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Microbiological, physicochemical and organoleptic attributes of yoghurt enriched with lotus (*Nelumbo nucifera*) seed milk

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Lotus (*Nelumbonucifera*) seed is an important raw material for dessert and versatile medicine. It has been considered as a vital ingredient for numerous traditional desserts. It contains abundant amount of phytochemical components as a functional food. This research evaluated the partial replacement of cow milk by lotus seed milk in yoghurt production. Lotus seed at milk-ripe stage was peeled, cored, soaked 8 hours, cooked at 100 ºC for 45 minutes, mashed, filtered, homogenized 30 MPa, and added 5 % of saccharose, pasteurized at 90 ºC for 5 minutes, cooled to ambient temperature. The lotus seed milk was then mixed with pasteurized milk and skim milk powder in different percentage (0, 3.85, 7.41, 10.71, 13.79, and 16.79 %). These mixtures were heated at 80ºC for 15 minutes and cooled to 42 ºC before adding 0.1 % of *Lactobacillus bulgaricus* as starter culture. The fermentation was terminated at pH 4.6 and the enriched yoghurt was stored at 4 ºC for 28 days. Viability of starter culture (log cfu/g), syneresis (%), water-holding capacity (%), viscosity (mPas), overall acceptance of the enriched yoghurt were carefully examined in 7 day-interval for 28 days of storage. Results showed that 10.71 % of lotus milk could be incorporated into milk fermentation to improve microbiological, physicochemical and organoleptic properties of the enriched yoghurt.

Keywords: Enriched yoghurt, Lactobacillus bulgaricus, lotus seed milk, microbiological, organoleptic, physicochemical

INTRODUCTION

Lotus (*Nelumbonucifera*) seed consists three parts: epicarp, embryo, and cotyledons (Mingzhi et al. 2016). It is utilized in different cuisine and traditional medicine. It can be processed into different forms like tea, jam, juice, oil, loaf, and cake (Abdoul-Azize, 2016). It's a rich source of vitamins, dietary fibers and proteins (Soumya et al. 2018). Various alkaloids like nuciferine, neferine, lotusine, isoliensinine, quercitin, isoquercitrin, and flavinoids were detected in lotus seed (Rajalakshmy, 2019). It's commonly available in shelled and dried form. It can be eaten raw, roasted or boiled into a paste/syrup/ground after being peeled and cored. Different health benefits such as anti-ischaemic, anti-inflammatory, hepatoprotective, anti-fibrosis, diuretic, anticancer, anti-obesity, antioxidant from lotus seed were reported (Ushimaru et al. 2001; Liu et al. 2004; Kuo et al. 2005; Ono et al. 2006; Chen et al., 2007; Sridhar and Bhat, 2007; Guo et al. 2015; Soumya et al. 2018).

*Lactobacillus bulgaricus*is neccessary to enhance the sensory, hygienic and functional attributes of yoghurt and fermented milk (Erdorul and Erbilir, 2006). *Lactobacillusbulgaricus*grown in dairy products mainly rely on its capability to effectively utilize carbon (lactose) and nitrogen (casein) sources in medium, and to synthesize nucleotide bases.*Lactobacillusbulgaricus*has better proteolytic activity than *Streptococcus thermophilus* (Courtin and Rul, 2004).

*Lactobacillusbulgaricus*has the essential peptidolytic and transport pathways for casein utilization. It metabolizes casein into free amino acids, hence acomplishing its protein synthesis demand.*Lactobacillus bulgaricus*begins at a high inoculum ratio,*Streptococcus thermophilus* has a numerical advantage over *Lactobacillus bulgaricus*by the end of fermentation (Courtin andRul 2004, Herve-Jimenez et al. 2008, Ben-Yahia et al. 2012). *Streptococcus thermophilus* seems to be superior to *Lactobacillus bulgaricus* in dairy products. *Lactobacillus bulgaricus* begins to proliferate exponentially. The proliferation of *Lactobacillusbulgaricus* extends to pH 4.4 (Beal and Corrieu 1991).*Lactobacillus bulgaricus*is superior to *Streptococcus thermophilus* in the acidic environment due to its ability to convert ornithine into putrescine to accelerate the intracellular pH (Azcarate-Peril et al. 2004). Yoghurt flavor results from a combination of the main flavor compounds and fatty-acid derivatives (Beshkova et al. 1998).

