

## Microbiological, physico-chemical, and sensory quality of yoghurt supplemented with gelatinized pigeon pea (*Cajanus cajan* (L.) Millsp.) flour

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### Abstract

Recent advances in functional food research fostered new food ingredients that can alleviate the properties already found in these foods. Among them, product diversification in fermented foods totally benefitted because of the array of raw materials that can be mixed with milk-based fermented milk products like yoghurt. Legumes were reported to have bioactive ingredients that can potentially increase the beneficial effects of yoghurt by increasing the viability of yoghurt starter cultures. In this study, the effect of supplementation with gelatinized pigeon pea flour on the microbiological, physico-chemical, and sensory properties of yoghurt during 14 days of refrigerated storage was determined. Supplementation of yoghurt with gelatinized pigeon pea flour significantly increased the viability of *Lactobacillus delbrueckii* subsp. *bulgaricus*, fermentation rate and total titratable acidity, and significantly lowered the pH of the supplemented yoghurt. However, no significant effect on the viability of *Streptococcus thermophilus* was found. Results of sensory evaluation showed that the addition of gelatinized pigeon pea flour to yoghurt significantly affected the colour, texture and consistency of yoghurt, and imparted a perceivable aftertaste, but did not significantly affect the aroma, sourness, sweetness, and overall acceptability of the product. The findings suggested that pigeon pea flour can be considered a potential ingredient for yoghurt supplementation.

## 1. Introduction

Yoghurt is a fermented dairy product known to have various beneficial effects on health owing to the functional properties of the viable lactic acid bacteria it contains. Traditionally, starter cultures used to produce yoghurt are *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB). Studies claim that these lactic acid bacteria provide health benefits to consumers, although both microorganisms do not inhabit the intestine naturally and cannot survive the conditions of the gastrointestinal tract. One of the health claims for yoghurt is the improvement of lactose digestion for individuals who are unable to digest lactose (European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010).

The enrichment of food matrices with supplements, such as prebiotic components, has been done by several studies such as fortification in dairy products, cereals, beverage supplementation and infant formula

supplementation and most of these studies aimed at enhancing the growth and efficacy of lactic and probiotic cultures (Gibson *et al.*, 2004). This in turn provides opportunities for research to develop an almost infinite array of new functional food concepts. Furthermore, product supplementation can result in an increase in the nutritional properties of several food products, including yoghurt and novel foods (Zare *et al.*, 2012).

The supplementation of prebiotics and other potential ingredients to improve the nutritional properties of yoghurt and other fermented dairy products has become a noteworthy trend. Several plant-based materials contain significant amounts of prebiotic compounds. Various food crops contain prebiotic carbohydrates, and it has been suggested that legumes can serve as a potential prebiotic ingredient that can possibly alter intestinal microbiota, affecting the production of gut hormones and therefore affecting the appetite (Foyer *et al.*, 2016).

Pigeon pea (*Cajanus cajan* (L.) Millsp.), known in

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the Philippines as *kadios*, is an indigenous and underutilized legume that has not been fully explored. Over the years, pigeon pea has been considered a "poor man's crop", particularly in the Philippines. However, recent studies have explored the potential of the crop in the form of value-added processed products. Barroga (2007) tested various recipe formulations to develop new processed products from pigeon peas, including pigeon pea cakes, cookies, and polvoron. The study concluded that pigeon pea processing has a potential and a high prospect as an income-generating venture in the agri-based micro-small and medium-scale enterprises of the country.

The dry edible seeds of legumes called pulses which include pigeon peas are excellent food sources with several health benefits. Further, pigeon peas are good sources of protein and complex carbohydrates, including resistant starch, dietary fibres, and oligosaccharides, and contain important vitamins and minerals (Khan *et al.*, 2007; Sandhu and Lim, 2008; Kasote *et al.*, 2014; Talari and Shakappa, 2018). Thus, this food ingredient can serve as a valuable source of nutrients and prebiotic components and may be added to several existing food products. Trinidad *et al.* (2010) suggested that pigeon peas can be considered a functional food ingredient to supplement rice, bread, and other food products.

Although various efforts were done to explore the potential of the pigeon pea crop in the form of value-added processed products, the application of pigeon pea flour as a potential ingredient in yoghurt and its effect on the viability of the starter bacteria, as well as the physico-chemical and sensory characteristics of the fermented dairy product, have been less reported. Thus, the effect of the supplementation of yoghurt with gelatinized pigeon pea flour was explored in this study.

