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Development of Set Type Yoghurt Using Juice of Local Watermelon Varieties of Sri Lanka

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ABSTRACT

The objective of the current study was to develop a set-type yoghurt incorporating the juice of three watermelon varieties including 'Thilini', 'Kinaree C28' and 'Rocky 475' which are most commonly grown in the dry zone of Sri Lanka. "Initially, to identify the best watermelon variety for set-yoghurt production, the yoghurts were made separately using each watermelon variety at 25% (v/v) juice concentration. Next, to determine the appropriate inclusion level, batches of yoghurt were made using the selected best watermelon variety at the four concentration levels of 15% (T1), 20% (T2), 25% (T3) and 30% (T4) (v/v). A batch of yoghurt made without watermelon was used as the authenticated control. Sensory evaluations were performed by 30 untrained panelists. Microbiological and physiochemical properties of the watermelon juice incorporated set-yoghurts were analyzed during the storage period of 21 days at 4℃. Incorporation of 'Rocky 475' variety at 20% level resulted the best sensory attributes for colour, taste, texture and aroma. Both fat and protein content of the yoghurts were gradually decreased with the increased levels of watermelon juice. Highest syneresis was observed in the T4 yoghurts which contained the highest level of watermelon juice and the lowest syneresis was observed in the control. In all treatments, total plate count and yeast and mold counts were within the acceptable level during the storage period. This study results revealed that by using up to 20% (v/v) of the juice extracted from the 'Rocky 475' watermelon variety, the organoleptic properties of set-yoghurt could enhance while ensuring microbiological safety.

1. Introduction

As the burden of non-communicable diseases is rapidly growing, consumers are conscious about the health benefits of their food. Recent dietary patterns show new trends, such as 'functional foods' in the food industry. A functional food Item is engineered to deliver health promoting substances in addition to the traditional nutrients of the food. Yoghurt is a widely used functional dairy product as it is popular among consumers as a highly nutritive fermented food item. Yoghurt delivers many health benefits including improved lactose tolerance, strengthening the immune system, anti-carcinogenic effect, prevention of osteoporosis and a variety of health attributes associated with probiotic bacteria [1].

Many recent studies have shown that the consumption of fruits coupled with yoghurt may offer additional nutritional and physiological benefits through the improved prebiotic and probiotic phenomena. Fruit juice is a better source than milk for the growth and survival of probiotic bacteria since fruit juice provide favorable acidic pH required for the growth of probiotic bacteria. Additionally, when incorporated into fermented yoghurts, fruit juices can significantly promote the growth of probiotic bacteria by serving as a media for cultivating [2]. Also, the high levels of saccharides and prebiotic fibers available in fruits support the survival of probiotic organisms. Further, fruits are considered excellent sources of antioxidants and polyphenols. Antioxidants in foods help prevention of many degenerative diseases such as cancer, coronary heart diseases, type 2 diabetes and obesity [3]. Nevertheless, the consumption of fruit in combination with yoghurt provide strong support for the maintenance of cardiometabolic health and prevention of many other diet related diseases [4].

Watermelon (*Citrullus lanatus*) is an economically important fruit species of Cucurbitaceae family cultivated over the world; usually grown in tropical and subtropical areas [5]. Watermelon is rich in phytochemicals such as lycopene and β-carotene which shows antioxidant properties; epidemiological studies have suggested that lycopene may have protective effects against certain types of cancers and cardiovascular diseases. The lycopene rich nature and health benefits associated with watermelon juice make it an excellent choice for the development of functional food products [6].

Commercial cultivation of watermelon is widely done with irrigation facilities in dry zone of Sri Lanka. Watermelon is cheaply available in Sri Lanka during the harvesting seasons and excess harvest is highly subjected to post-harvest losses due to inadequacy of the market. However, there is a great potential for watermelon to be utilized in the food processing industry in Sri Lanka. It can be effectively used in the development of various value-added food products including cordial, juice, ice-cream, yoghurts and other dairy based beverages [7]. Thus, the objective of this study was to develop a set-yoghurt by incorporating extracted juice of three different watermelon varieties; namely 'Thilini', 'Kinaree C28' and 'Rocky 475'. These are the most commonly cultivated watermelon varieties in the dry zone of Sri Lanka and find out the best variety to be incorporated in development of set-yoghurt, and to evaluate the effect addition of watermelon addition on physicochemical, microbiological and sensory attributes of set type yoghurts.

