PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY ATTRIBUTES OF PROBIOTIC YOGHURT INCORPORATED WITH *KITHUL* (*Caryota urens*) FLOUR

K.R.M Peiris[1], P.H.P. Prasanna[2], D.V.P. Chandramali[1], D.C.S. Gunasekara[3] and P.M.V. Madhusanka[4]

[1]*Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura.*
[2]*Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya,*
[3]*CIC Dairies (Pvt.) Ltd., Ibbankatuwa, Dambulla.*
[4]*Food and Nutrition Research Center, CIC Agribusiness (Pvt.) Ltd., Palwehera.*

Abstract

In this study, physicochemical, microbiological and sensory differences of probiotic set yoghurt incorporated with different concentration of Kithul flour (KF) were evaluated, as well as the changes taking place during storage at 4°C for 21 days. Gelatine incorporated yoghurt and yoghurt without any added stabilisers (control) were used to compare the results. During the storage, the addition of KF improved survival of B. animalis ssp. lactis BB-12 (BB12). All yoghurts exhibited a decrease in pH followed by an increase in titratable acidity during storage. Most of yoghurts with KF except incorporation level of 1.5% showed low syneresis than the control yoghurt. Results of the sensory evaluation showed that yoghurt incorporated with KF (0.5%) had improved sensory attributes compared to other treatments. Therefore, this study revealed that KF could be used to produce probiotic set yoghurt with improved physicochemical, microbiological and sensory attributes.

Keywords*: B. animalis ssp. lactis BB-12, Kithul flour, Syneresis, Probiotic, Yoghurt*

INTRODUCTION

Kithul (Caryota urens) is an underutilized palm species which is native to India, Sri Lanka, Malaysia and Indonesia (Wimalasiri et al., 2016). It is recorded as a multipurpose tree species cultivated for preparation of toddy, sago, jaggery and fiber. One of the main edible food product yield from the *Kithul* palm is the starch, which is stored in the trunk. *Kithul* flour is a cheap, readily available source of food starch and the palm reported to produce 24 tonnes/hectare of starch per year (Rajyalakshmi, 2004). *Kithul* flour has higher gel-forming ability than other flour in the food industry (Kuhanneya et al., 2016). Stabilizers are the chemical substances which used as food additives and they help to improve the structure and texture of the food products. Sometimes these are considered as the hydrocolloids which have the ability to make a network linkage between the milk constituents (Tamime and Robinson, 1999). Most of the stabilizers (Gelatine) are animal origin and few plant-based stabilizers are available in the market like sea weeds and pectin. However, in Sri Lanka, the availability of those plant-based stabilizers is lower and prices are somewhat higher than gelatine (Wijesingha et al., 2015). At present there is a trend created toward the consumption of non-animal origin food ingredients which are low cost. In this scenario, *Kithul* flour can be considered as a low cost and good nutritious alternative for gelatine like synthetic stabilizers because this flour has a good gel forming ability when compared with other types of flours.

Probiotics are described as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill et al., 2014). Probiotics supply beneficial effects on the gut and the immune system. Dairy products such as yoghurt, cheese and fermented milk are the most important foods in the current probiotic food industry (Nicklas et al., 2011). Probiotics must be present at a minimum level, ranging from 106 to 109 CFU/mL of the product, to be beneficial. The best-known probiotic microorganisms include strains within *Lactobacillus* and *Bifidobacterium* genera (Vasiljevic and Shah, 2008). Prebiotics are non-digestible food ingredients which can stimulate the growth of probiotic bacteria (Gibson et al., 2017). Among the food ingredients, non-digestible carbohydrates such as oligosaccharides and polysaccharides, some peptides and proteins and certain lipids (both ethers and esters) are used as prebiotics (Gibson et al., 1995). According to Rajyalakshmi (2004), *Kithul* flour contains 0.2-3.5% amount of crude fiber, and could act as prebiotic which may help to increase the viability of probiotic bacteria. Thus, this research was designed to evaluate the incorporation of *Kithul* flour as a stabilizer on physicochemical, microbiological and sensory properties of probiotic set yoghurt.