## 2. Materials and methods

### 2.1 Experimental design

A completely Randomized Design (CRD) with three replications for each treatment was used for the physicochemical, microbiological, and sensory analyses.

### 2.2 Preparation of pigeon pea flour

Mature pigeon pea seeds (Farmer's Variety: Bangluwan) were obtained from Salcedo, Ilocos Sur, Philippines. Pigeon pea flour was prepared by washing and soaking the seeds in potable water, followed by dehulling, and drying using a cabinet dryer at 60°C for 16 hrs. The dried seeds were then ground using a pin mill and passed through a 100-mesh sieve to obtain the flour. The flour was then stored in a clean, air-tight plastic container at room temperature until needed for analysis.

### 2.3 Preparation of gelatinized pigeon pea flour

The technology for producing gelatinized pigeon pea flour was developed in this study. Pigeon pea flour was gelatinized using a 1:3 flour-water ratio, at 74°C for 15 mins. The gelatinized flour was then cooled to room temperature before it was used in yoghurt production.

### 2.4 Microbial cultures

Commercially available freeze-dried yoghurt starter culture (YF-L812) manufactured by Chr. Hansen was used for the experiment.

### 2.5 Yoghurt production

Yoghurt was produced following the methods of Chen *et al.* (2018) with modifications. Milk was standardized by the addition of skim milk powder and refined sugar to obtain the following desired composition: 3.5% fat, 12% milk solids-not-fat MSNF and 7% sugar. The mixture was stirred until the dry ingredients were completely dissolved. Approximately 5.17% of gelatinized pigeon pea flour was then added to the yoghurt mix. The mixture was pasteurized at 85 – 90°C for 10 mins, then cooled to 45°C in a chilled water bath. The cooled yoghurt mix was then inoculated with the freeze-dried starter cultures, then fermented at 42°C. The pH of the yoghurt mix was monitored after 4 hrs of incubation until pH 4.5 was reached. Without disturbing the gel, the yoghurt was cooled to 7°C and stored at this temperature for 14 days. Control yoghurt sample without gelatinized pigeon pea flour was prepared following the same procedure. The viability of the starter cultures, as well as the changes in the rate of fermentation, pH and total titratable acidity, were monitored at 0, 1, 2 and 14 days of refrigerated storage. The treatments were replicated three times.

### 2.6 Microbiological quality of the pigeon pea flour - supplemented yoghurt

The determination of the viable counts of the yoghurt starters was done following the procedure by Guevarra and Barraquio (2015) with modifications. Enumeration of the starter microorganisms was performed using the pour plate technique. LB and ST were enumerated on Lee's Agar.

### 2.7 Physico-chemical analyses

The pH values of the yoghurt samples were measured in triplicate using a Milwaukee pH pen. The total titratable acidity of the samples was determined following the standard AOAC Method 947.05 (1990) and expressed as % lactic acid.

## 2.8 Sensory evaluation

Prior to the conduct of the sensory evaluation of the samples, approval was sought from the Research Ethics Review Committee of the University of San Agustin. During the sensory evaluation sessions, panellists were asked to evaluate yoghurt samples with and without gelatinized pigeon pea flour based on its colour (0 – creamy white, 15 – yellowish); aroma (yoghurt): (0 – weak, 15 – strong); texture (0 – gritty, 15 – smooth); consistency (0 – runny, 15 – thick); sourness (0 – weak, 15 – strong), sweetness (0 – weak, 15 – strong), after-taste (0 – none, 15 – strong); and overall acceptability (0 – not acceptable, 15 – very acceptable) using a 15-cm horizontal line scale.

## 2.9 Statistical analysis

All analyses were performed in triplicates. Experimental results were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) was used to analyze the significant differences among the treatment means. All statistical analyses were carried out using Statistica version 10.

