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NUTRITIONAL, PHYSICOCHEMICAL STABILITY, MICROBIAL SURVIVABILITY AND SENSORIAL EVALUATION OF LEGUME YOGURTS

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ABSTRACT

Legumes and probiotics are versatile food ingredients that can be incorporated into nutritious food products. In this study, legume yogurts from legume flours (8% w/w) and legume milks (16% w/w) were formulated using soybean (*Glycine max*), pigeon pea (*Cajanus cajan*), and mung bean (*Vigna radiata*). The formulations were incorporated with sugar (7%), xanthan gum (0.5%), and commercial mix cultures containing starter cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and probiotics (*L. acidophilus*, *L. Casei*, and *Bifidobacterium longum*), followed by fermentation at 37°C until they reached pH 4.5. Legume yogurts were analyzed for their chemical compositions, amino acid content, and sensory evaluation. Changes in pH, titratable acidity, color, water holding capacity (WHC), rheological properties, and microbial survivability were also evaluated during storage at 4°C for 28 days. Total solid (≥11.63%), moisture (≥81.44%), and protein (1.62 to 5.12%) contents were recorded, while glutamic acid, aspartic acid, and lysine were the most prevalent amino acids detected in the legume yogurts. During storage, the pH significantly decreased (P>0.05) while the titratable acidity increased. The legume yogurts showed shear-thinning properties (n<1) with reduced WHC during storage. The lactic acid bacteria survivability remained ≥7 log cfu/ml until the end of storage, except for *B. longum*. Desirable consistency, sweetness, and sourness were obtained from the sensorial evaluation. However, there was also an unfamiliar taste that developed from the products which affected their overall acceptability. In conclusion, legumes are great lactic acid bacteria carrier and their utilization provide a promising alternative for dairy substitutes.

Keywords: Non-dairy yogurt, Legumes, Lactic acid bacteria

INTRODUCTION

Consumption of plant-based products has grown worldwide as people contemplate healthier food alternatives. Changing perceptions of consumers on dairy products due to lactose intolerance, milk protein allergy, cholesterol content, and lifestyle changes also contributed to this trend **(Granato *et al.*, 2010)**. Consequently, the dairy alternatives market offers great appeal to a growing market segment and it is estimated to grow up to USD 14.36 billion by 2022 **(Markets and Markets, 2017)**. Furthermore, consumers are captivated by having functional foods such as plant-based yogurt with probiotics in their daily diet as the foods provide rich nutritional content and exert health benefits effects upon consumption **(Martins *et al.*, 2013)**. Soybean is the most common source used for yogurt-like products **(Cruz *et al.*, 2007; Ferragut *et al.*, 2009)**, while there are also studies conducted on cereals, grains, fruits, and vegetables **(Martins *et al.*, 2013; Mridula and Sharma, 2015; Russo *et al.*, 2016)**. Besides, legumes can also be a good potential source for plant-based yogurt production.

Legumes are recognized as a sustainable ingredient with excellent sources of protein, dietary fibers, carbohydrates, oligosaccharides, minerals, and vitamins **(Tiwari *et al.*, 2011)**. Several studies have utilized legumes milk from African yam bean **(Amakoromo *et al.*, 2012)**, *Lupinus campestris* seed **(Jiménez-Martínez *et al.*, 2003),** and peanut **(Bansal *et al.*, 2016)** in yogurt formulations, but there is limited literature on the utilization of legume flours. There are also yogurt formulations that incorporate dairy, dairy-derived, and/or animal-based ingredients such as lactose, whey protein, sodium caseinate, and gelatin which can compromise the authenticity of the plant-based foods.

Besides providing health benefits, consumers‘ acceptances of new products are also dependent on their texture and taste **(Hickisch *et al.*, 2016)**. Legumes have good techno-functional properties like emulsification and gelling abilities which make them suitable for non-dairy yogurt production. For food to provide gastrointestinal health benefits, probiotics viability should be at least 6 log cfu/ml prior to ingestion, but preferable at 7 – 9 log cfu/ml **(Martins *et al.*, 2013; Bernat *et al.*, 2015)**. Hence, legumes need to provide an optimal environmental condition that supports the growth and survival of the probiotics during storage. Fortunately, the presence of resistant starch in legumes can act as a prebiotic which can induce a symbiotic interaction with probiotics in the food matrix **(Martins *et al.*, 2013; Mridula and Sharma, 2015)**. Thus, in this work, legume yogurts were developed from legume milks and legume flours using only plant-based ingredients in the formulation. Soybean (*Glycine max*), pigeon pea (*Cajanus cajan*), and mung bean (*Vigna radiata*) were used to further explore their potential as functional and nutritional ingredients in the food system. They also have huge potential as probiotic vehicles in the plant-based food system. In addition, they are considered important food crops that play an important role in global food and nutrition security. In addition, the sensory evaluation, physicochemical and microbial performance of legume yogurts during storage were evaluated.

MATERIAL AND METHODS

Materials

Mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), sugar, and xanthan gum were purchased from a local market in Serdang, Selangor, Malaysia. Yogurt culture containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *L. acidophilus*, *L. Casei,* and *Bifidobacterium longum* (Yogourmet) was obtained from Lyo-San Inc. (Canada). Internal standard (L-2-Aminobutyric acid, AABA) was purchased from Sigma-Aldrich Co. All chemicals used were analytical grades.

Preparation of legume flour and legume milk

The legume seeds were washed and air-dried at 40°C overnight. The seeds were ground (Waring, New Hartford, USA) into course size before lipid extraction by soaking them in n-hexane for 1 hour, with ratio of legume seeds to n-hexane being 1:3 **(Ugwuona and Suwaba, 2013)**. The solvent was decanted from the mixture and the process was repeated twice. The remaining n-hexane presence in the seeds was eliminated through overnight dried off in a fume hood. The defatted legume seeds were finely ground to pass through a 0.21 mm sieve to form legume flour. Besides, legume milk was prepared by blending the mixture of deionized water (84% w/w) and legume flour (16% w/w) at the maximum speed (BB250S, Waring, USA) for 3 minutes. The resulting slurry was filtered by muslin cloth, and the liquid phase produced was referred to as legume milk.

Preparation of legume yogurt

Legume yogurts were produced from flour and milk of soybean, mung bean, and pigeon pea as the main ingredient. Both ingredients required different amount of legume contents due to their least gelation properties, where the values were 8% (w/w) and 16% (w/w) for legume flour-based yogurts and legume milk-based yogurts, respectively. All legume yogurt managed to show yogurt-like consistency although lesser legume contents were used in legume flour-based yogurts than legume milk-based yogurts. In addition, fixing the legume contents to 16% (w/w) in both ingredients resulted in paste-like consistency in legume flour-based yogurt due to the presence of insoluble carbohydrate and protein in the legume composition.

Initially, legume flours (8% w/w) were incorporated with deionized water (83.5% w/w) and mixed to form suspension. Meanwhile, legume milks (91.5% w/w) containing 16% w/w of legume flour can be readily used to produce yogurts. Legume flour suspensions and legume milks were heated under continuous stirring until 60°C. Sugar (7.5% w/w) and xanthan gum (0.5% w/w) were added followed by homogenization (IKA T25, Ultra-Turrax, USA) at 5000 rpm for 3 minutes. The mixtures were further heated at 95°C for another 15 minutes for pasteurization and gelatinization processes. Prior to starter culture inoculation (0.5% w/w), the mixtures were let to cool to approximately 40°C. The fermentation process was carried out at 37°C until they reached pH 4.5. Yogurts produced were refrigerated at 4°C until further analyses. Control yogurt was prepared from full fat soybean to mimic the commercial soy yogurt production as prepared by **Ferragut *et al.* (2009**) with slight modification. The beans were soaked with water at ratio 1:2 (soybean: water) for 14 hours. The water used was discarded and milk was obtained by blending the hydrated beans (16% w/w) with water (84% w/w), followed by filtration via muslin cloth. Control yogurt was prepared accordingly to the same methods described before. The flow process for yogurts production was illustrated in Figure 1.