Yoghurt is a functional beverage as fermented dairy product prepared from milk fermentation (Gao et al. 2018). It is a rich source of macronutrients (casein and lactose) and micronutrients (B vitamins, calcium and phosphorus) (Lourens-Hattingh and Viljoen, 2001; Staffolo et al., 2004). Peptides and amino acids derived from lactic fermentation greatly contributed to health effects (Ye et al. 2013). It is highly prefer to milk, especially for people encoutering lactose intolerant because lactose with the support of bacterial starter culture has been fermented to lactic acid (Heyman, 2000; Vesa et al., 2000). Yoghurt has been considered as functional probiotic carrier beneficial for gastro intestinal disorders (Lourens-Hattingh and Viljoen, 2001; Mazahreh and Ershidat, 2009). It significantly enhances gum health, suport calcium absorption; avoid osteoporosis (Kerry et. al., 2001). There has a potential trend of consuming symbiotic yoghurt including prebiotics and probiotics to facilitate human health and well-being. Yoghurt could be enriched from adundant bioactive sources such as coconut (Joel et al. 2014), spirulina (Priyanka et al. 2013), dietary fiber (Adriana et al. 2018), date (Hashim et al., 2009), milk protein (Berrakand Tulay, 2017), strawberry pulp (Henrique et al. 2018), apple pomace flour (Marina et al. 2020), artichoke flour (Tatjana et al. 2017), olive fruit polyphenol (Konstantinos et al. 2012), soy bean flour (Amove et al. 2019), *Hibiscus sabdariffa* (Se-Hyung et al. 2019). Incorporation of yogurt with proper ingredients could enhance not only the quality characteristics but also the acceptabilities of yogurt with limited deffects (Gahruie et al. 2015; Liu and Lv., 2019). Yoghurt is manufactured in different styles and varieties (Weerathilake et al. 2014). Objective of our study was to study the possibility of partial replacement of the pasteurized milk by lotus seed milk during yoghurt production. iability of starter culture (log cfu/g), syneresis (%), water-holding capacity (%), viscosity (mPas), overall acceptance of the enriched yoghurt were carefully examined in 7 day-interval for 28 days of storage.

MATERIALS AND METHODS

2.1 Material

Lotus seeds at milk-ripe stage (70% maturity) were collected from Nga Nam district, SocTrang province, Vietnam. Pasteurized milk and skim milk powder were purchased from grocery store. *Lactobacillus bulgaricus*from Vinmec was utilized as starter culture for yoghurt fermentation. MRS agar was purchased from Merck (Germany). Chemical reagents obtained from Merck (Germany) were all analytical grade.

2.2 Researching method

Lotus seedswithout core were deeply soaked in fresh water including 0.05 % w/w of citric acid for 8 hours. The soaked lotus seeds were then washed under potable water 3 times before cooking at 100 ^oC for 45 minutes, mashing, diluting with water (1:10), filtering, homogenizing at 30 MPa, adding 5 % of saccharose, pasteurizing at 90 ^oC for 5 minutes, cooling to ambient temperature. 1000 mL of pasteurized milk and 250 gram ofskim milk powder were primarily mixed together. An aliquot of 50, 100, 150, 200, 250 mL of lotus seed milk was added into the prepared cow milk. Percentage of lotus seed milk existed in the prepared cow milk were 3.85, 7.41, 10.71, 13.79, 16.79 % in equivalent. These mixtures were heated at 80^{o}C for 15 minutes and cooled to 42 ^oC before adding 0.1 % of *Lactobacillus bulgaricus*as starter culture. The fermentation was terminated at pH 4.6 and the samples stored at 4^{o}C for 28 days. The control group was similarly prepared as the above protocol without lotus seed milk. All produced yoghurt samples were examined viability of starter culture (log cfu/g), syneresis (%),water-holding capacity (%), viscosity (mPas),overall acceptance.

2.3 Microbiological, physicochemical and organoleptic determination

Viability of starter culture (log cfu/g) was determined via the pour plate protocol. 10 g of yogurt was diluted with sterile peptone water (0.1 g/100 mL, 90 mL), and serial dilutions were prepared. Viability of *Lactobacillus bulgaricus* cells were enumerated under anaerobic condition at 37°C for 48 h.Syneresis (%) was evaluated bydraining 50 mL of unstirred yoghurt spread evenly on filter paper at 4 ◦C for 6 hours.The obtained whey volume multiplied by 2 was considered as syneresis (Sidira et al. 2017). Water-holding capacity (%) was determined by method described by Ilyasoglu et al. (2015).25 g of yoghurt (M_1) was centrifuged for 2.5 min at 4,000 rpmat 20 °C. The whey (M_2) was separated and weighed. The water-holding capacity (%) was expressed as: $WHC=(M_1-M_2)*100/M_1$**.** Viscosity (mPas) of yoghurt was measured by rheometer (Anton Paar, Germany), after stirring the product for 60 s (Donkor et al. 2007)**.** Overall acceptance was evaluated by a group of panelist using 9 point-Hedonic scale.