MATERIALS AND METHODS

Preparation of *Kithul* flour

Kithul flour was extracted according to the method explained by Rajyalakshmi (2004) with some modifications. Pith of the palm was cut into 1 cm 2 pieces and crushed by using a clean mortar. The crushed pith was mixed with water and filtered by using a clean muslin cloth into a pot, and was kept for 12 hours in room temperature to facilitate to settle flour into the bottom of the pot. Then, it was dried by using oven at 105ºC for 4 hours, and was ground and sieved by 100 µm sieve to get *Kithul* flour.

Proximate analysis

Moisture content, crude protein, crude fat, ash content and crude fiber content of raw *Kithul* flour and yoghurts were analysed according to the AOAC (2005) techniques.

Preparation of yoghurt inoculum

Working cultures of thermophilic yoghurt bacteria and *B. animalis* ssp. *lactis* BB-12 were prepared and in the production of the working thermophilic yoghurt culture, sterilised milk was inoculated with thermophilic yoghurt cultures (YC-X11 YoFlex, Chr. Hansen, Hoersholm, Denmark), consisting *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *bulgaricus* at a rate of 1% (w/v) and incubated at the 42°C for ∼4 h. In the case of *B. animalis* ssp. *lactis* BB-12, the sterilised milk was supplemented with 0.5% filter sterilised yeast extract (0.5%, v/v) and inoculated with the culture (Chr. Hansen, Hoersholm, Denmark) at a rate of 1% (w/v). The mixture was incubated at 37°C under anaerobic conditions for 18 h.

Preparation of probiotic set yoghurt by incorporating *Kithul* flour

Cow milk was preheated at 55℃ and *Kithul* flour was added (0.25%, 0.5%, 1%, 1.5%) to the milk and homogenized for 15 minutes (10-20 MPa). The mixture was pasteurized at 85°C for 30 min and cooled to 43°C and inoculated with the standard working thermophilic yoghurt culture and the working culture of *B. animalis* ssp. *lactis* BB-12 at the level of 1% (v/v). The mixture was incubated at 42 ± 2°C until the pH reached 4.5. The samples were collected from each yoghurt on 1, 7, 14, 21 and 28 d of storage for analysis.

Measurement of pH and titratable acidity of yoghurts

pH of the sample yoghurts was measured using a pH meter (Mettler Toledo, UK) during the storage. In the case of titratable acidity, 10 g of yoghurt sample was taken in to a conical flask and 2-3 drops of phenolphthalein were added as an indicator. It was titrated with 0.1 N NaOH solution until it turned to permanent pale pink colour and calculation was done as explained by Dave and Shah (1997).

Determination of syneresis

Syneresis of yoghurt was measured by centrifugal method as described by Lobatocalleros et al. (2014). Briefly, 14g of yoghurt was measured and placed in centrifugal tube and centrifuged at 222g for

10 minutes, at 4℃. The weight of supernatant was measured. The syneresis value was recorded as weight of released whey per 100 g of yoghurt.

Determination of viability of bifidobacteria

The growth and viability of bifidobacteria were studied using the spread plate technique. For this purpose, 1 g of yoghurt was mixed with 9 mL of sterile phosphate-buffered saline (PBS) (Oxoid, UK) and subsequently samples were serially diluted using PBS for the selective enumeration of bifidobacteria. 100 µL aliquots were plated on BSM agar (Sigma- Aldrich, UK) to enumerate the viable bacterial counts. The plates were incubated under anaerobic conditions at 37°C for 72 h.

Sensory evaluation

Sensory evaluation of the final product was conducted by using 30 untrained panellists. The panellists were analysed 5 sensory attributes as colour, odour, flavour, texture and overall acceptability according to the nine-point hedonic scale for the newly prepared yoghurts.

Statistical analysis

There were six treatments and four replicates. Completely Randomized Design (CRD) was used to analyse significant difference in physiochemical, microbiological properties among yoghurt samples. Data was analysed using Analysis of Variance (ANOVA) in Statistical Software Analysis (SAS) programme version 9.2 (SAS Institute Inc., Cary NC, USA) with 95% confidence interval and mean separation was done by Tukey's Range Test. Sensory evaluation data was analysed using Friedman method with MINITAB software with 95% confidence interval.