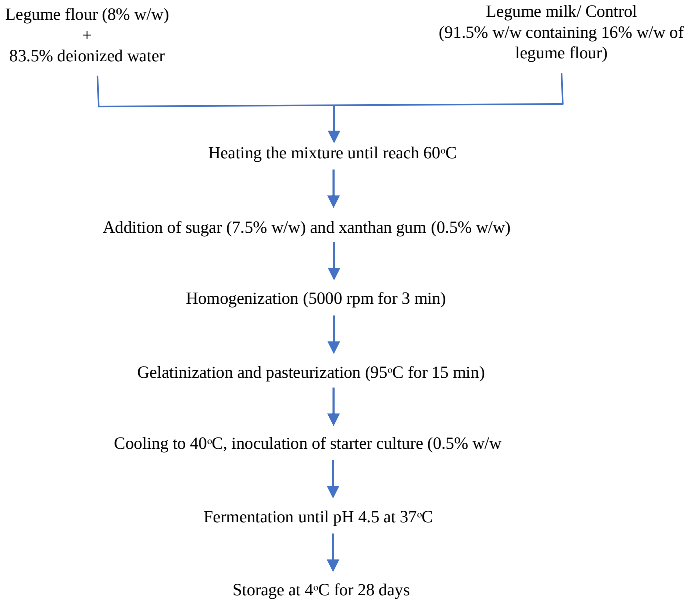


Figure 1 Flow process of legume yogurt production

Chemical composition

Legume seeds and legume yogurts were analyzed for moisture, total solids, ash, fat and protein based on method of **AOAC (2005)** at Day 0 of storage. Moisture content and total solid were determined by drying the samples in an oven at 105°C until constant weight achieved. Ash content was obtained by heating the residues at 550°C using muffle furnace. Gerber method was used to evaluate the fat content in legume yogurts, while Soxhlet was used to determine fat content in legume seeds. Kjedahl method was used to determine crude protein (conversion factor: 6.25).

Amino acid profiling

The amino acid profile of the samples was determined based on method described by **Goh *et al.* (2017)** with some modifications. Legume yogurts were measured (0.2g), added to 5 ml 6N HCl and heated at 110°C for 24 hours for hydrolysate process. The internal standard (2.5 mM L-2-aminobutyric acid, AABA) (4 ml) was added into the samples and further diluted with water in 100 ml volumetric flask. The diluted samples (1.5 ml) were filtered through 0.45 µm PTFE syringe filter.

Derivatization process was carried out by mixing 70 µl borate buffer (AccQ Fluor Borate Buffer) and 10 µl of filtered samples. Then, 20 µl AccQ Fluor reagent was added prior heating at 55°C for 10 minutes. The samples were injected (10 µl) while the column was kept at 36°C. AccQ tag column (3.9 x 150 mm) on HPLC system (Waters, Model Alliance e2695, Massachusetts, USA) with fluorescence detector (Waters, Model 2475, Massachusetts, USA) with $E_x = 250$ nm, $E_m = 395$ nm was used to separate the amino acid contains in the samples. The mobile phases consist of eluent A (AccQ Tag Eluent A: deionized water, 1:10), eluent B (acetonitrile) and eluent C (water) was injected at a flow rate of 1 ml/min throughout the analysis. The amino acids contents in the samples were presented in g/100g.

Determination of pH and titratable acidity

The pH values were measured using pH meter (Mettler Toledo, Switzerland) at room temperature (24°C). Titratable acidity was determined on 9 g of samples diluted with distilled water (18 g), and subsequent titration with 0.1N NaOH using 0.1% phenolphthalein as indicator. The volume of NaOH used was recorded when the pH reached 8.3. The results were expressed as lactic acid percentage following this equation:

$$\text{Titratable acidity (\% lactic acid)} = \frac{\text{Volume of 0.1N NaOH} \times 0.009}{\text{Weight of sample}} \times 100$$

Water holding capacity

The water holding capacity was determined as described by **Ferragut *et al.* (2009)** with slight modifications. The samples were weighted (30 g) and inserted in 50 ml polypropylene centrifuge tubes, followed by centrifugation at 480 g for 10 min at 4°C to obtain the expelled whey. WHC was calculated according to the following equation.

$$\text{Water Holding Capacity (\%)} = \frac{(\text{Weight of sample} - \text{Weight of whey})}{\text{Weight of sample}} \times 100$$

Color

The colors of samples were measured by Hunter Lab colorimeter (HunterLab, USA). Color was determined as lightness (L*), red/greenness (a*), and yellow/blueness (b*) **(Zare *et al.*, 2011)**.

Rheological properties

Rheological properties were determined based on method by **Hickisch *et al.* (2016)** with little modification using rheometer (Haake RheoStress 6000, USA) equipped with 35 mm parallel serrated plates (PP35 Ti L S), and gap setting of 1 mm at controlled temperature (4°C). Samples were gently stirred for 10 seconds before carefully placed in the inset plate and left rest for 5 minutes. Flow curves were obtained through upward (0 to 300 s^{-1}) and downward (300 to 0 s^{-1}) flow shear rate at a linear ramp within 3 minutes for each flow. The apparent viscosity with shear rate of 50 s^{-1} was calculated with Rheowin software (ThermoHaake GmbH,). Herschel-Bulkley model was used to generate flow behavior parameters following this equation:

$$\sigma = \sigma_y . + K . \dot{\gamma}^n$$

where σ is the shear stress, σ_y is the yield stress, K is the consistency index, n is the behavior index and γ is the shear rate.

The viscoelasticity properties of samples were determined by frequency sweep test from 0.1 Hz to 10 Hz at constant shear strain of 0.5%. Measurements were conducted within the linear viscoelastic range, previously determined by amplitude sweep test from 0 to 50% strain at 1 Hz **(Yang *et al.*, 2012)**. Storage modulus (G'), loss modulus (G'') and tan δ were calculated with Rheowin software (ThermoHaake GmbH).

Enumeration of microbes

Ten-fold serial dilutions were prepared in 0.1% (w/v) peptone water (Oxoid, UK), spread plate technique was applied by spreading 0.1 ml samples of appropriate dilution on the surface of agar plate. Total *Lactobacillus* sp. (*L. delbrueckii subsp. bulgaricus, L. casei and L. acidophilus*) and *S. thermophilus* were enumerated based on method from **Süle *et al.* (2014)** with some modifications. Total *Lactobacillus* sp. were enumerated by *Lactobacilli* MRS agar, and incubated anaerobically at 37°C for 72 hours; *S. thermophilus* was enumerated by ST agar at 45°C for 24 hours under aerobic condition. BSC agar supplemented with 0.05 g/L mupirocin was used to count *B. longum* via pour plate technique, incubated at 37°C for 72 hours under anaerobic condition **(Kim *et al.*, 2010)**. Colony forming units (cfu) were enumerated in plates containing 30 to 300 colonies, and cell concentration in the samples were expressed as log cfu/mL.

The possible contaminants present in the samples were evaluated by the Yeast and Mold Petrifilm$^{\text{TM}}$ and Coliform Petrifilm$^{\text{TM}}$, incubated at 21°C (3 to 5 days), and 37°C (48 hours), respectively **(Walsh *et al.*, 2010)**.

Sensory evaluation

The commercial soy yogurt (Kingland, Australia) was used as a commercial sample. Fifty untrained panelists were invited to evaluate the legume yogurts using a 9-point hedonic scale, ranging from extremely dislike (1) to extremely like (9). They were served at 7-10 °C in paper cups and were coded with three-digit numbers. Blueberry filling was added to each sample cup before serving to mimic the commercial yogurt used in this evaluation and mineral water was also provided to cleanse the mouth between testing. The test comprising 8 attributes namely, aroma, flavor, consistency, sweetness, astringency, sourness, aftertaste and overall acceptance were used to determine consumers preferences on the legume yogurts prepared **(Zare *et al.*, 2011)**.

Statistical analysis

The significant differences of the mean values ($n = 3$) were analyzed by two-way ANOVA followed by Tukey's HSD *posthoc* test ($p < 0.05$) using Minitab 17 (Minitab Inc., State College, PA, USA). All data were presented as mean value ± standard deviation.

RESULTS AND DISCUSSION

Chemical composition of legume flours and yogurts

The chemical compositions of legume flours and legume yogurts were presented in Table 1 and Table 2, respectively. The moisture contents of defatted flours (defatted soybean flour (DSBF), defatted pigeon pea flour (DPPF) and defatted mung bean flour(DMBF)) were lower (P<0.05) than whole soybean flour (WSBF) due to the overnight drying process to remove the remaining solvent used during defatting process. Meanwhile, there were variations in the protein, ash and fat values recorded by the legume flours which ranged between 19.35% to 40.21%, 3.31% to 5.33% and 0.96% to 21.72%, respectively. DSBF had the highest protein and ash contents, where the values were significantly different (P<0.05) from other legume flours. Besides, fat removal had increased the relative proportion of protein and ash in DSFB as compared to WSBF. The variation inchemical compositions among type of legume flours are largely attributed by their genetics, varieties and growth environment (**Kaur and Sigh, 2007**). It is crucial to remove the fat to obtain a more concentrated protein content in the flours which helps in improving the flours' functionality. Besides, elimination of fat provides the flours better stability because it removed the possibility of fat hydrolysis and oxidative rancidity.