2.4 Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS AND DISCUSSION

Fig 1 showed the effect of lotus seed milk supplementation (0, 3.85, 7.41, 10.71, 13.79, 16.79 %) to the viability of *Lactobacillus bulgaricus* (log cfu/g). The number of *Lactobacillus bulgaricus* tended to increase with increasing lotus seed milk. It's clearly noticed that there was not significant reduction of viability of *Lactobacillus bulgaricus*during 28 days of storage in all treatments except at 13.79 % and 16.79 %. There was significant difference of viability of starter culture by replacement of pasteurized milk by lotus seed milk at 13.79 % and 16.79 %. Meanwhile, there was no significant difference of viability of starter culture by replacement of pasteurized milk by lotus seed milk from 0-10.71 %. Polyphenol in apple supplemented into yogurt could accelerate the proliferation of starter culture (Sun-Waterhouse et al. 2012). Although the polyphenol content in lotus seed milk was quite high 33.59 mg/g extract (Yan-Bin et al. 2011), different processing steps such as cooking, diluting, filtering, homogenizing, pasteurizing might seriously decomposed polyphenol so its content was not enough to contribute to the viability of starter culture. The more lotus seed milk supplemented, the more water incorporated into yoghurt gel structure. Hence *Lactobacillus bulgaricus*has not enough carbon (lactose) and nitrogen (casein) from cow milk to grow. 10.71 % of lotus seed milk could be considered as threshold for partial replacement to the pasteurized milk to ensure a normal proliferation of *Lactobacillus bulgaricus*during yoghurt production.

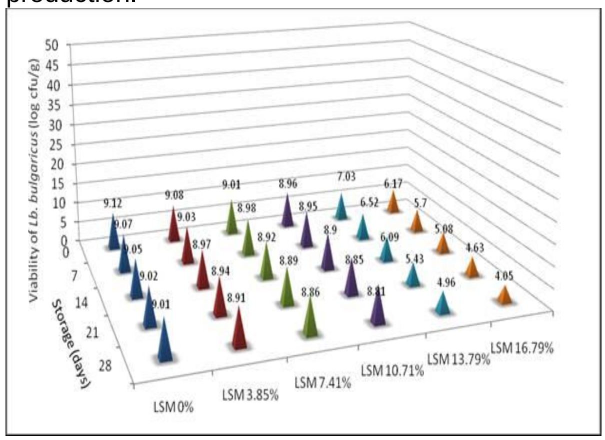

Figure 1: Viability of starter culture (log cfu/g) of the enriched yoghurt by lotus seed milk replacement (LSM, %) during 28 days of storage

Syneresisresulted from of separating yoghurt gel to retain the serum phase (Vital et al. 2015). The strength of coagulum and its stability during storage reflected the syneresis of yoghurt. Fig 2 exhibited the influence of lotus seed milk supplementation (0, 3.85, 7.41, 10.71, 13.79, and 16.79 %) to the syneresis (%) of the enriched yoghurt. Control yoghurtshowed the highest level of syneresis throughout the storage, whereas the lowest level syneresis wasnoticed by incorporation with 16.79 % of lotus seed milk. There was not significant difference of syneresis by replacement of the pasteurized milk with lotus seed milk at 0-10.71%. There was a trend of syneresis degradation during during 28 days of storage in replacement of the pasteurized milk with lotus seed milk at 13.79 % and 16.79 %. Meanwhile, there was a minor degradation of syneresis in replacement of the pasteurized milk with lotus seed milk at 0-10.71 % during 28 days of storage. Major problems of yogurt were low viscosity and syneresis (Vital et al. 2015). Hydrocolloids provided much more total solids content into milk materialtoretard the defects of yogurt and enhance its structure (Nguyen et al. 2017). Polyphenol would be beneficial for enhancing the structure and syneresis of yogurt (Donmez et al. 2017). Low polyphenol content remaining in the lotus seed milk after passing different thermal treatment could not support to improve syneresis of the enriched yoghurt. Contrary, the more lotus seed milk supplemented, the more water incorporated into yoghurt gel structure. This resulted to the low syneresis of yoghurt. Moreover, starch in lotus seed milk could not reinforce gel of casein micelle.