RESULTS AND DISCUSSION

Proximate composition of the extracted *Kithul* flour and yoghurt

The proximate composition of the extracted *Kithul* flour and different yoghurts is shown in Table 1.

Table 1: Proximate composition of raw *Kithul* flour and different yoghurt types

Treatment		Moisture content %	Ash %	Fat %	Protein %	Crude Fiber %	Carbohydrates By difference %
Raw *Kithul* flour		12.1±1.85	0.56±0.03	0.47±0.07	0.93±0.03	1.77±1.1	84.17±1.01
***Kithul* flour yoghurt**	0.25%	80.75±1.50^{a}	0.89±0.22^{a}	3.60±0.10^{a}	3.42±0.02^{a}	ND	ND
	0.5%	81.25±4.27^{a}	1.03±0.42^{a}	3.53±0.20^{a}	3.39±0.04^{ab}	ND	ND
	1.0%	83.00±0.00^{a}	0.73±0.26^{a}	3.57±0.15^{a}	3.40±0.04^{ab}	ND	ND
	1.5%	84.00±4.24^{a}	1.29±0.61^{a}	3.57±1.06^{a}	3.44±0.02^{a}	ND	ND
Gelatine yoghurt		84.50±1.73^{a}	1.05±0.55^{a}	3.67±0.10^{a}	3.33±0.03^{b}	ND	ND
Control yoghurt		83.75±2.87^{a}	1.05±0.59^{a}	3.57±0.06^{a}	3.33±0.02^{b}	ND	ND

Values are means ± standard deviations of four replicate measurement. Means with the same letters in the same column are significantly different (P<0.05). Control made by without adding *Kithul* flour or gelatin. ND: Not determined.

The results showed that moisture content of Kithul flour was 12.1% while protein content was around 0.9%. In addition, Kithul flour had very low amount of fat. These results are similar to the values reported by Wijesinghe et al. (2015). This ensures that the extraction method used in the study was successful and could be used to extract this valuable food resource. There was no significant difference (P<0.05) among treatments for moisture, dry matter, fat and ash content. However, water content showed a slight increase with the increase the level of incorporation of Kithul flour in yoghurt. In addition, there was a significant difference (P<0.05) of in protein content among treatments. This may due to the incorporation of Kithul flour which can increase protein content of yoghurts.

Changes of pH and titratable acidity

There was a decrease in pH (Figure 1) and increase in titratable acidity (TA) (Figure 2) during the storage for all the treatments. However, the drop in pH and increase in TA were higher for the samples containing *Kithul* flour up to 0.5% compared to those of the control yoghurt and this is due to continued residual fermentation at the refrigerated storage. The higher decrease in pH and concurrent increase in acidity observed in the *Kithul* flour containing treatments may be due to higher availability of carbohydrate sources from flour to the metabolic activity of both yoghurt starter cultures and *B. animalis* ssp. *lactis* BB-12 resulting higher level of production of organic acids. The similar results have been previously reported by Coman et al., (2013) for fermented milk supplemented with buck wheat flour and oat bran. In addition, Cruz et al., (2010) reported that pH decrease of yoghurt is mainly due to post acidification caused by the uncontrolled growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* under refrigerated conditions.