## 3. Results and discussion

The supplemented yoghurt had LB and ST viable counts that were higher (Figure 1a and Figure 1b) than the recommended minimum level which is  $10^7$  CFU/mL following the Codex Stan 243-2003 (Codex Alimentarius, 2003). Viable counts of LB and ST in the supplemented yoghurt increased from 5.19 to 8.45 log CFU-mL<sup>-1</sup> and 6.12 to 8.31 log CFU/mL, respectively, throughout the 14 days of refrigerated storage. Results showed that the cell density of LB in the supplemented yoghurt was significantly higher compared to the control after two days of refrigerated storage. At the end of the storage period, viable counts of LB in the control sample significantly decreased and is significantly lower compared to viable counts of LB in the supplemented sample. These observed reductions in the viable counts of LB in the control sample were similar to those reported by Zare *et al.* (2012) and Chen *et al.* (2018). Unlike the control sample, the viability of LB in the supplemented yoghurt remained stable until the 14th day of refrigerated storage. On the other hand, viable counts of ST in the supplemented yoghurt were not significantly different from the control. The significantly higher viable counts of LB in the supplemented yoghurt compared to the control indicates that supplementation of yoghurt with pigeon pea flour significantly enhanced the growth and improved the viability of the microorganism during refrigerated storage. - Similarly, some studies reported that the addition of pulse flours to yoghurt mix enhanced the growth of probiotic bacteria specifically during 7-day

storage (Agil *et al.*, 2013) and promoted the viability of LB (Zare *et al.*, 2012; Chen *et al.*, 2018).

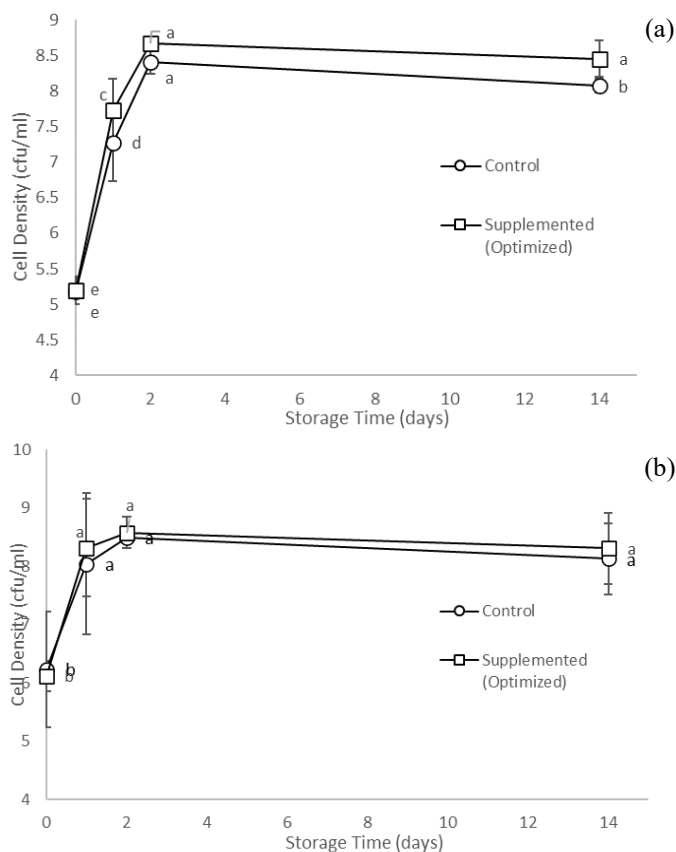


Figure 1. Cell density of (a) *Lactobacillus delbrueckii* subsp. *bulgaricus* and (b) *Streptococcus thermophilus* in yoghurt during refrigerated storage. Means with different notations are statistically significantly different ( $p < 0.05$ ).

The specific component of pigeon pea flour that may have contributed to the beneficial effect particularly the improved viability of the starter cultures remains unclear since this was outside the scope of the study. However, pigeon pea contains high levels of carbohydrates and can act as a potential prebiotic source as it contains a significant amount of resistant starch, dietary fibre and  $\alpha$ -galactoside oligosaccharides such as raffinose, stachyose, and verbascose which can favour the growth of the starter cultures and other probiotic microorganisms (Parra *et al.*, 2013). Carbohydrates have also been shown to contribute to the improvement of the stability of yoghurt bacteria during storage (Silva *et al.*, 2004) which suggests that the carbohydrate fraction of pigeon pea may have contributed to the improvement of the viability of the microorganisms. The improvement in the growth of LB and ST during refrigerated storage may also be due to several other stimulatory factors present in pigeon pea flour, which include protein, micronutrients, and B vitamins. However, further research is needed to determine which specific components are involved in improving the viability of the starter cultures in the pigeon pea-supplemented yoghurt. Although the control sample had bacterial counts within the given limit,

yoghurt supplemented with gelatinized pigeon pea flour demonstrated viable counts higher than the minimum level (8 log CFU/mL) after 14 days of refrigerated storage.