Table 1 Chemical compositions of legume flours

Flour	Chemical composition			
	Moisture (%)	Protein (%)	Ash (%)	Fat (%)
WSBF	11.17 ± 0.29^{A}	30.51 ± 0.91^{B}	4.62 ± 0.05^{B}	21.72 ± 0.28^{A}
DSBF	8.23 ± 0.23^{B}	40.21 ± 0.93^{A}	5.33 ± 0.01^{A}	7.44 ± 0.55^{B}
DPPF	8.31 ± 0.33^{B}	19.35 ± 0.43^{D}	3.31 ± 0.01^{D}	1.11 ± 0.04^{C}
DMBF	8.38 ± 0.14^{B}	23.82 ± 0.17^{C}	4.19 ± 0.17^{C}	0.96 ± 0.19^{C}

Means with different letter in the same column are significantly different (p<0.05). (Abbreviation: WSBF, whole soybean flour; DSBF, defatted soybean flour; DPPF, defatted pigeon pea flour; DMBF, defatted mung bean flour). Based on dry matter/dry weight basis.

Table 2 Chemical compositions of legume yogurts

Yogurt	Chemical composition				
	Moisture (%)	Total solid (%)	Protein (%)	Ash (%)	Fat (%)
SBFY	84.95 ± 0.04^{B}	15.05 ± 0.04^{C}	3.45 ± 0.03^{BC}	0.43 ± 0.01^{D}	0.60 ± 0.04^{C}
PPFY	82.46 ± 0.40^{C}	17.54 ± 0.40^{B}	2.67 ± 0.12^{CD}	0.27 ± 0.01^{G}	0.09 ± 0.01^{D}
MBFY	84.78 ± 0.03^{B}	15.22 ± 0.03^{C}	1.62 ± 0.10^{E}	0.34 ± 0.01^{F}	0.08 ± 0.02^{D}
SBMY	81.44 ± 0.05^{D}	18.56 ± 0.05^{A}	5.12 ± 0.39^{A}	0.64 ± 0.01^{A}	0.89 ± 0.07^{B}
PPMY	82.24 ± 0.04^{C}	17.76 ± 0.04^{B}	3.72 ± 0.27^{B}	0.40 ± 0.01^{E}	0.13 ± 0.01^{D}
MBMY	82.51 ± 0.09^{C}	17.49 ± 0.09^{B}	3.75 ± 0.35^{B}	0.50 ± 0.02^{C}	0.12 ± 0.02^{D}
CY	88.37 ± 0.02^{A}	11.63 ± 0.02^{D}	1.97 ± 0.71^{DE}	0.55 ± 0.01^{B}	2.61 ± 0.03^{A}

Means with different letter in the same column are significantly different (p < 0.05).
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

In Table 2, the moisture (81.44% - 88.37%) and solid contents (11.63% - 18.56%) of all legume yogurts except for control yogurt (CY) (P<0.05) were comparable with other findings, indicating that the typical ranges of yogurts are between 80% and 85% and 12% to 15% w/v, respectively **(Rinaldoni *et al*., 2012; Santillán-Urquiza *et al*., 2017)**. Weak gel-like texture was observed in CY due to its low solid content (11.63%). This indicated that solid content in yogurt formulation is crucial to form stable structures with adequate viscosity **(Rinaldoni *et al.*, 2012)**. Besides, legume yogurts from legumes milk formulation (soybean milk yogurt (SBMY), pigeon pea milk yogurt (PPMY), mung bean milk yogurt (MBMY)) had higher total solids and protein contents than legume flours formulation of similar legume varieties (soybean flour yogurt (SBFY), pigeon pea flour yogurt (PPFY), and mung bean flour yogurt (MBFY)). These happened due to differences in legume concentrations (8% and 16%) used in both formulations. Legume milk yogurt formulation required higher legume concentration to imitate gel-like consistency of yogurt, while legume flour formulation able to form similar consistency with lesser legume concentration. The ability to form gel like texture at different concentration can be linked to their least gelation properties. Generally, the expected chemical compositions of legume yogurt can be calculated based on the legume flour chemical compositions presented in Table 1. The protein content in CY was lower than its expected values (4.88%). It can be due to leaching of chemical components into soaking medium prior to yogurt production (**Ogundipe *et al*., 2021**). Similar trend was observed on legume milk yogurts formulation, where the values of protein contents were slightly lower than their expected amounts (3.10% - 6.43%). Some of the residues that were trapped on the muslin cloth during filtration process contained insoluble materials that affected the chemical composition of the legume milks. Meanwhile, incorporation of legume flour in legume flour yogurt formulation gave higher protein values than their expected values (1.55% - 3.22%). Intense proteolytic activity during fermentation process helps to increase the protein contents in the yogurts. Similar observation was recorded by **Lim *et al*. (2019)** when legume-based yogurt was prepared using water kefir as starter culture. In addition, the protein contents of legume yogurts varied between 1.62% to 5.12%, where the value in SBMY was significantly higher (P<0.05) compared to the others. Based on **Tiwari *et al*. (2011)**, soybean has high protein content (35% - 43%). Thus, having higher amounts of soybean in the formulation will contribute to higher protein content per gram of flour in contrast to pigeon pea and mung bean flours. CY had the highest fat content (2.61%) (P<0.05) among all the legume yogurts (<0.89%) as whole fat bean was used to prepare the CY, while others had gone through defatted process prior to utilization into yogurt production. The ash contents (0.27% - 0.64%) for all legume yogurts were in accordance with values reported in sprouted cereals probiotic drinks (0.33% - 0.48%) **(Mridula and Sharma, 2015)** and almond fermented product (0.325%)**(Bernat *et al*., 2015)**, while higher values were obtained from garbanzo chickpeas and yellow soybean beverages (0.15% and 0.22%, respectively) **(Wang *et al*., 2018)**. In addition, the variation of chemical compositions obtained from legume yogurts were influenced by their inherited nutritional differences among legume species **(Du *et al*., 2014)**.

Amino acids content

The amino acids contents of legume yogurts were depicted in Table 3. Glutamic acid, aspartic acid and lysine were the main amino acids found in all legume yogurts, ranging from 0.237 to 0.620 g/100g, 0.140 to 0.413 g/100g and 0.105 to 0.289 g/100g, respectively. Sulphur-containing amino acids such as methionine and cysteine are limited, whereas cysteine was only detected in MBFY (0.059 g/100g) and MBMY (0.183 g/100g). Similarly, yogurt produced from *Lupinus campestris* seeds also had inadequate sulphur-containing amino acids **(Jiménez-Martínez *et al.*, 2003)**, which is a hereditary trait of legumes **(Vaz Patto *et al.*, 2015)**. The total amino acids present in legume yogurts were from 1.121 g/100g to 3.057 g/100g. Low total amino acids content in CY may be due to loss of soluble proteins and amino acids during soaking process of whole fat soybean prior to yogurt preparation. This observation was in accordance with **de Lima *et al.* (2014)**, and showed that soaking treatment had led to leaching of water-soluble compounds (protein, carbohydrates and others) in soybean.