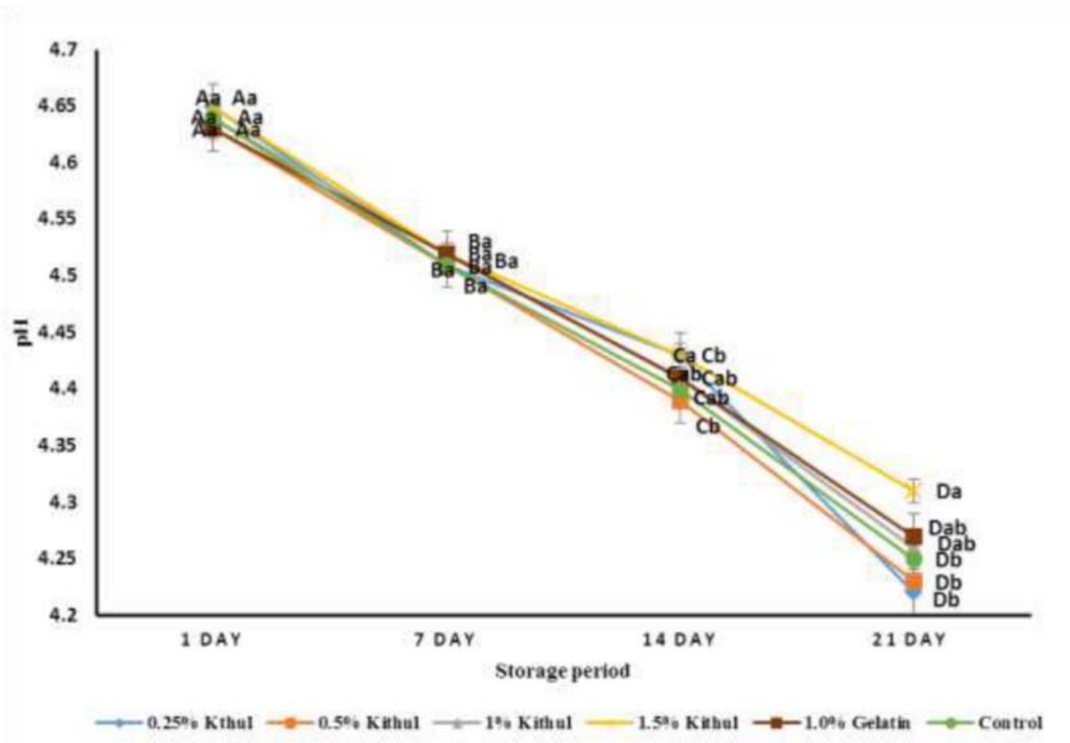

Figure 1: Variation of pH of different yoghurts during the storage period

Vertical lines represent standard deviations. A, B, C, D Means with different uppercase are significantly (P<0.05) different between each day, for each type of yoghurt during the storage. a, b, c, d Means with different lowercase are significantly (P<0.05) different between each type of yoghurt, for a particular day of storage.

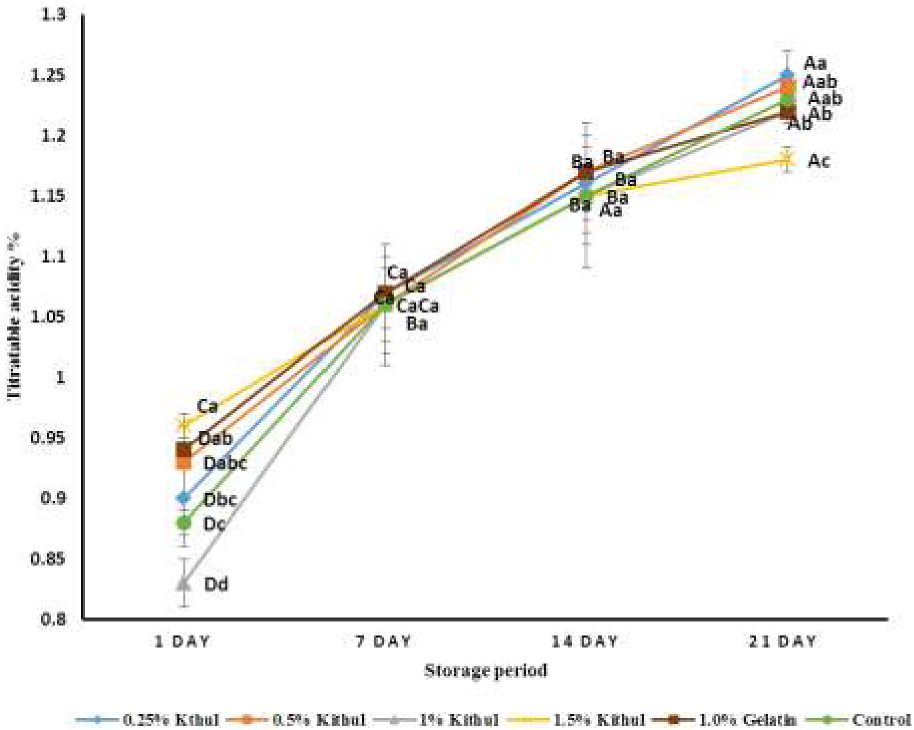

Figure 2: Changes in titratable acidity of yoghurts during the storage period

Vertical lines represent standard deviations. A, B, C, D Means with different uppercase are significantly (P<0.05) different between each day, for each type of yoghurt during the storage. a, b, c, d Means with different lowercase are significantly (P<0.05) different between each type of yoghurt, for a particular day of storage.