The rate of fermentation considerably affects the processing time for yoghurt production which is directly correlated to economic viability (Zare, 2012). The growth rate of the yoghurt starters is generally related to acidification and the rate of fermentation is linked to the decrease in pH of yoghurt due to the production of lactic acid from lactose by the starter cultures during incubation at 42°C (Chen *et al.*, 2018). The fermentation rate of yoghurt starters in the control and supplemented yoghurt are shown in Figure 2.

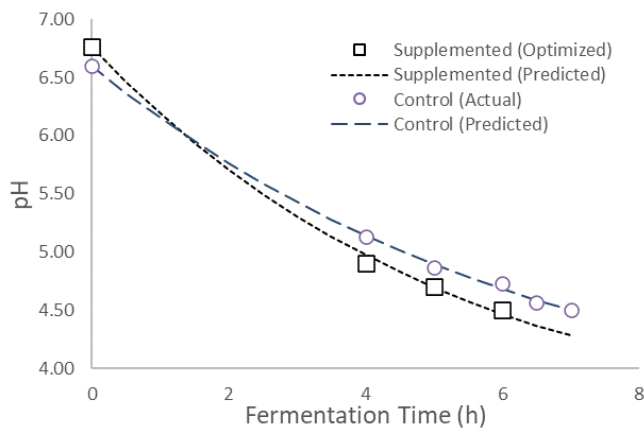


Figure 2. Change in pH as a function of incubation time during fermentation of yoghurt at 42°C

As both supplemented and control samples reached the endpoint pH of 4.5, it can be considered that the lactose fermentation to lactic acid was not affected by the supplementation of yoghurt with pigeon pea flour. The supplemented yoghurt reached the endpoint of pH in 6 hrs compared to 7 hrs for the control. This would mean that the activity of the yoghurt bacteria was not inhibited by the supplementation with pigeon pea flour as the supplemented yoghurt reached the endpoint pH faster than the control. Furthermore, the addition of gelatinized pigeon pea flour to yoghurt resulted in an increased rate of fermentation and consequently decreased the processing time for yoghurt production. Chen *et al.* (2018) also reported an increase in the fermentation rate of yoghurt fortified with chickpea flour. Similarly, it has also been reported that the rate of acidification of probiotic-fermented milk is increased when pulse flours are added (Zare *et al.*, 2012). In some studies, modifying the composition of the carbohydrates in milk resulted in an increase in the rate of acidification of yoghurt starters (Tamime and Robinson, 1999). As previously mentioned, pigeon pea contains high levels of carbohydrates, particularly resistant starch, dietary fibre and  $\alpha$ -galactoside oligosaccharides such as raffinose, stachyose, and verbascose. This would suggest that the

carbohydrate fraction of pigeon pea may have promoted the metabolic activity of the yoghurt microorganisms (Chen *et al.*, 2018).

Following fermentation, the supplemented yoghurt had a significantly more acidic pH relative to the control after a day of refrigerated storage (Figure 3). A significant reduction in the pH of the supplemented yoghurt was observed as the storage time increased which is related to the increase in the activity of the microorganisms in the presence of pigeon pea flour. On the other hand, a slow decrease in the pH of the control was observed. At the end of the storage period, there was a significant difference in the pH values of the supplemented and the control yoghurt. The supplemented yoghurt had lower final pH than the control, which indicates that supplementation with pigeon pea flour resulted in an increase in the acidifying ability of the starter cultures during storage.

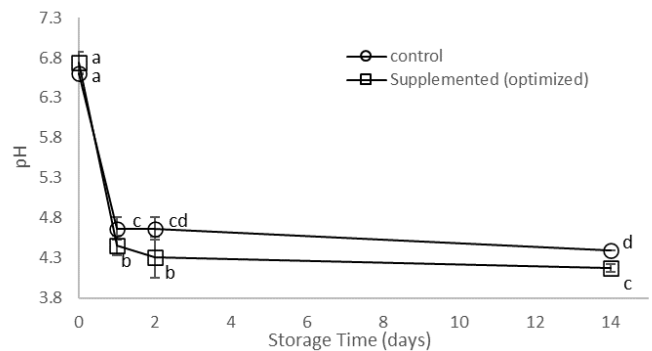


Figure 3. Change in pH as a function of storage time during refrigerated storage of yoghurt. Means with different notations are statistically significantly different ( $p < 0.05$ ).