2. Material and Methods

Preparation of watermelon juice and set-type yoghurt

Fruits of three watermelon (*Citrullus lanatus*) varieties were collected from the local market in Anuradhapura, Dambulla and Kekirawa areas in dry zone of Sri Lanka. Size of the fruit, shape and the different coloured rinds of the fruits were used as indicators for distinguishing among the three varieties. Technical assistance was obtained from Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka during identification of the watermelon fruits.

Watermelon fruits were washed with clean water and peeled manually. Seeds were removed and the watermelon pieces were blended using a food processor (CML-7360065, India). Filtration of the juice was done using clean muslin clothe. Then the filtrate was heated up to 90 °C for 5 minutes in order to facilitate pasteurization. Pasteurized watermelon juice was analyzed for pH, titratable acidity, moisture content and total soluble solids.

Cow's milk was subjected to pre-heating at 55-60 °C followed by homogenization at 65℃ for about 15 minutes under 10-20 MPa. Then the pasteurization was done at 80-85 °C for 30 minutes. Sugar (10%) and gelatin (0.7%) were mixed thoroughly with milk followed by addition of watermelon juice to the heated mixture. Samples of watermelon juice obtained from three different varieties were added to the heated milk at 25% level. Freeze dried yoghurt starter culture (YC-X11, Thermophilic Yoghurt Culture, Chr. Hansen Company, Denmark) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was added to the mixture after cooling to 42 °C. Prepared set-yoghurts were incubated at 45 ° C for 4 h and stored at 4℃ until used for further experiments. After selecting the best watermelon variety for development of set-yoghurts, further analysis was conducted to verify the best incorporation level of watermelon juice for set-yoghurt production. Four incorporation levels were used in yoghurt production with selected best watermelon variety as 15% (T1), 20% (T2), 25% (T3) and 30% (T4) (v/v) while zero inclusion of watermelon juice in set-yoghurt was used as authenticated control (T5).

Sensory evaluation

Initial sensory evaluation was conducted to identify the best variety of watermelon for inclusion in set-yoghurts while the second sensory evaluation was conducted to find out the optimum level of watermelon juice to be incorporated in set-yoghurts. Thirty (30) untrained panelists participated for each. evaluation. Evaluation was done using five-point hedonic scale based on the acceptance of different attributes of the product as color, aroma, taste, texture and overall acceptability.

Physicochemical property analysis

Proximate analysis was conducted to determine the protein and fat content of the yoghurts according to AOAC [8] protocols. Titratable acidity, pH and syneresis were determined using standard procedures at weekly intervals up to 21 days of storage. The pH was measured using a pH meter (SENSION pH meter, Hach, USA) whereas the titratable acidity of the set-yoghurt was estimated by titration with 0.1 N NaOH solution using phenolphthalein as the indicator. Syneresis of set-yoghurts was determined according to the method described by Prasanna et al. [9].

Microbiological analysis

In order to determine the shelf life of the set-yoghurt, total bacterial count and yeast and mould

counts were determined using a serial dilution of yoghurts with spread plate technique. Total plate count was determined using plate count agar (Sigma-Aldrich, Saint Louis, USA) and plates were incubated at 37℃ for 24 hours. Potato dextrose agar media (Sigma-Aldrich, Saint Louis, USA) was used to enumerate yeast and mold count of yoghurts while the plates were incubated at 25℃ for 5 days. Number of colonies were counted using colony counter (Rocker 330, Taiwan) and expressed as colony forming units CFU/ mL.

Statistical analysis

Parametric data were analyzed using one-way analysis of variance (ANOVA) procedure in statistical software for data analysis (SAS) with software package version of 9.0 with 95% confidence interval. Data from sensory evaluation were analyzed using Friedman non parametric test in MINITAB software by package version of 17.1.