Syneresis

Syneresis is a major visible defect in commercial yoghurt manufacturing which leads accumulation of whey on the surface of the gel, which can lead to poor acceptability of the product by consumers (Amatayakul et al., 2006). Fig. 3 shows syneresis of different yoghurts during the storage. Incorporation of *Kithul* flour resulted yoghurt with low syneresis compared to the control and gelatin incorporated yoghurt. Previous researches showed that addition of modified starches and dried dairy ingredients increase in the density of protein matrix in the gel microstructure that helps to decrease syneresis of yoghurts (Wijesingha et al., 2015). The observed results in the present study agree with the findings of Kumari et al. (2015) who showed that the rice incorporation led for lower syneresis in symbiotic yoghurts. Similarly, Zare et al. (2012) reported that addition of lentil flour resulted probiotic yoghurt with lower syneresis. However, yoghurt containing 1.5 % (w/v) flour level showed sudden higher syneresis when compared to other three levels which may be due to disruption of textural properties of yoghurt as observed by Guggisberg et al. (2009) who reported no effect of addition inulin beyond 2% (w/v) in reduction of syneresis.

Viability of *B. animalis* ssp. *lactis BB-12* during storage

Figure 4 shows the cell concentration of *B. animalis* ssp. *lactis* BB-12 in the different yoghurts produced, as well as during refrigerated storage for 21 days. During storage for 21 days, all yoghurt showed a significant (P<0.05) decrease in the number of bifidobacteria. However, all yoghurts containing *Kithul* flour showed significantly higher population of BB-12 compared with the control and gelatine containing yoghurt. These results indicate that *Kithul* flour could stimulate the growth of BB-12 during the storage period. The improvement of the growth of BB- 12 probiotic bacteria in *Kithul* flour incorporated yoghurts could be related to the compounds that present in the *Kithul* flour. *Kithul* flour contains higher level of carbohydrates and fiber which can have an effect on growth of bifidobacteria. In addition, *Kithul* flour also contains considerable amount of calcium, potassium, sodium, iron, zinc and magnesium (Wijesingha et al., 2015), which may stimulate growth and performances of bifidobacteria. These compounds may be beneficial for the BB-12 because probiotic bacteria required high nutrients to full fill their growth. Similarly, Casarotti et al. (2014) showed that addition of quinoa flour had the same trends for the viability of BB-12 during the storage period of fermented milk.

Sensory evaluation of yoghurts

Figure 5 shows results of sensory evaluation of different yoghurts. There was a significant difference (P<0.05) in sensory attribute namely appearance, colour, taste, texture and overall acceptability except odour.

0.5% *Kithul* flour incorporated yoghurt showed the highest rank for texture, taste and overall acceptability while 0.25% *Kithul* flour incorporated yoghurt showed the highest rank for appearance and odour. Gelatin incorporated yoghurt showed the highest rank for the colour. 1.5% *Kithul* flour incorporated yoghurt had a light pinkish colour compared with other *Kithul* flour incorporate yoghurts and 0.25% and 0.5% *Kithul* flour incorporated yoghurt had a very similar colour to the control. When the concentration of the *Kithul* flour increases, the sour taste of the yoghurts decreases than the control and gelatin incorporated yoghurts.