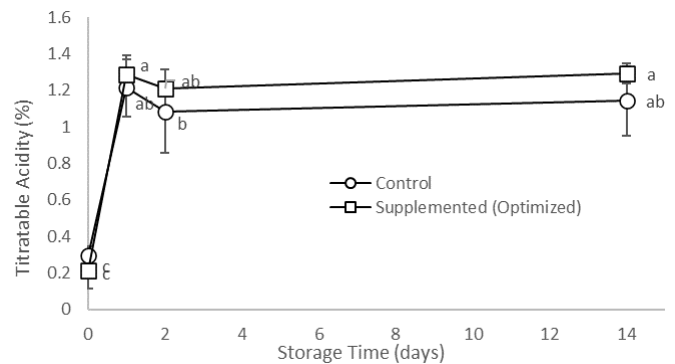


Figure 4. Change in titratable acidity as a function of storage time during refrigerated storage of yoghurt. Means with different notations are statistically significantly different ( $p < 0.05$ ).

The decrease in pH of the yoghurt samples resulted in a concurrent increase in the titratable acidity of yoghurt over storage time (Figure 4). The titratable acidity values of both control and supplemented yoghurt generally increased across the 14-day refrigerated storage. After fermentation and a day of refrigerated storage, both control and supplemented samples had titratable acidity values above the recommended limit

which is a minimum of 0.6% according to Codex Stan 243-2003 (Codex Alimentarius, 2003). Initially, the titratable acidity of the control sample was significantly higher than the supplemented yoghurt. The slow increase in the acidity of the supplemented yoghurt was probably due to the higher protein content of the product. The buffering action of proteins stabilizes the acidity of the yoghurt system (Zanhi and Jideani, 2012). However, as the storage time increased, the titratable acidity of the supplemented yoghurt significantly increased and was significantly higher than the titratable acidity of the control sample. This indicates that supplementation with pigeon pea flour enhanced the metabolic activity of the yoghurt bacteria over time, which resulted in the increased titratable acidity of the product towards the end of storage. Similarly, Agil *et al.* (2013) observed that yoghurt samples containing 0 – 4% lentils have significantly higher titratable acidity levels than their respective controls towards the end of the 21-day refrigerated storage.

Thus, supplementation of yoghurt with pigeon pea flour significantly decreased the pH and increased the titratable acidity compared to the control towards the end of the refrigerated storage. Pulse flours were observed to encourage the growth of yoghurt bacteria, particularly LB which is known to produce high amounts of acid (Zare *et al.*, 2012). Enhanced growth and metabolic activity of the said microorganism may have mainly contributed to the significant decrease in pH and increase in titratable acidity of the supplemented yoghurt. In addition, the decreased pH and increased titratable acidity at the later storage periods are also consistent with the results of the viable counts of LB and ST, whereby the viable counts of both microorganisms increased at these same storage times in the presence of pigeon pea flour. The high viable counts of the starter cultures in the supplemented yoghurt even at refrigerated storage showed that the microorganisms remained active and continued to break down lactose to lactic acid which resulted in a faster drop in pH and an increase in titratable acidity compared to the control.

Results showed no significant difference ( $p>0.05$ ) for scores on aroma, sourness, sweetness, and the overall acceptability between the control sample and the pigeon pea – supplemented yoghurt (Table 1). On the other hand, scores on colour, texture, consistency and aftertaste were found to be significantly different.

The supplemented product had a mean colour score higher than the control sample. Higher colour scores indicate a higher degree of yellowness. Raw pigeon pea flour has a very light yellow color similar to skim milk. However, gelatinization of the flour enhanced its colour,

increasing the degree of yellowness and this contributed to a more intense yellow colour of the pigeon pea – supplemented yoghurt compared to the control. The enhanced colour of the flour after gelatinization was due to the reaction of the protein and carbohydrate components of pigeon pea upon the application of heat (Maillard reaction).

The texture of the yoghurt samples was evaluated in terms of smoothness. The supplemented yoghurt had a lower mean score than the control which indicates that the supplemented sample has a relatively gritty texture. The grittiness in the texture of the supplemented yoghurt was due to the insoluble dietary fibres of the pigeon pea flour.