Table 3 Amino acids contents of legume yogurts

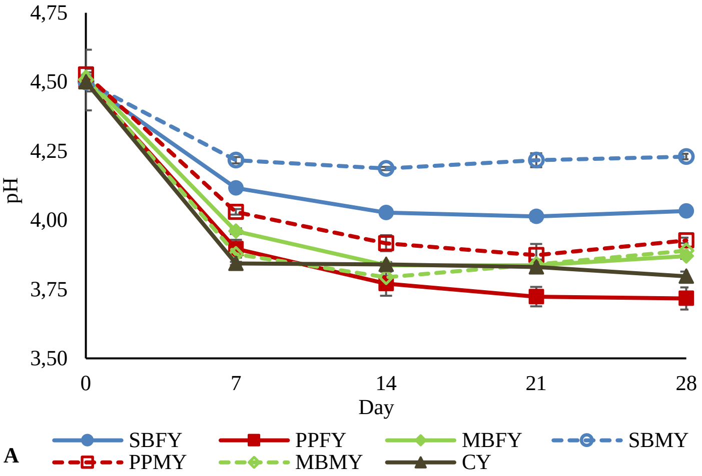
Amino Acid (g/100g)	Type of yogurt						
	SBFY	PPFY	MBFY	SBMY	PPMY	MBMY	CY
Hydroxyproline	0.026±0.003^{b}	0.035±0.010^{ab}	0.045±0.010^{ab}	0.046±0.007^{a}	0.034±0.005^{ab}	0.037±0.006^{ab}	0.027±0.005^{ab}
Aspartic acid	0.214±0.005^{c}	0.134±0.005^{d}	0.154±0.004^{d}	0.413±0.013^{a}	0.204±0.006^{c}	0.353±0.005^{b}	0.140±0.044^{d}
Serine	0.082±0.003^{b}	0.062±0.006^{c}	0.059±0.004^{c}	0.159±0.006^{a}	0.088±0.009^{b}	0.142±0.011^{a}	0.054±0.004^{c}
Glutamic acid	0.352±0.006^{c}	0.271±0.009^{d}	0.242±0.012^{d}	0.620±0.060^{a}	0.401±0.010^{c}	0.526±0.006^{b}	0.237±0.004^{d}
Glycine	0.062±0.006^{c}	0.040±0.007^{de}	0.038±0.006^{e}	0.118±0.006^{a}	0.058±0.005^{cd}	0.094±0.009^{b}	0.040±0.006^{de}
Histidine	0.047±0.003^{c}	0.045±0.010^{c}	0.035±0.006^{c}	0.091±0.004^{a}	0.068±0.012^{b}	0.087±0.006^{ab}	0.031±0.006^{c}
Arginine	0.101±0.003^{c}	0.061±0.007^{d}	0.063±0.008^{d}	0.192±0.007^{a}	0.098±0.011^{c}	0.158±0.005^{b}	0.067±0.012^{d}
Threonine	0.058±0.003^{c}	0.039±0.005^{d}	0.034±0.003^{d}	0.107±0.006^{a}	0.058±0.004^{c}	0.083±0.004^{b}	0.038±0.004^{d}
Alanine	0.081±0.004^{bc}	0.065±0.007^{bc}	0.065±0.012^{bc}	0.149±0.011^{a}	0.095±0.022^{b}	0.139±0.006^{a}	0.056±0.005^{c}
Proline	0.099±0.010^{b}	0.068±0.007^{c}	0.063±0.004^{c}	0.166±0.007^{a}	0.058±0.005^{c}	0.111±0.012^{b}	0.063±0.005^{c}
Cysteine	ND	ND	0.059±0.007^{b}	ND	ND	0.183±0.006^{a}	ND
Tyrosine	0.042±0.009^{b}	0.029±0.008^{b}	0.029±0.004^{b}	0.073±0.007^{a}	0.041±0.004^{b}	0.062±0.004^{a}	0.028±0.007^{b}
Valine	0.079±0.011^{ab}	0.054±0.003^{b}	0.060±0.007^{ab}	0.140±0.078^{a}	0.082±0.009^{b}	0.141±0.005^{a}	0.052±0.004^{b}
Methionine	0.022±0.006^{ab}	0.014±0.008^{b}	0.015±0.008^{ab}	0.033±0.012^{ab}	0.021±0.004^{ab}	0.035±0.007^{a}	0.014v0.004^{b}
Lysine	0.146±0.010^{c}	0.131±0.011^{c}	0.136±0.007^{c}	0.287±0.004^{a}	0.183±0.006^{b}	0.289±0.007^{a}	0.105±0.007^{d}
Isoleucine	0.076±0.004^{c}	0.047±0.009^{e}	0.050±0.011^{de}	0.138±0.010^{a}	0.070±0.003^{cd}	0.117±0.007^{b}	0.051±0.005^{de}
Leucine	0.125±0.009^{b}	0.084±0.004^{c}	0.084±0.005^{c}	0.216±0.008^{a}	0.129±0.005^{b}	0.206±0.008^{a}	0.079±0.007^{c}
Phenylalanine	0.062±0.010^{d}	0.081±0.007^{c}	0.047±0.005^{de}	0.109±0.005^{b}	0.134±0.006^{a}	0.121±0.004^{ab}	0.039±0.007^{e}
Total Amount Amino Acid	1.672±0.063^{b}	1.259±0.057^{c}	1.278±0.054^{c}	3.057±0.127^{a}	1.820±0.045^{b}	2.884±0.029^{a}	1.121±0.048^{c}

(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

pH and titratable acidity

Figures 2A and 2B show changes in pH and titratable acidity of legume yogurts during 28 days of storage, respectively. The acidity in the legume yogurts were determined based on pH and titratable acidity, which the latter was expressed as lactic acid percentage. Lactic acid is produced as primary metabolite on proliferation of lactic acid bacteria during fermentation process, thus resulting in pH reduction in the legume yogurt cultures. In this study, the incubation process was stopped once the legume yogurts reached pH 4.5 (Figure 2A). However, lactic acid percentages (Day 0) differed significantly (P<0.05), ranging between 0.40% and 0.92% (Figure 2B). Besides, the result showed that legume yogurts from milk legume formulation had higher lactic acid percentage than their respective legume yogurts from flour legume formulation, and followed this sequence: SBMY > MBMY > PPMY > SBFY > PPFY > CY > MBFY.

Reduction of pH in legume yogurts once fermentation ended had shown that all legume substrates used in this study managed to support the growth of lactic acid bacteria with sufficient amount of potentially vital nutrients without dairy-based ingredients (lactose, whey protein and sodium caseinate) supplementation. Type of legumes and formulations had different ability to stimulate lactic acid production in the cultures, thus suggesting wide variations of lactic acid percentages in legume yogurts. This trend can be linked to acid-buffering capacity (also referred as buffering capacity) of legumes. The buffering capacity is positively influenced by solid and protein content of food formulation, particularly with high amount of glutamic acid and aspartic acid **(Kizzie-Hayford *et al.*, 2016)**. High buffering capacity in SBMY, MBMY and PPMY resulted in low acidification rate. Therefore, they required longer time to reach pH 4.5 and provide more incubation time for lactic acid bacteria proliferation which led to higher lactic acid percentage. Previous study by **Almnura and Arabia (2011)** also found that buffering capacity in different sesame yogurt formulations had affected the titratable acidity values (0.52% - 1.00%) after 8 hours of fermentation process.



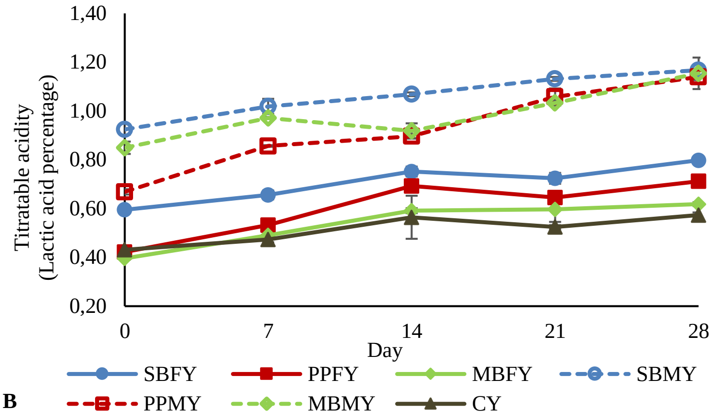


Figure 2 pH (A) and titratable acidity (% lactic acid) (B) of legume yogurts during storage (Error bars represent standard deviations of the mean (n = 3))
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

Throughout the storage, the pH of legume yogurts on Day 7 had reduced significantly (P<0.05), followed by minimal reduction (P>0.05) on Day 14 until Day 28. Conversely, lactic acid percentages had increased gradually along the storage, and significant increments (P<0.05) were observed on Day 7. Maximum amount of lactic acid percentages were observed on Day 28 in all legume yogurts, ranging between 0.57% and 1.17%. This trend had suggested that post-acidification process occurred in legume yogurts during storage **(Bedani *et al.*, 2014; Walsh *et al.*, 2010)**.

Color

The changes in color parameters (L*, a* and b*) in legume yogurts were shown in Table 4. On Day 0, the L*, a* and b* values ranged from 68.26 to 77.07, -0.90 to 4.64 and 14.38 to 27.44, respectively. The L* values of legume yogurts were highly influenced by their type of legume used compared to their formulation, where yogurts from pigeon pea (PPFY and PPMY) had higher L* than soybean (SBFY and SBMY) and mung bean (MBFY and MBMY) . CY had the highest L*, suggesting that CY had better gel homogeneityity that provided better light reflection on the gel surface than other legume yogurts. The L* values recorded in legume yogurts were higher than L* of yogurt-like product from marble variety of Africa yam bean (60.1 – 63.5) **(Aminigo *et al.*, 2009)**, but lower L* when compared with soy yogurt prepared by ultrahigh-pressure homogenization (UHPH) (84.89 – 86.41) treatment **(Ferragut *et al.*, 2009)**. Minimal changes of L* were recorded on Day 28 in legume yogurts, but the values were significantly different (P<0.05). The fluctuations of L* in legume yogurts during storage can be related to the aggregation level of particles that changed the opacity of the food system **(Bernat *et al.*, 2015)**. However, the changes of L* of legume yogurts during storage can be negligible as the differences were only 1-unit values and probably undetected by consumers' naked eyes **(Ferragut *et al.*, 2009)**.