3. Results and Discussion

Sensory evaluation

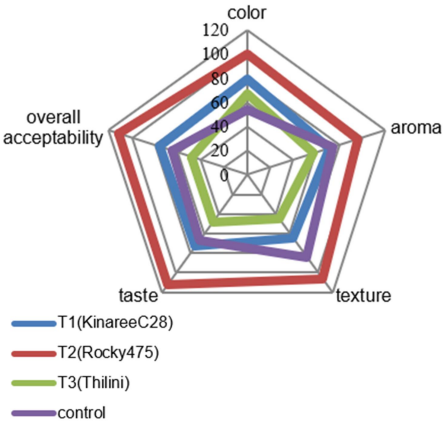

Figure 1: Preliminary sensory evaluation: Variation of sensory properties of set-yoghurts developed by incorporating watermelon extract (25% v/v) of three different varieties ('Thilini', 'Rocky 475' and 'Kinaree C28')

The initial sensory analysis results revealed that there was a significant difference between three yoghurt treatments for appearance, color, texture, taste and overall acceptability of final product (Figure 1). Among three fruit yoghurt types, 'Rocky 475' watermelon variety incorporated yoghurts received the highest scores for all sensory attributes while 'Thilini' watermelon variety incorporated yoghurt resulted the lowest scores for all the tested sensory attributes. The highest colour score was observed in set-yoghurts incorporated with 'Rocky 475' variety which appeared in moderate red colour. However, dark red colour was observed in 'Kinaree C28' watermelon variety incorporated set-yoghurts while 'Thilini' watermelon variety incorporated set-yoghurts appeared to be light red in colour. Set-yoghurts incorporated with 'Thilini' watermelon variety had the lowest texture score. The highest moisture content and lowest total solids contents (Table 1) could be attributed to the poor texture of the 'Thilini' variety incorporated set-yoghurts.

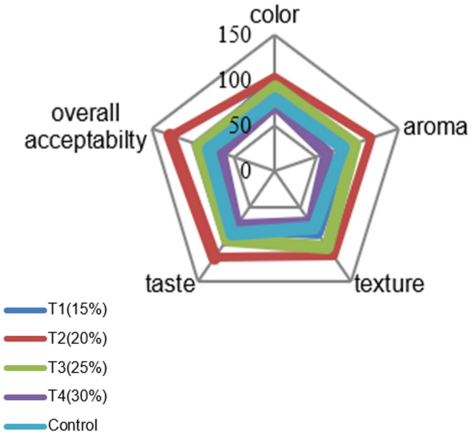

Figure 2: Secondary sensory evaluation: Variation of sensory attributes for set-yoghurts developed by using different incorporation levels (v/v= 15%: T1, 20%: T2, 25%: T3, 30%: T4, 0%: T5) of 'Rocky 475' watermelon variety

Figure 2 depicts that set-yoghurt developed incorporating 20% (v/v) watermelon (T2) scored higher average from the panelists compared to the control yoghurt in terms of appearance, color, texture, taste and overall acceptability. This may be due to the combined effects from flavor compounds of watermelon juice and enhanced growth of *S. thermophilus*, *L. delbrueckii* ssp. *Bulgaricus* resulting higher production of flavor compounds. The major chemical compounds which are responsible for the flavor of the watermelon are 9-carbon alcohols and aldehydes [10]. Further, Ranadheera et al. [1] have reported that, higher natural sugar content in fruit yoghurts due to the addition of fruit base material may leads to higher consumer demand. Warakaulle et al. [11] have reported that the addition of watermelon juice at 25% level in fruit yoghurt had the highest sensory scores. The lowest overall acceptability was observed for set-yoghurts prepared by incorporating 30% watermelon (T4). Nevertheless, there were significant difference ($p<0.05$) among the different treatments for appearance, color, texture, taste and overall acceptability.