Table 1. Mean sensory scores of control and pigeon pea flour – supplemented yoghurt.

| Parameters                          | Control | Supplemented |
|-------------------------------------|---------|--------------|
| Colour*                             | 2.90    | 5.00         |
| Aroma <sup>ns</sup>                 | 8.13    | 7.33         |
| Texture*                            | 12.93   | 10.18        |
| Consistency*                        | 6.95    | 9.68         |
| Sourness <sup>ns</sup>              | 5.28    | 6.17         |
| Sweetness <sup>ns</sup>             | 5.78    | 5.05         |
| Aftertaste*                         | 2.88    | 6.25         |
| Overall Acceptability <sup>ns</sup> | 10.78   | 10.15        |

Values are means of three independent determinations, n = 10. \*Means are statistically significantly different ( $p<0.05$ ), <sup>ns</sup>not significant

Similarly, supplementation with gelatinized flour significantly affected the consistency of the yoghurt. Judges gave the control sample a mean consistency score that is neither too runny nor too thick. On the other hand, the supplemented yoghurt was given a higher mean consistency score which means that its consistency is thicker than the control sample. Pigeon pea has relatively high carbohydrate content, particularly their starch and fibre fractions which mainly contributed to the thick consistency of the supplemented product. Subjecting the flour to the gelatinization – retrogradation process and further heating during pasteurization of the yoghurt mix resulted in the gelatinization of its starch component (Singh *et al.*, 2003). The starch fraction of the pigeon pea flour was solubilized in the milk, resulting in viscous yoghurt. Starch granules become embedded in the continuous protein network during the pasteurization of the yoghurt mix prior to the start of the fermentation process (Oh *et al.*, 2006). The gelatinized starch granules are then increased in size, resulting in an increased concentration of the protein network and large protein particle formation, and thus, a thick consistency (Singh *et al.*, 2003; Oh *et al.*, 2006).

Sensory evaluation results showed that the

supplemented yoghurt had a significantly perceivable aftertaste. The non-volatile compounds that contribute to the aftertaste of pigeon pea – supplemented yoghurt are less well understood. According to studies, bitterness and astringency are the common aftertastes perceived. The small peptides present, as well as the various secondary compounds such as flavonoids, phenols and saponins, may have contributed to the bitterness of the product (Kinney, 2003), which in turn led to the formation of astringent aftertaste in the supplemented yoghurt (Zhang et al., 2012). The aftertaste perceived by the panellists may have been also due to the beany flavour associated with the gelatinized pigeon pea flour. Supplementation with 5.17% gelatinized pigeon pea flour resulted in a dominant beany flavour in the product.

Although the mean scores for aroma, consistency and aftertaste of the control and supplemented yoghurt were found to be significantly different, this did not greatly affect the overall acceptability of the product. Results showed that the overall acceptability of the control sample was not significantly different from the supplemented product. Some modifications in the yoghurt formulation could help improve some sensory aspects, particularly the perceivable aftertaste and beany flavour, especially at high levels of supplementation. Nevertheless, the supplemented yoghurt is comparable to the control sample in overall acceptability, thus, it is reasonable to say that yoghurt can be supplemented with pigeon pea flour to enhance the nutritional quality of the product without affecting the aroma, sourness, sweetness, and overall acceptability significantly ( $p < 0.05$ ).

#### 4. Conclusion

Supplementation of yoghurt with pigeon pea flour resulted in significantly higher viable counts of LB compared to the control throughout the 14 days of refrigerated storage which indicates that the growth and viability of the microorganism were significantly enhanced. Furthermore, the addition of gelatinized pigeon pea flour to yoghurt resulted in a significantly increased rate of fermentation, which consequently decreased the processing time for yoghurt production. A significant decrease in the pH and a concurrent increase in the titratable acidity in the supplemented sample were also observed. The enhanced growth and metabolic activity of LB in the supplemented yoghurt may have mainly contributed to the significant decrease in pH and increase in titratable acidity of the product. Results of sensory evaluation showed that the pigeon pea – supplemented yoghurt is comparable to the control in terms of the aroma, sourness, sweetness, and overall acceptability. Overall, based on the microbial, physico-

chemical and sensory properties of the pigeon pea – supplemented yoghurt, the results suggest that pigeon pea flour can be considered a potential ingredient for yoghurt supplementation.

#### Conflict of interest

The authors declare no conflict of interest.

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