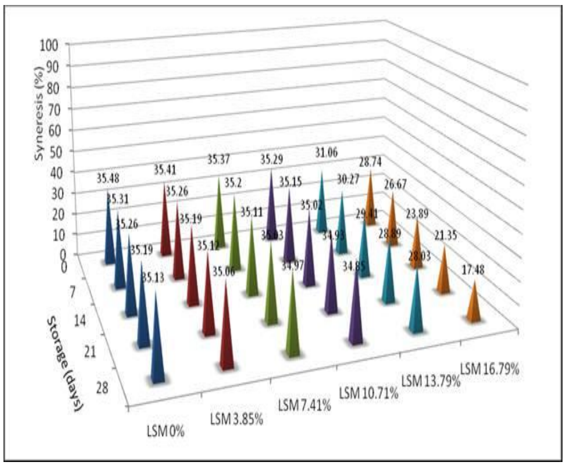

Figure2: Syneresis (%) of the enriched yoghurt by lotus milk replacement (LSM, %) during 28 days of storage

Water-holding capacityis correlated to the capacity of protein to capturemoisture in the yoghurt gel structure (Kinsella and Moor, 1984).Fig 3 proved the impact of lotus seed milk supplementation (0, 3.85, 7.41, 10.71, 13.79, 16.79 %) to the water-holding capacity (%) of the enriched yoghurt. It's clearly relealized that control yoghurtshowed the highest level of water-holding capacity throughout the storage, whereas the lowest water-holding capacity werenoticed by incorporation with 16.79 % of lotus seed milk. There was no significant difference of water-holding capacity by replacement of the pasteurized milk with lotus seed milk at 0-10.71%. There was a trend of water-holding capacity degradation during during 28 days of storage in replacement of the pasteurized milk with lotus seed milk at 13.79 % and 16.79 %. Meanwhile, there was a minor degradation of water-holding capacity in replacement of the pasteurized milk with lotus seed milk at 0-10.71 % during 28 days of storage. A high water-holding capacityimproved curd stability and accelerated viscosity (Srısuvor et al. 2013). In one research, incorporation of lotus leaf extractimproved the water-holding capacity of yoghurt by at least 1.5-fold than that of control during storage (Da-Hee et al. 2019). Dietary fibers could retain moistutre, increasing the water holding capacity of yogurt (Choe et al. 2011; Barkallah et al.2017). Polyphenol combined with casein resultingadundant cages in preventing serum emission from the yogurt gel network (Donmez et al.2017). Low polyphenol content left in the lotus seed milk after passing different thermal treatments could not support to improve water-holding capacity of the enriched yoghurt. Contrary, the more lotus seed milk supplemented, the more water incorporated into yoghurt gel structure. This resulted to the low water-holding capacity of the enriched yoghurt.Moreover, low dietary fiber in lotus seed milk could not reinforce gel of casein micelle.

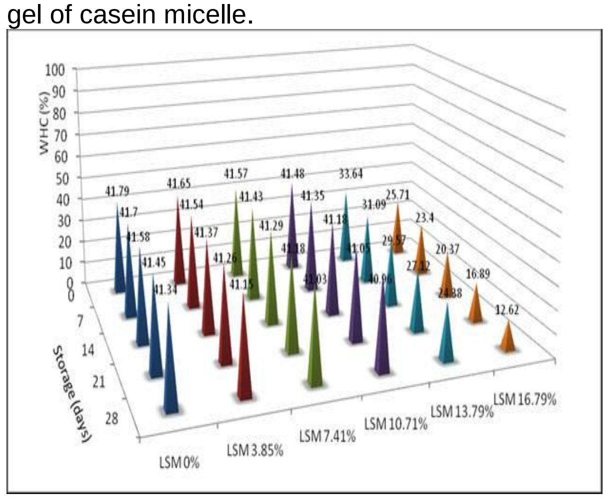

Figure3: Water-holding capacity (WHC, %) of the enriched yoghurt by lotus milk replacement (LSM, %) during 28 days of storage