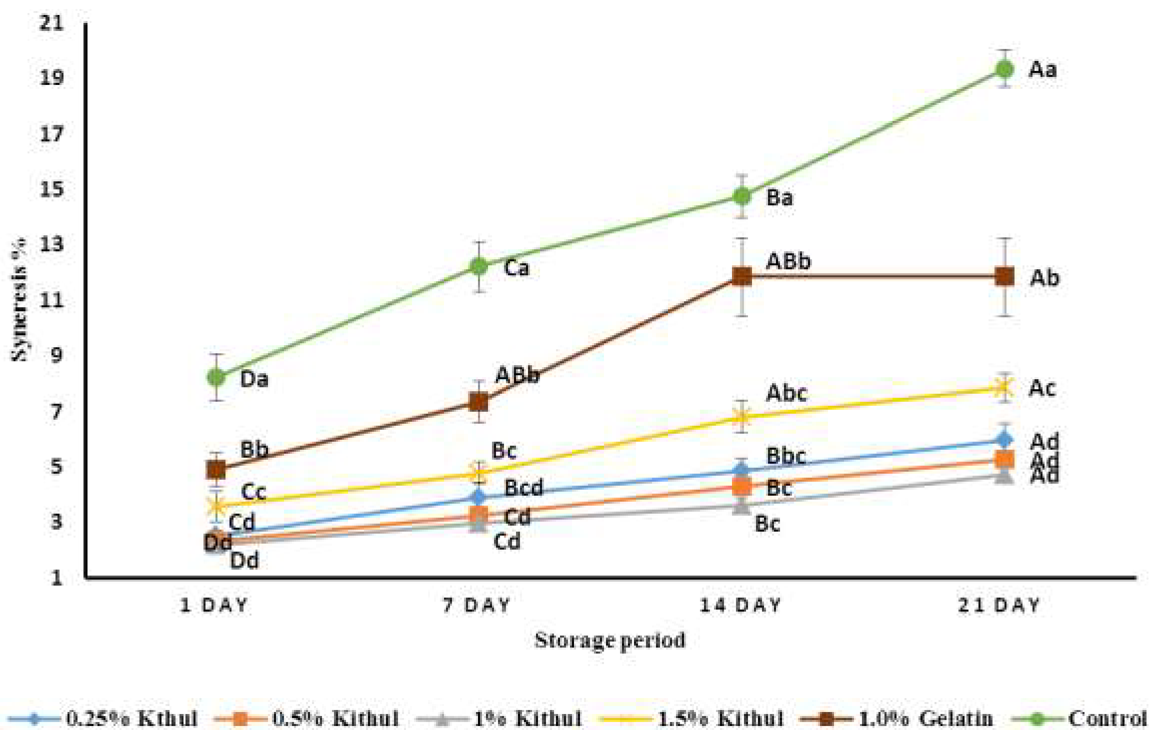

Figure 3: Variation of syneresis of different yoghurts during the storage period

Vertical lines represent standard deviations. A, B, C, D Means with different uppercase are significantly (P<0.05) different between each day, for each type of yoghurt during the storage. a, b, c, d Means with different lowercase are significantly (P<0.05) different between each type of yoghurt, for a particular day of storage.