Table 4 Color changes of legume yogurts during storage

Yogurt	Day				
	0	7	14	21	28
L* value					
SBFY	68.26 ± 0.69^{aD}	67.38 ± 0.08^{bF}	67.40 ± 0.11^{bF}	67.45 ± 0.06^{abF}	67.33 ± 0.08^{bG}
PPFY	74.23 ± 0.24^{aB}	74.23 ± 0.25^{aB}	73.83 ± 0.28^{aB}	73.93 ± 0.04^{aB}	73.79 ± 0.10^{aB}
MBFY	68.13 ± 0.13^{aD}	67.98 ± 0.15^{abE}	67.86 ± 0.08^{abEF}	67.67 ± 0.14^{bEF}	67.72 ± 0.12^{bF}
SBMY	70.43 ± 0.40^{aC}	70.47 ± 0.23^{aD}	70.45 ± 0.43^{aD}	70.23 ± 0.03^{aD}	70.29 ± 0.06^{aD}
PPMY	73.39 ± 0.30^{aB}	72.78 ± 0.12^{bC}	72.69 ± 0.20^{bcC}	72.22 ± 0.16^{cC}	72.22 ± 0.06^{cC}
MBMY	68.31 ± 0.02^{aD}	68.37 ± 0.01^{aE}	68.34 ± 0.13^{aE}	68.03 ± 0.08^{bE}	68.04 ± 0.16^{bE}
CY	77.07 ± 0.23^{aA}	76.90 ± 0.19^{aA}	76.90 ± 0.07^{aA}	76.59 ± 0.34^{aA}	76.64 ± 0.11^{aA}
a* value					
SBFY	3.90 ± 0.05^{aB}	3.89 ± 0.13^{aB}	3.85 ± 0.05^{aB}	3.84 ± 0.09^{aB}	3.78 ± 0.03^{aB}
PPFY	-0.90 ± 0.05^{cE}	-0.88 ± 0.04^{cF}	-0.67 ± 0.02^{bE}	-0.56 ± 0.03^{aF}	-0.57 ± 0.05^{abF}
MBFY	0.82 ± 0.05^{cC}	0.84 ± 0.10^{cCD}	0.91 ± 0.03^{bcC}	1.10 ± 0.05^{aC}	1.05 ± 0.02^{abC}
SBMY	4.64 ± 0.08^{aA}	4.35 ± 0.03^{bcA}	4.41 ± 0.07^{bA}	4.24 ± 0.03^{cA}	4.35 ± 0.03^{bcA}
PPMY	0.46 ± 0.03^{cD}	0.58 ± 0.03^{bE}	0.73 ± 0.01^{aD}	0.77 ± 0.03^{aD}	0.75 ± 0.04^{aD}
MBMY	0.82 ± 0.01^{dC}	0.93 ± 0.02^{cC}	1.01 ± 0.04^{bcC}	1.07 ± 0.01^{abC}	1.11 ± 0.05^{aC}
CY	0.72 ± 0.02^{aC}	0.71 ± 0.02^{aDE}	0.69 ± 0.07^{abD}	0.60 ± 0.03^{bE}	0.64 ± 0.02^{abE}
b* value					
SBFY	21.36 ± 0.18^{aC}	21.86 ± 0.40^{aB}	21.50 ± 0.17^{aBC}	21.81 ± 0.36^{aB}	21.63 ± 0.05^{aB}
PPFY	21.91 ± 0.07^{aC}	21.94 ± 0.15^{aB}	20.90 ± 0.29^{bC}	20.73 ± 0.03^{bC}	20.93 ± 0.02^{bC}
MBFY	14.54 ± 0.11^{aD}	14.60 ± 0.17^{aCD}	14.25 ± 0.16^{aE}	14.33 ± 0.07^{aE}	14.60 ± 0.15^{aE}
SBMY	23.01 ± 0.35^{aB}	21.92 ± 0.22^{bB}	21.98 ± 0.21^{bB}	21.66 ± 0.21^{bB}	21.76 ± 0.07^{bB}
PPMY	27.44 ± 0.38^{aA}	27.29 ± 0.14^{aA}	25.95 ± 0.38^{bA}	25.42 ± 0.19^{bA}	25.44 ± 0.20^{bA}
MBMY	14.38 ± 0.15^{aD}	14.22 ± 0.08^{aD}	14.18 ± 0.02^{aE}	14.20 ± 0.01^{aE}	14.33 ± 0.03^{aE}
CY	14.77 ± 0.16^{cD}	15.09 ± 0.10^{bC}	15.21 ± 0.03^{abD}	15.34 ± 0.08^{aD}	15.38 ± 0.02^{aD}

Means in lower case with different alphabet in the same row are significantly different ($p < 0.05$). Means in upper case with different alphabet in the same column are significantly different ($p < 0.05$). L*[L* = 0 (black) and L* = 100 (white)], a*(-a* = greenness and +a* = redness), b* (-b* = blueness and +b* = yellowness).
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

Only PPFY obtained negative a* value (-0.90) on Day 0, indicating the presence of a slightly green hue in the legume yogurt. While other legume yogurts were basically in red hue (0.48 – 4.64), where SBFY had the highest a* value ($P<0.05$). The b* values recorded among them were between 14.38 to 23.01, implying that they were in the yellow color range. Besides, the stability of a* and b* of legume yogurts were different from each other during storage. On Day 28, deviations of a* in legume yogurts were less than 0.5-unit value which corresponded to values on Day 0. Meanwhile, slightly higher differences in b* were observed in PPMY and SBMY during the end of storage where the values reduced to 2-unit and 1.25-unit values, respectively. The differences in color parameters of legume yogurts were attributed to colored pigments of legume flours, based on their species hereditary trait **(Joshi *et al.*, 2015)**. In addition, probiotics drinks incorporated with various sprouted cereals had illustrated diverse color attributes **(Mridula and Sharma, 2015)**.

Water holding capacity

Water holding capacity (WHC) of legume yogurts throughout 28 days of storage were depicted in Figure 3. During Day 0, the WHC of legume yogurts ranged between 46% and 100%, where MBFY and CY had the maximum and minimum ability to hold water in the matrix, respectively. Protein is an important component that helps to strenghten the microstructure network in yogurt and consequently improved the WHC by entraping water within its three-dimensional network **(Yang and Li, 2010)**. However, legume yogurts from soybean (SBFY and SBMY) had lower WHC than mung bean (MBFY and MBMY) and pigeon pea (PPFY and PPMY) despite having higher protein content than others. Therefore, we suggested that starch and other carbohydrate components (hemicellulose and fiber) may help to enhance WHC in MBFY, MBMY, PPFY and PPMY as pigeon pea and mung bean had high amount of these components. Based on **De Pasquale *et al.* (2020)**, gelatinization process had enhanced water binding capacity in flour due to disruption of the internal structure of starch granules during thermal treatment. Previous studies on plant-based materials (rice bran, oat and barley) had shown that these plant-based materials provided more water binding ability and texture firmness due to high water holding capacity and swelling capacity of hemicellulose and dietary fibers content in the materials **(Ozcan and Kurtuldu, 2014; Demirci *et al.*, 2017)**.

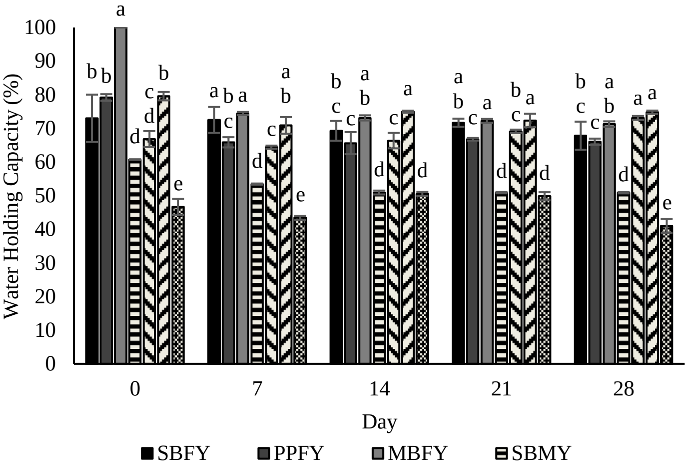


Figure 3 Water holding capacity (%) of legume yogurts during storage (Error bars represent standard deviations of the mean (n = 3))
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

During storage, WHC of MBFY had shown 20% reduction ($P<0.05$) on Day 7, while PPFY and SBMY had minimal reduction ($P>0.05$) on Day 7 and onwards. No significant changes ($P>0.05$) was showed by SBMY on Day 14 until Day 28. WHC had declined steadily for CY, MBFY and PPMY throughout the storage. These results indicated that each of legume yogurt had perceived different capability to retain water molecules in their matrix based on their gel strength **(Sah et al., 2016)**. Presence of starch in legume yogurts can induce retrogradation upon cooling which lead to water expulsion from gel, and resulted in reduced WHC during storage **(Vaz Patto *et al.*, 2015)**. Degree of retrogradation in legume yogurts might vary depending on the amylose/amylopectin ratio and starch concentration of the legume used. Besides, extensive storage time can initiate water loss in the food system due to passive diffusion **(Cruz *et al.*, 2007)**. This suggests that similar process could have happened in legume yogurts as clear phase separation was observed at the end of the storage study.