Viscosity was an important characteristic reflecting the texture of yoghurt. Viscosity increased with lowering shear rate (Isanga and Zhang, 2009). The higher viscosity values were obtained when the cow milk enriched with lotus seed milk. Fig 4 demonstrated the effectiveness of lotus seed milk supplementation (0, 3.85, 7.41, 10.71, 13.79, and 16.79 %) to the viscosity (mPas) of the enriched yoghurt. It's obviously noticed that the more lotus milk replaced to the pasteurized milk, the higher viscosity was recorded.The was not significant reduction of viscosity during 28 days of storage. The highest viscosity of yoghurt was found at16.79 % of lotus milk replacement. The lowest viscosity of yoghurt was realized at control. In one report, yogurt enriched by lotus leaf extract had better viscosity than that of control. 0.2% of lotus leaf extracts maintained viscosity of yoghurt until the end of the storage (Da-Hee et al. 2019). Polyphenol existing in lotus seed can combine with proteins to create protein–polyphenol complex (Vital et al. 2015).The available phenolic in lotus seed milk complexed with casein in the yogurt matrix, causing higher viscosity than that in the control (Limwachiranon et al. 2018). This explained the increase of viscosity of the enriched yoghurt when adding lotus seed milk. Yogurt viscosity declined during storage due to whey separation (Al Mijan et al. 2014). Yogurt was a gel of casein micelle with captured serum. Both viscosity and water-holding capacity were correlated to the gel structure of yogurt (Achanta et al. 2006).

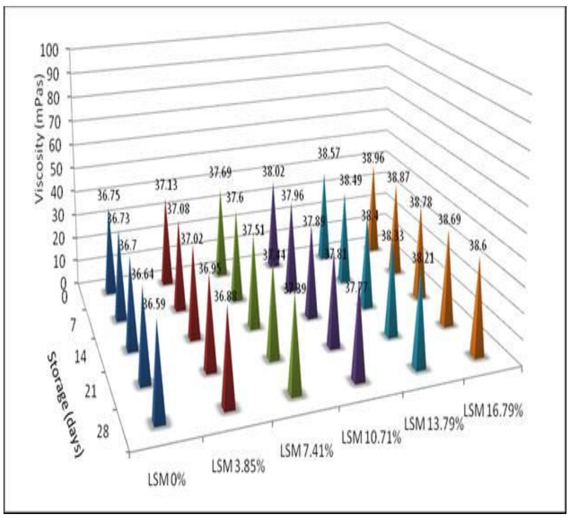

Figure4:Viscosity (mPas)of the enriched yoghurt by lotus milk replacement (%) during 28 days of storage

Overall acceptance indirectly reflected the quality of yogurts (Coggins et al. 2010). The appropriate physicochemical property in yogurt, like proper structure and non-serum removal, contributed an important decision in consumer acceptability (Cardines et al. 2018). Fig 5 presented the efficacy of lotus seed milk supplementation (0, 3.85, 7.41, 10.71, 13.79, 16.79 %) to the overall acceptance of the enriched yoghurt. There was no significant difference of overall acceptance by replacement of the pasteurized milk with lotus seed milk at 0-10.71%. There was dramatically reduction of overall acceptance during during 28 days of storage in replacement of the pasteurized milk with lotus seed milk at 13.79 % and 16.79 %. Meanwhile, there was a slight reduction of overall acceptance in replacement of the pasteurized milk with lotus seed milk at 0-10.71 % during 28 days of storage. In another study, ice cream flavored with lotus seed was prepared. The overall acceptance was not significantly different based on the amount of lotus seed (Eunhee et al. 2012).

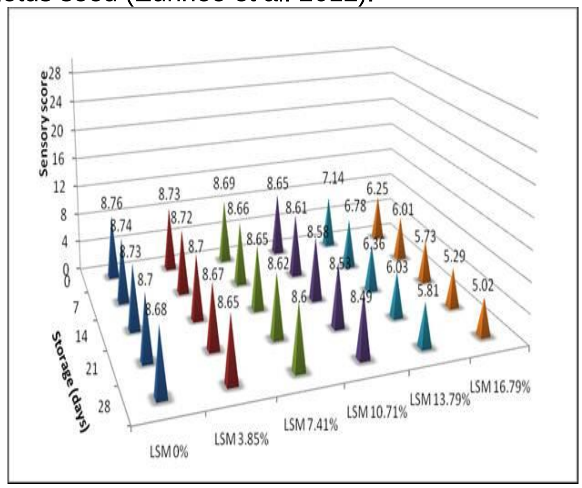

Figure5 :Overall acceptance (sensory score) of the enriched yoghurt by lotus milk replacement (%) during 28 days of storage

CONCLUSION

Yoghurt enriched with lotus seed milk is highly evaluated due to its excellent phytochemical constituents acceptable for therapeutic benefits. In this research, lotus seed milk added into yoghurt in different percentage. Results showed that cow milk could be replaced 10.71 % by lotus seed milk to obtain the best quality of the enriched yoghurt. Lotus seed would be a promising carbohydrate source incorporated into milk fermentation.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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