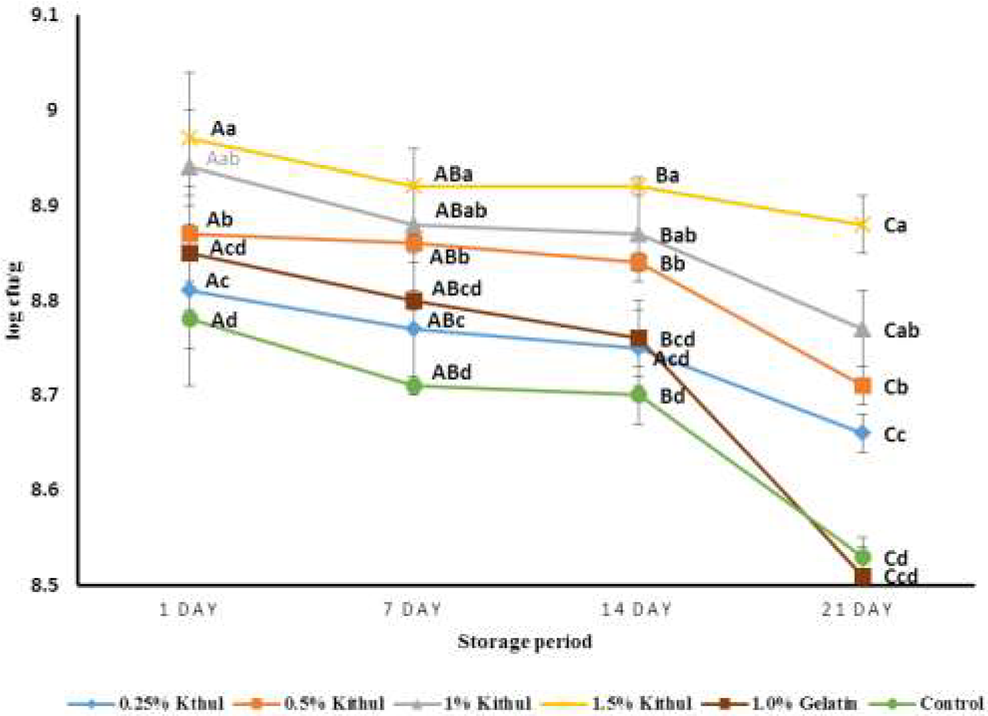

Figure 4: Viability of *B. animalis ssp. lactis* BB-12 in different yoghurts during the storage period

Vertical lines represent standard deviations. A, B, C, D Means with different uppercase are significantly (P<0.05) different between each day, for each type of yoghurt during the storage. a, b, c, d Means with different lowercase are significantly (P<0.05) different between each type of yoghurt, for a particular day of storage.

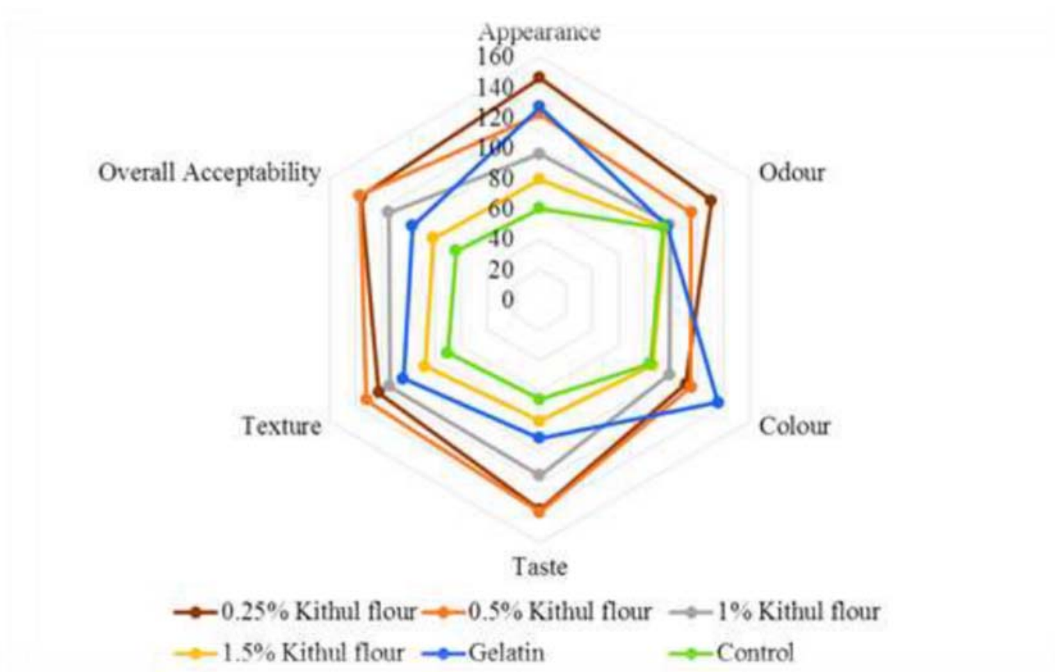

Figure 5: Pattern of variation of sensory properties of *Kithul* flour incorporated probiotic set yoghurts

CONCLUSION

Kithul flour was successfully used to produce probiotic set yoghurts with *B. animalis* ssp. *lactis* BB-12. *Kithul* flour incorporation resulted significantly lower pH and higher titratable acidity of yoghurt during storage. Yoghurt containing *Kithul* flour showed lower syneresis compared to control and gelatine containing yoghurt during the storage. Addition of *Kithul* flour supported the growth and viability of *B. animalis* ssp. *lactis* BB-12 in yoghurt during the storage. The panellists recorded the highest scores for texture, taste and overall acceptability to the yoghurt containing *Kithul* flour. Therefore, it can be concluded that *Kithul* flour can be used in manufacturing a yoghurt with good physicochemical properties, sensory attributes, and probiotic count during the storage.