Rheological properties

Flow curves of legume yogurts were described by Herschel-Bulkley model (Table 5) and it satisfactorily fitted the downward flow curves for all samples with minimum correlation coefficient (R^2) of 0.991. Yield stress were linked to legume yogurts firmness and this is due to the presence of crosslinked structure in the food system **(Yang *et al.*, 2012)**. Throughout the study, MBFY and MBMY demonstrated higher yield stress than other legume yogurts. During Day 0, MBFY had the highest yield stress (23.91 Pa) and K (2.17). The values were significantly different ($P<0.05$) from other legume yogurts, indicating that MBFY had high crosslinked network in its structure. On Day 28, MBMY had notable increased of yield stress, ($P<0.05$) suggesting that a firmer gel structure network during storage. In addition, all legume yogurts exhibited various capacities of shear thinning properties ($n<1$) during storage. Lesser shear thinning behavior is illustrated when the n value approaches 1. SBMY had the weakest shear thinning bahavior (0.84), reflecting that it had lower length of molecular chains and cross linking of protein micelles compared to other legume yogurts **(Yang *et al.*, 2012)**. Similarly, shear thinning behavior were also reported in other fermented products such as lupin yogurt **(Hickisch *et al.*, 2016)** and germinated soy yogurt **(Yang *et al.*, 2012)**. Meanwhile, the apparent viscosity of legume yogurts on upward flow curves were higher than their respective downward flow curves due to limited structure rebuilding time after applying continuous shear rate. As expected, higher apparent viscosity were also observed in MBFY and MBMY samples compared to other legume yogurts. According to **Akin and Ozcan (2017)**, viscosity can be influenced by the changes of protein matrix density inside the microstructure at different rates of plant proteins. Presence of starch in legume yogurts formulations also helps to strenghten gel network, contributing to gelation **(Masiá *et al.*, 2021)**. Thus, composition of protein and starch in mung bean had demonstrated stable and rigid gel structure with suitable rheological properties for legume yogurts development compared to other type of legumes used in this study.

The extend and strength of legume yogurts internal structure during storage can be described by the viscoelastic properties (Figure 4). The G' (Figure 4A and 4C) values in legume yogurts were continuously higher than G'' (Figure 4B and 4D) with varied viscoelasticity intensity ($P<0.05$) between samples on Day 0 and Day 28, respectively. This result showed a predominantly elastic behavior (G' > G'') among legume yogurts. Besides, the values of G' and G'' in legume yogurts formulated with flour (MBFY and PPFY) were higher compared to CY and milk formulation (MBMY and PPMY), except in soybean (SBFY and SBMY). High G' is influenced by better protein-protein interaction which happened after protein rearrangement **(Doleyres *et al.*, 2005)**. There is also a possible protein-carbohydrate interaction in the system, especially in legume flours formulation that help to strengthen gel network **(Sendra *et al.*, 2010)**. Furthermore, tan δ corresponded to G''/G' and the value closer to 0 represent more-solid like behavior. Tan δ values for the legume yogurts on Day 0 and Day 28 ranged from 0.13 to 0.76 and 0.10 to 0.75, respectively (Table 5), confirming that solid-like properties predominated over liquid-like properties.

Table 5 Rheological properties of legume yogurts

	R^2	τ_0 (Pa)	K ($Pa.s^n$)	n	Apparent viscosity (mPa.s)		
					Upward curve	Downward curve	Tan δ
Day 0							
SBFY	0.988	1.53 ± 0.06^{F}	0.47 ± 0.03^{DE}	0.62 ± 0.01^{D}	165.43 ± 8.92^{F}	142.72 ± 5.05^{G}	0.76^{A}
PPFY	0.994	16.44 ± 0.33^{B}	0.93 ± 0.13^{C}	0.73 ± 0.02^{BC}	1343.50 ± 30.24^{C}	669.45 ± 11.59^{B}	0.13^{F}
MBFY	0.991	23.91 ± 0.77^{A}	2.17 ± 0.21^{A}	0.60 ± 0.02^{D}	1669.87 ± 38.33^{A}	945.08 ± 20.16^{A}	0.18^{DE}
SBMY	0.993	4.71 ± 0.54^{D}	0.34 ± 0.10^{E}	0.84 ± 0.05^{A}	862.18 ± 36.53^{D}	279.59 ± 4.50^{F}	0.30^{B}
PPMY	0.998	3.13 ± 0.07^{E}	1.00 ± 0.01^{C}	0.71 ± 0.01^{C}	864.22 ± 32.29^{D}	395.82 ± 4.11^{D}	0.21^{D}
MBMY	0.994	10.65 ± 0.43^{C}	0.72 ± 0.10^{CD}	0.79 ± 0.02^{AB}	1478.17 ± 18.99^{B}	548.10 ± 4.11^{C}	0.18^{E}
CY	0.992	5.39 ± 0.17^{D}	1.48 ± 0.19^{B}	0.50 ± 0.02^{E}	338.96 ± 5.26^{E}	315.84 ± 6.16^{E}	0.26^{C}
Day 28							
SBFY	0.979	1.50 ± 0.30^{D}	0.43 ± 0.12^{D}	0.64 ± 0.05^{C}	158.20 ± 4.56^{E}	138.03 ± 3.13^{F}	0.75^{A}
PPFY	0.995	15.04 ± 0.92^{B}	0.88 ± 0.12^{BC}	0.74 ± 0.03^{AB}	1263.00 ± 50.25^{B}	631.58 ± 12.28^{B}	0.14^{EF}
MBFY	0.994	25.64 ± 1.34^{a}	1.49 ± 0.11^{A}	0.70 ± 0.01^{BC}	1782.80 ± 74.74^{A}	994.68 ± 25.05^{A}	0.15^{E}
SBMY	0.996	2.88 ± 0.07^{D}	0.46 ± 0.02^{D}	0.77 ± 0.01^{A}	470.88 ± 13.00^{CD}	255.34 ± 1.39^{E}	0.36^{B}
PPMY	0.999	3.05 ± 0.03^{D}	0.60 ± 0.01^{CD}	0.79 ± 0.01^{A}	589.96 ± 16.77^{C}	332.14 ± 2.22^{D}	0.19^{D}
MBMY	0.994	27.33 ± 0.74^{A}	0.98 ± 0.07^{B}	0.78 ± 0.01^{A}	1878.53 ± 80.45^{A}	998.27 ± 20.20^{A}	0.10^{F}
CY	0.996	6.95 ± 0.54^{C}	1.79 ± 0.22^{A}	0.49 ± 0.02^{D}	421.30 ± 4.48^{D}	387.98 ± 4.88^{C}	0.26^{C}

Means in upper case with different alphabet in the same column are significantly different ($p < 0.05$).
R^2 = correlation coefficient, τ_0= yield stress, K = consistency index and n = behavior index.
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt)

Microbials survivability

The common starter culture used in commercial yogurt contained the combination of *S. thermophilus* and *L. bulgaricus* **(Granato *et al.*, 2010)**. In recent years, incorporation of probiotics bacteria from *Lactobacillus* and *Bifidobacterium* species together with starter culture has gained so much interest to further exert overall health benefits to consumers, especially the gut system. An appropriate amount of viable probiotics is necessary to ensure continuing positive well-being upon consumption and it is recommended to be between 6 – 10 log cfu/ml **(Martins *et al.*, 2013)**. Thus, selective enumeration of *S. thermophilus*, *Lactobacillus* sp. and *B. longum* in legume yogurts were done throughout the 28 days of storage (Table 6). In this study, Day 0 is defined as the time legume yogurts reached pH 4.5 after incubation process. On Day 0, viable cell counts of *S. thermophilus* and *Lactobacillus* sp. in all legume yogurts were between 7.96 log cfu/ml to 8.67 log cfu/ml and 8.21 log cfu/ml to 8.91 log cfu/ml, respectively, while *B. Longum's* viability ranged from 4.97 log cfu/ml to 5.29 log cfu/ml. Higher viability of *S. thermophilus* and *Lactobacillus* sp. than *B. longum* in legume yogurts were attributed by the differences in initial count of bacteria before inoculation, where the amount of *B. longum* (~4.00 log cfu/ml) was much lower than the others (~6.00 log cfu/ml) (data not shown). Nutrient competition among bacteria and low substrate suitability of *B. longum* in legume yogurts might also contribute to this result. This finding is in agreement with previous studies which suggested that excellent growth performance and high cell viability in yogurt culture can be obtained by using high concentration of probiotics inoculation (> 7 log cfu/ml) **(Hickisch *et al.*, 2016; Russo *et al.*, 2016)**.

