showed that both sonication time and temperature did not significantly affect the particle size of milk-soymilk yogurt. Although there was reduction in particle size of sonicated samples, it was insignificant. On the other hand, viscosity of milk-soymilk quality was significantly affected by sonication time and temperature. Viscosity of the yogurt sample increased with increasing sonication time. Besides that, there was a significant effect of sonication temperature on yogurt texture quality including firmness, consistency, cohesiveness, and index of viscosity. These textural qualities were also significantly affected by sonication time. There was also a significant interaction between the effects of sonication temperature and time on yogurt firmness, cohesiveness, consistency, and index of viscosity. Sonication time and temperature also significantly affected the syneresis of milk-soymilk yogurt where it increased as compared to control. It was also found that sonication condition (time and temperature) did not significantly affect the particle size of milk-soymilk yogurt but significantly affected the viscosity, texture, and syneresis of milk-soymilk yogurt.

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