Fat content is one of the most important quality factors of flavored yoghurt. It mainly depends on the quality of the milk, amount of incorporated fruit pulp or juice and fruit variety [12]. Fat also acts as a principal flavor solvent and therefore, it has an important effect on flavor release in yoghurts.

Current study showed that addition of watermelon juice had a significant ($p<0.05$) effect on the fat content of the set-yoghurts. Table 2 shows that, the highest fat content (3.71± 0.03) was observed in the control set-yoghurt while the lowest fat content was observed in T4 set-yoghurt (3.32 ± 0.02) which contained the highest amount of watermelon juice. Similar observations were reported by Tarakçi et al. [13], where addition of fruit in the forms of pulps and marmalade into fresh plain yoghurt significantly reduces the fat content in contrast to the control sample. Since watermelon generally has a lower fat content compared to fresh milk, the addition of watermelon juice to the yoghurt has contributed to decrease the final fat content of the prepared set-yoghurt samples. In general, total fat content of watermelon juice per 100 g is around 0 - 0.15 g [14] and consumption of watermelon incorporated dairy products are desirable by the consumers for a healthy diet due to fewer quantities of fat and cholesterol.

Table 1: Chemical properties of watermelon juice of 'Thilini', 'Rocky 475' and 'Kinaree C28' varieties

Physicochemical Property	Watermelon Variety		
	Thilini	Rocky 475	Kinaree C28
pH	5.42±0.05	5.53±0.07	5.69±0.02
Titratable acidity (%)	0.1	0.09	0.06
Moisture (%)	94.12	93.88	93.05
Total soluble solids (Brix value%)	12	13	14

Addition of watermelon juice had a significant effect ($p<0.05$) on protein content of all set-yoghurts (Table2) and it was greatly influenced by the incorporation level of watermelon. The control sample (T5) had the highest protein content (3.72 ± 0.06) while T4 set-yoghurts had the lowest protein content. These results showed that the protein content of the set-yoghurts get decreased with the increased incorporation levels of watermelon. This is attributed to the fact that watermelon juice has a comparatively low protein content (0.6 - 0.9 g/100 g) than cow milk [14]. Therefore, when milk is replaced with higher amounts of fruit juice, the protein content of the final product is also decreased. Similar behavior has been observed by Roy et al. [18] when watermelon pulp was incorporated into cow milk yoghurt at three different levels (5%,10% and 15%), total protein content of yoghurt was significantly ($p<0.05$) decreased with the escalated amounts of watermelon pulp.

Table 2: Variation of fat and protein contents of set-yoghurts incorporated with different level of 'Rocky 475' watermelon variety

Treatment	Fat (%)	Protein (%)
T1 (15%)	3.62^{b} ± 0.04	3.53^{b}± 0.03
T2 (20%)	3.51^{b} ± 0.03	3.42^{c} ± 0.02
T3 (25%)	3.43^{c} ± 0.06	3.34^{c}± 0.05
T4 (30%)	3.32^{c} ± 0.02	3.26^{c} ± 0.02
Control	3.71^{a} ± 0.03	3.72^{a} ± 0.06

Variation of pH, titratable acidity and syneresis of watermelon incorporated set-yoghurts

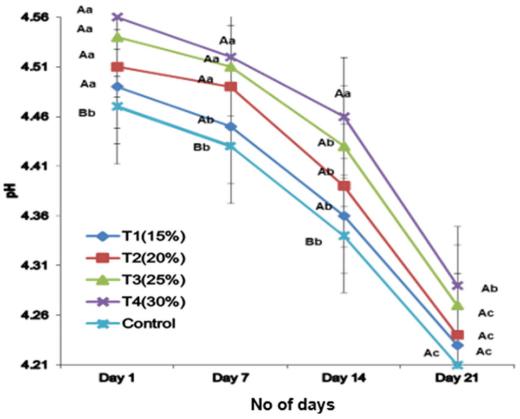

Figure 3: Variation of pH in watermelon incorporated set-yoghurts during the storage period of 21 days at refrigerated condition of 4±2 ºC. (Vertical lines represent standard deviations. ^{AB}Means with different uppercase letters differ significantly ($p<0.05$) between each set-yoghurt for a particular day of storage. ^{abc}Means with different lowercase letters significantly differ ($p<0.05$) between each day for each treatment of set-yoghurt)