Furthermore, legume yogurts prepared from legume milk (SBMY, PPMY and MBMY) produced significantly higher ($P<0.05$) viable cell counts compared to their respective legume yogurts from legume flour formulation (SBFY, PPFY and MBFY). This could be linked to higher soluble nutrients content, as legume yogurts from milk formulation contained double the percentage of legume composition and most of the nutrients available were water soluble. Same observation had been shown by **Wang *et al.* (2018)**, indicating the fermentability of lactic acid bacteria can be enhanced through water soluble carbohydrate and protein content. Besides, different type of legumes used have their own unique chemical composition that would display diverse substrate capability and eventually affect the probiotics bacteria proliferation **(Sanders and Marco, 2010)**. High viability of *S. thermophilus* and *Lactobacillus* sp. in legume yogurts were maintained until Day 28, ranging from 7.26 to 8.52 log cfu/ml and 7.45 to 8.58 log cfu/ml, respectively. CY had the lowest viable cell count ($P<0.05$) of *S. thermophilus* and *Lactobacillus* sp. among the legume yogurts, but it was above the minimal recommendation value (>6 log cfu/ml) for potential therapeutic properties. Legume yogurts had shown great probiotics bacteria survival throughout the storage, similarly exhibited by other dairy alternative products from fermented emmer beverages **(Coda *et al.*, 2011)** and lupin-based yogurt **(Hickisch *et al.*, 2016)**. Although there were slight deviation of bacteria enumeration in legume yogurts throughout the storage, the differences were possible and can be considered as microbiologically insignificant as the deviations were below 0.5 log cfu/ml **(Bedani *et al.*, 2014)**.

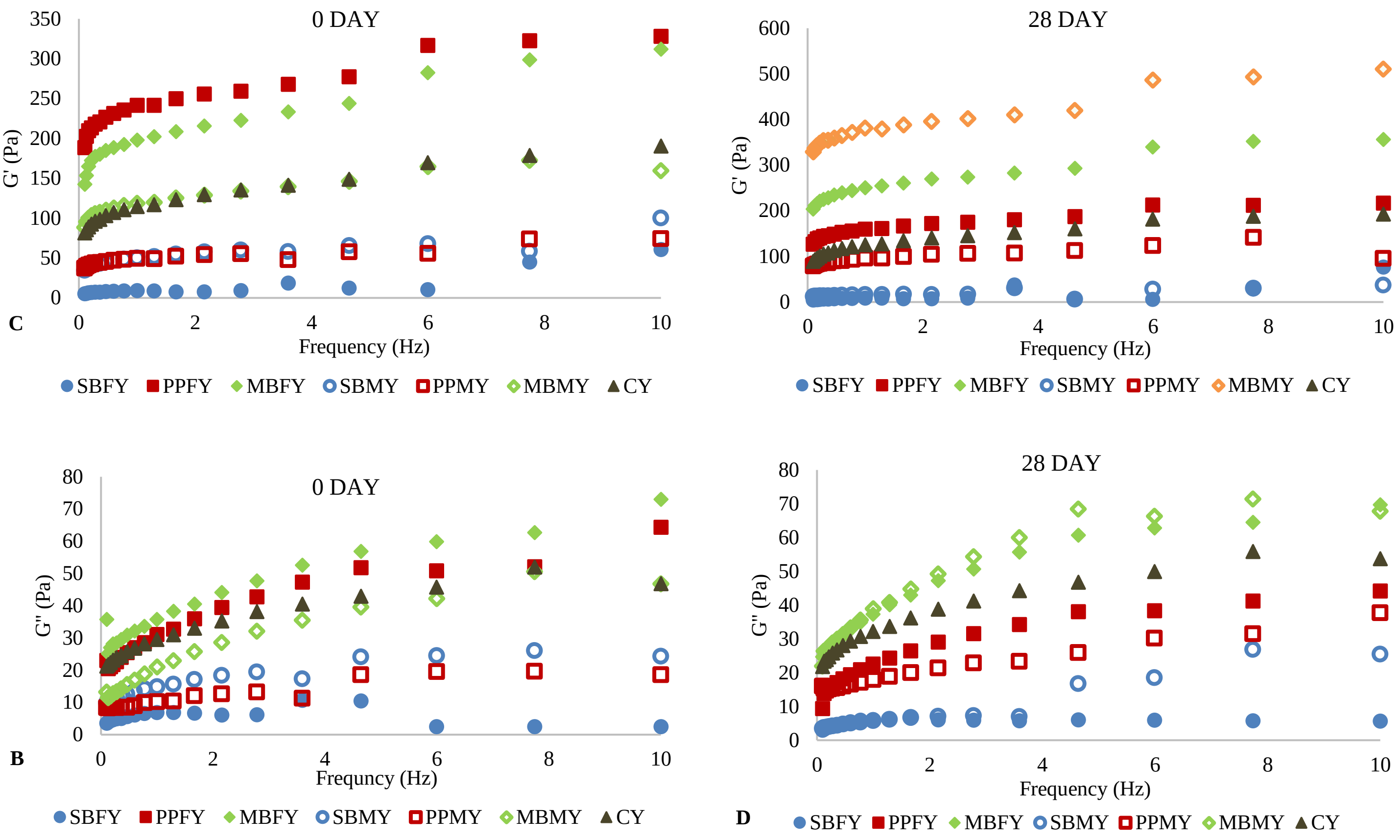


Figure 4 G' (Pa) and G'' (Pa) of legume yogurts during 0 (A and B) and 28 (C and D) days of storage.
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt)

Table 6 Microbes viability of legume yogurts during storage

Type of yogurt	Day				
	0	7	14	21	28
Viability of *S. thermophilus* (log CFU/ml)					
SBFY	8.52 ± 0.08^{aAB}	8.45 ± 0.15^{abAB}	8.36 ± 0.08^{abB}	8.33 ± 0.05^{abB}	8.27 ± 0.04^{bBC}
PPFY	8.35 ± 0.24^{aB}	8.30 ± 0.09^{aBC}	8.12 ± 0.07^{aC}	8.40 ± 0.03^{aAB}	8.21 ± 0.04^{aC}
MBFY	8.29 ± 0.01^{aB}	8.02 ± 0.06^{bDE}	8.03 ± 0.03^{bC}	7.95 ± 0.07^{bC}	7.85 ± 0.15^{bD}
SBMY	8.67 ± 0.05^{aA}	8.56 ± 0.08^{aA}	8.67 ± 0.06^{aA}	8.24 ± 0.12^{bB}	8.48 ± 0.01^{aA}
PPMY	8.67 ± 0.08^{aA}	8.51 ± 0.04^{abAB}	8.47 ± 0.07^{bAB}	8.59 ± 0.05^{abA}	8.52 ± 0.07^{abA}
MBMY	8.37 ± 0.06^{abcB}	8.18 ± 0.05^{bcCD}	8.44 ± 0.10^{aB}	8.17 ± 0.15^{cC}	8.41 ± 0.01^{abAB}
CY	7.96 ± 0.05^{aC}	7.81 ± 0.10^{bE}	7.21 ± 0.06^{cD}	7.29 ± 0.01^{cD}	7.26 ± 0.01^{cE}
Viability of *Lactobacillus sp.* (log CFU/ml)					
SBFY	8.73 ± 0.07^{aAB}	8.66 ± 0.05^{abA}	8.36 ± 0.08^{dB}	8.53 ± 0.08^{bcA}	8.45 ± 0.03^{cdA}
PPFY	8.50 ± 0.12^{aC}	8.56 ± 0.11^{aA}	8.36 ± 0.10^{abB}	8.16 ± 0.12^{bcC}	8.00 ± 0.01^{cB}
MBFY	8.21 ± 0.04^{aD}	7.98 ± 0.04^{bC}	7.84 ± 0.02^{cC}	7.94 ± 0.04^{bD}	7.89 ± 0.05^{bcB}
SBMY	8.91 ± 0.04^{aA}	8.71 ± 0.03^{bA}	8.75 ± 0.04^{bA}	8.51 ± 0.05^{cAB}	8.58 ± 0.07^{cA}
PPMY	8.77 ± 0.07^{aAB}	8.66 ± 0.04^{abA}	8.67 ± 0.15^{abA}	8.51 ± 0.02^{bAB}	8.46 ± 0.06^{bA}
MBMY	8.65 ± 0.06^{aBC}	8.34 ± 0.12^{bB}	8.57 ± 0.05^{aAB}	8.34 ± 0.07^{bBC}	8.58 ± 0.07^{aA}
CY	7.69 ± 0.05^{aE}	7.40 ± 0.02^{bD}	7.23 ± 0.05^{cD}	7.23 ± 0.04^{cE}	7.45 ± 0.06^{bC}
Viability of *B. longum* (log CFU/ml)					
SBFY	5.07 ± 0.12^{ABC}	ND	ND	ND	ND
PPFY	5.20 ± 0.17^{AB}	ND	ND	ND	ND
MBFY	4.83 ± 0.16^{C}	ND	ND	ND	ND
SBMY	4.87 ± 0.15^{BC}	ND	ND	ND	ND
PPMY	5.15 ± 0.05^{ABC}	ND	ND	ND	ND
MBMY	4.97 ± 0.07^{ABC}	ND	ND	ND	ND
CY	5.29 ± 0.01^{A}	ND	ND	ND	ND