REFERENCES

Amatayakul, T., Sherkat, F. and Shah, N.P., 2006. Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. International Journal of Dairy Technology, 59(3), 216-221.

AOAC 2005. 'Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals', *AOAC International*. 1–38.

Casarotti, S.N., Carneiro, B.M. and Penna, A.L.B., 2014. Evaluation of the effect of supplementing fermented milk with quinoa flour on probiotic activity. Journal of Dairy Science, 97(10), 6027-6035.

Cruz, A, G., Walter, E. H. M., Cadena, R.S., Faria, J.A.F., Bolini, M. A. H., Pinheiro, H. and Santana, A. S., 2010. 'Survival analysis methodology to predict the shelf- life of probiotic flavoured yogurt', Food Research International, 43, 1444-1448.

Dave, R.I. and Shah, N.P., 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. International Dairy Journal, 7(1), 31-41.

Gibson, G.R. and Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition, 125(6), 1401-1412.

Gibson G R, Hutkins R, Sanders M E et al. (2017) Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology, 14, 491–502.

Guggisberg, D., Cuthbert-Steven, J., Piccinali, P., Bütikofer, U. and Eberhard, P., 2009. Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. International Dairy Journal, 19(2), 107-115.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S. and Calder, P.C., 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology, 11(8), 506-514.

Kuhanneya, K., Marapana, R.A.U.J., Ranasinghe, P., Lanka, S., Section, H.T., Lanka, S., 2016. Global journal of advanced engineering technologies and sciences development of cinnamon flavoured instant porridge powder using amylase treated kithul flour (*Caryota urens*) Herbal Technology Section , Industrial Technology Institute , Sri Lanka 3, 14–22.

Lobato-Calleros, C., Ramírez-Santiago, C., Vernon-Carter, E.J. and Alvarez-Ramirez, J., 2014. Impact of native and chemically modified starches addition as fat replacers in the viscoelasticity of reduced-fat stirred yogurt. Journal of Food Engineering, 131, 110-115.

Nicklas, T.A., O'Neil, C.E., Liska, D.J., Almeida, N.G., Fulgoni III, V.L., 2011. Modeling dietary fiber intakes in US adults: implications for public policy. Food and Nutritional Science 2 (09), 925-931.

Sendra, E, Fayos, P., Lario, Y., Fermandez-Lopez, J., Sayas-Barbera, E., and Alvarez, J. A. (2008) 'Incoporation of citrus fibers on fermented milk containing probiotic bacteria', *Food Microbiology*, 25, 13-21.

Shah, N. (2007) 'Functional cultures and health benefits', *International Dairy Joutnal*, 17, 1262-1277.

Staffolo, M.D., Bertola, N. and Martino, M., 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. International Dairy Journal, 14(3), 263-268.

Tamime, A. Y. and Robinson , R. K. (1999) 'Yoghurt Science and Technology', Wood head publishing, London, 35-108.

Rajyalakshmi, P., 2004. Caryota Palm Sago―A potential yet underutilized natural resource for modern starch industry. Natural Product Radiance 3, 144–149.

Vasiljevic, T., and Shah, N. P. (2008). 'Probiotics—From Metchnikoff to bioactives'. International Dairy Journal, 18, 714–728.

Wijesinghe, J.A.A.C., Wicramasinghe, I. and Saranandha, K.H., 2015. Kithul flour (Caryota urens) as a potential flour source for food industry. American Journal of Food Science and Technology, 2015, 3(I), 10-18

Wimalasiri, G.E.M., Ranasinghe, P., Gunaratne, D.M.A. and Arachchi, L.V., 2016. Antioxidant and anti-diabetic properties of Caryota urens (Kithul) flour. Procedia Food Science, 6, 181-185.

Zare, F., Orsat, V., Hx, Q.C., Champagne, C., Js, Q.C., Simpson, B.K., 2012. Microbial and Physical Properties of Probiotic Fermented Milk Supplemented with Lentil Flour. Journal of Food Research, 1, 94–109.