As illustrated in the Figure 3, pH was significantly different ($p<0.05$) among the four different treatments and control during the storage period of 21 days. The initial pH of all the set-yoghurts ranged between 4.47- 4.56 while the initial pH of the watermelon incorporated set-yoghurts was significantly higher ($p<0.05$) than the control. During the storage period, pH gradually decreased in all treatments. The final pH of the set-yoghurts ranged between 4.21- 4.29 after 21 days storage period. However, there was no significant difference among the treatments at the end of the storage period. These results align with the findings of

Garcı´a-Pe´rez et al. [15], who observed that pH decrease in orange fiber added yoghurt in the first 14 days is more evident. In a similar experiment, it was revealed that the initial mean pH value and mean acidity of untreated yoghurt samples were lower than those of yoghurts with fruits (osmo-dried mulberry), since fruits naturally show a little higher acidity and a lower pH. The main reason for the gradual decrease of pH is the process of lactic acidification which is initiated by associative growth of thermophilic, homofermentative lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus* [16].

Figure 4 shows that, titratable acidity of the control yoghurt was significantly higher ($p<0.05$) than the other four treatments. The addition of watermelon juice decreased the acidity of set-yoghurts. However, the trend of increment in acidity continued up to the 21 days of storage period, although the acidity of the treated samples showed a constant rate of acceleration during the storage period of 21 days unlike the control sample, which reported the highest titratable acidity at the day 21 (1.01%). In contrast, the lowest titratable acidity was reported from the 30% watermelon incorporated yoghurt throughout the storage period of 21 days

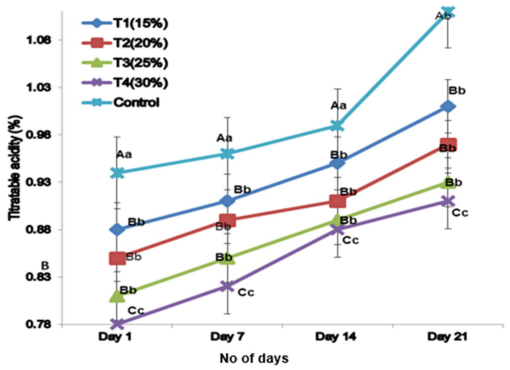

Figure 4: Variation of titratable acidity in watermelon incorporated set-yoghurts during the storage period of 21 days at refrigerated condition of 4±2 ºC. (Vertical lines represent standard deviations. [ABC]Means with different uppercase letters differ significantly ($p<0.05$) between each set-yoghurt for a particular day of storage. [abc]Means with different lowercase letters significantly differ ($p<0.05$) between each day for each treatment of set-yoghurt)

(T4). The increment of acidity goes hand in hand with the lactic fermentation which is occurred due to the special synergism between *Lactobacillus* spp. and *Streptococcus* spp. These results align well with the findings of Öztürk & Öner, [17], where it was discovered that the titratable acidity of concentrated grape juice incorporated yoghurt remained nearly constant following the second and third weeks with a small increase after 14[th] day of storage.

Syneresis is a crucial factor when it comes to determination of the quality of set-yoghurts. Higher syneresis levels indicate lower quality of yoghurt leading to poor consumer acceptance. The syneresis of yoghurts with added fruits is partially affected by the concentration of the fruits added [18].