Means in lower case with different alphabet in the same row are significantly different ($p < 0.05$). Means in upper case with different alphabet in the same column are significantly different ($p < 0.05$).
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, CY: Control yogurt).

However, the viability of *B. longum* in all legume yogurts can only be detected on Day 0 and the amount was below the detection limits after Day 7 and onwards. This finding suggested that *B. longum* has low stability and susceptibility towards legumes as the substrate. Lack of sulphur-containing amino acids in legumes can negatively influence the viability because *Bifidobacterium* species require considerable amount of these amino acids to support its population during growth and storage **(Shi *et al.*, 2020)**. Besides, *B. longum* is stricly anaerobe bacteria, and presence of oxygen in the legume yogurt packaging during storage would cause detrimental effect to its survival **(Arboleya *et al.*, 2016)**. Additionally, presence of mold, yeast and coliform were below the detection levels in the legume yogurts,indicating no contamination had occurred and the samples were stored properly throughout the storage (data not shown).

Sensory evaluation

The sensorial attributes of legume yogurts including color, viscosity, aroma, sweetness, sourness, astringency, aftertaste and overall acceptance are presented in Figure 5. During sensory evaluation, control yogurt was replaced with commercially available yogurt (Kingland, Australia) to evaluate and compare different sensorial attributes of legume yogurts. The commercial yogurt provides a better indicator on consumers acceptance of legume yogurts. Commercial soy yogurt obtained significantly higher score (8.18) on color attribute, followed by MBMY (6.12), SBMY (4.72), PPMY (4.52), SBFY (4.56), PPFY (4.48) and MBFY (4.42), respectively. Consumers were more familiar with the silky white color exhibited by commercial yogurt and thus gained better score, while light yellow and slight greenish color on the legume yogurts were perceived as lower visual quality. Commercial yogurt had 'moderately like' score (8) and the score was significantly different (P<0.05) from legume yogurts. Legume yogurts formulation from MBMY and PPMY were assumed to have desirable consistency as they received scores of over 5. While, the 'slightly dislike' score (4) obtained by SBFY, PPFY and MBFY could be due to the presence of lumpy and gritty material from the inhomogeneous texture of the samples. The fermentation process of legume yogurts will produce volatile organic compounds that may influence their aroma release **(Coda *et al.*, 2011)**. From the results, commercial yogurt scored 7.6 ('slightly like') (P<0.05) and PPFY was in 'neither like nor dislike' score (5). Meanwhile, aroma attributes for SBFY, SBMY, PPMY, MBFY and MBMY had below average scores, indicating low acceptance by the panelists due to the undesirable volatile compounds produced. Based on **Kaczmarska *et al.* (2018)**, green, beany and grassy odors of lupin and soybean are based on the production of hexanal, (E)-2-hexenal, 2-pentyfuran, 1-hexanol and 1- heptanol that will contribute to rancid and off flavors if present in high concentrations. Similar compounds could be present in legume yogurts since off flavors were detected during the sensory test. However, further studies are needed to verify the findings.

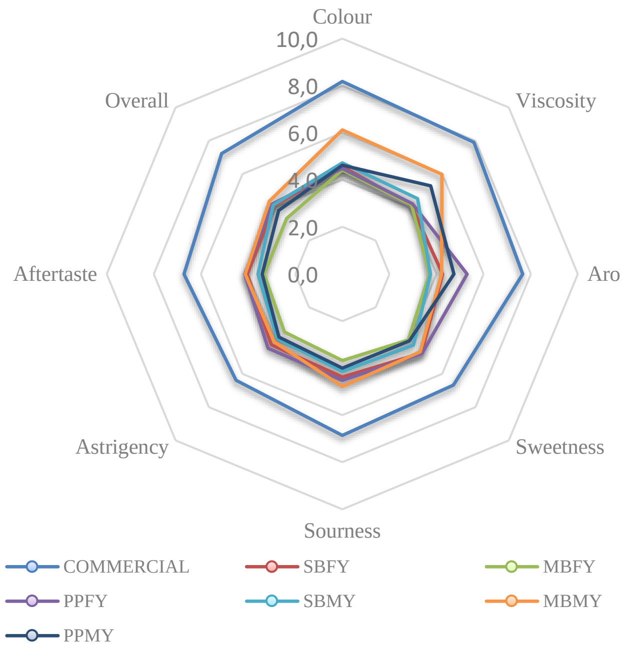


Figure 5 Sensory evaluation of legume yogurts
(Abbreviation: SBFY: Soybean flour yogurt, PPFY: Pigeon pea flour yogurt, MBFY: Mung bean flour yogurt, SBMY: Soybean milk yogurt, PPMY: Pigeon pea milk yogurt, MBMY: Mung bean milk yogurt, COMMERCIAL: Commercial yogurt).

Taste is a crucial key factor that determines the success of products. In all cases, the commercial yogurt had the highest score in sweetness (6.7), sourness (6.9), astringency (6.4) and aftertaste (6.7) attributes where the values were significantly different (P<0.05) from legume yogurts. MBFY had recorded the lowest acceptability values in all taste attributes, but they are not significantly different (P>0.05) among the legume yogurts. Addition of blueberry filling in legume yogurts were found to be insufficient to mask the unfavorable taste perceived by the panelists.A higher concentration of blueberry filling may be required in the samples. There were also great significant difference (P<0.05) in the overall acceptance between commercial yogurt (7.24) and legume yogurts (3.34 – 4.38). This study found that local consumers were not familiar with plant-based yogurt taste and this type of products were uncommon in the market. However, the impact of regular dairy yogurt consumption by consumers had resulted in diverse acceptability and perceptions on legume yogurts. Therefore, preference ratings of legume yogurts can be enhanced by increasing the exposure frequency through product awareness campaigns **(Stein *et al.*, 2003)**. In addition, repeated exposure helps to enhance familiarity to off flavors perception, which consequently influences consumer attitudes in a positive way including increase willingness to consume the products **(Granato *et al.*, 2010)**.

CONCLUSION

Legume yogurts produced from different formulations (flours and milks) and legume varieties (soybean, pigeon pea and mung bean) had shown promising utilization as fermented dairy alternative with great probiotic bacteria viability during storage (> 7 log cfu/ml), except for *B. Longum*. Legume milk yogurt formulation had higher nutritional composition than legume flour yogurt formulation, where the highest protein content was recorded by SBMY. Significant pH reduction during storage had led to an increase in lactic acid percentage in legume yogurts. Meanwhile, high buffering capacity in legume milk yogurt formulation managed to retain higher lactic acid percentage compared to legume flour yogurt formulation, indicating better viability of probiotic bacteria. All legume yogurts possessed shear thinning behaviour (n<1) with varied color (L*, a* and b*) compositions. These properties were largely influenced by their species hereditary chemical composition and colored pigments, respectively. Besides, smoother textured yogurts with no grittiness were obtained from legume milk yogurt formulation, especially with PPMY. Meanwhile, further improvements are necessary to enhance the WHC and consumer's acceptability on the legume yogurts.

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