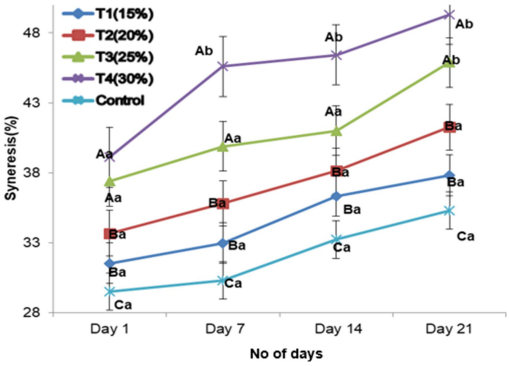

Figure 5: Variation of syneresis in watermelon incorporated set-yoghurts with storage period of 21 days at refrigerated condition of 4±2 ºC. Vertical lines represent standard deviations ([AB] Means with different uppercase letters differ significantly ($p<0.05$) between each set-yoghurt for a particular day of storage. [abc] Means with different lowercase letters significantly differ ($p<0.05$) between each day for each treatment of set-yoghurt.)

According to Figure 5, syneresis increased in both control yoghurt and watermelon juice added yoghurt samples during the storage period of 21 days, due to the weakening of the yoghurt micro-structure. Syneresis of control yoghurt was significantly lower ($p<0.05$) than the watermelon incorporated yoghurts. This could be attributed to the higher moisture content in watermelon juice (Table 1). Among the watermelon incorporated set-yoghurts, 15% (T1) watermelon incorporation level showed minimum syneresis and it has been found that syneresis becomes more prominent in increased-moisture yoghurt-based products. Furthermore, it is common to detect an increase of syneresis during the storage period and it is agreed that this phenomenon is generally associated with severe casein network rearrangements [19].

Microbiological analysis of watermelon incorporated set-yoghurts

According to Table 3, the lowest count of microbes on day 1 was recorded from 30% watermelon juice incorporated sample, which was 5.41 CFU/ mL. On the same day, the control sample recorded the highest microbial count which

was 5.52 CFU/ mL. The peak total microbial count was observed at day 21 in the control sample (5.91 CFU/ mL). During storage, the total bacterial counts increased up until day 21 in both control and watermelon incorporated yoghurts. These results are in agreement with the findings of Warakaulle et al. [11], who showed that the addition of watermelon juice to set-yoghurt resulted in a slight increase of the total microbial count during the storage period of 21 days.

Table 3: Total bacterial counts of watermelon incorporated set yoghurts

Total bacterial count of set-yoghurts(log CFU/mL)				
Treatment	**Storage period (Days)**			
	1	**7**	**14**	**21**
T1 (15%)	5.49±0.002	5.76±0.001	5.84±0.001	5.89±0.002
T2 (20%)	5.46±0.002	5.75±0.001	5.82±0.001	5.88±0.002
T3 (25%)	5.43±0.001	5.72±0.002	5.81±0.001	5.87±0.001
T4 (30%)	5.41±0.001	5.71±0.001	5.79±0.001	5.85±0.001
Control	5.52±0.002	5.79±0.001	5.86±0.002	5.91±0.002

According to a study conducted by Con et al, [20]it was concluded that addition of fruit flavors (sourced from pulps and concentrated juices) or sweeteners to yoghurt has no statistically significant effect on the presence of total bacteria and the current study presented similar results. Total bacterial counts of the watermelon incorporated set-yoghurts remained lower than those values of the control. The proven antibacterial properties of the watermelon due to the presence of different levels of phytochemicals could be attributed for these observations [21]. There was no yeast and mold growth up to the first week of storage in any treatment or control. Though, the rapid increase of yeast and mold count was observed in set-yoghurts during the third week of storage. None of the observed counts exceeded the maximum allowable count of 1×10^2 CFU/g which is recommended by Sri Lanka Standards Institution (SLSI) during the storage period of 21 days [22].

3. Conclusions

'Rocky 475' watermelon variety is the most preferred variety to be incorporated in set-yoghurt among the watermelon varieties used in the current study. Further, it can be recommended that incorporation of 20% watermelon juice in combination with 80% cow milk could be used as the best level of incorporation to develop set-yoghurt with higher consumer acceptance. Incorporation of watermelon in set-yoghurt significantly contributes to reduction of fat content of the yoghurt making promising effects as a low-fat dairy product. Nevertheless, the set-yoghurts developed incorporating 20% watermelon could be stored under refrigerated condition (4 ºC) up to 21 days without exceeding the standard levels of microbial counts.

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