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FORTIFICATION OF DRINKING YOGHURT WITH CARROT (*DAUCUSCARATA L*) PULP AND ORANGE (*CITRUS SINENSIS*) JUICE: A PRELIMINARY STUDY ON SENSORY EVALUATION, CHEMICAL COMPOSITION & MICROBIAL ANALYSIS

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ABSTRACT
Drinking yoghurt is a type of stirredyoghurt produced by incorporating artificial fruit flavor or natural fruit pulp/juice which is very popular in current Sri Lankan market. The main objective of this study was to develop a drinking yoghurt by utilization of carrot (*Daucuscarata L.*) pulp as the main beta carotene source and orange (*Citrus sinensis*) juice for enhanced sensory properties. The Yoghurt base was prepared according to the traditional procedure of yoghurt preparation with 3.5% fat and 8.5% MSNF. Carrot pulp and orange juice were mixed to 1:1 ratio and pasteurized at 105°C for 1 minute. The mixing ratio of yoghurt base and carrot-orange mix was 4:1 according to the data gathered from a preliminary conducted sensory trial using trained sensory panel with nine-point hedonic scale for the selection of best formula of yoghurt base, carrot pulp and orange juice. The developed drinking yoghurt was subjected to analysis of proximate composition, physiochemical properties, microbial properties and sensory properties for evaluation of shelf life.Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney test of Minitab 17 version was applied for the statistical analysis of gathered data. After analyzing the microbial, physiochemical and sensory properties for 35 days with 7 days' intervals, the shelf life of the product was detected with refrigerated storage condition. The total fat, total carbohydrate, total solid, crude protein, ash and beta carotene contents of developed drinking yoghurt were2.2±0.1%,4.59±0.18%, 18.27± 0.07%, 3.16±0.10%, 0.58±0.05% and 0.49478±0.000015 mg/100g respectively. These results collectively suggest that, the developed drinking yoghurt is rich withnutrients including beta carotene.

KEYWORDS: Yoghurt; Carrot; Orange; Beta-carotene; Milk

INTRODUCTION
Yoghurt is a most popular fermented milk product which is consumed all over the world due to its sensory properties and high nutritional value. Yoghurt fermentation process is carried out by lactic acid bacteria. Although yoghurt has own preservation due to low pH, the proper storage conditions should be maintained unless it may be spoiled since it is milk. The storage conditions are maintained for yoghurt throughout the distribution chain in order to avoid the risk of spoilage fromyeasts and also to prevent the further activity of starter culture [1].

Yoghurt is offered in different types such as set, drinking, frozen, concentrated etc. considering the fat and total solid percentage, the body structure, presence of additives, flavors or pulps with probiotic microflora. And also some bioactive compounds are used to fortify yoghurts [2]. Set type is incubated and cooled in the package. Stirred type is incubated in tanks and cooled before packing. Drinking type is similar to stirred type, but the coagulum is "broken down" to a liquid before being packed. Frozen type is incubated in tanks and frozen like ice cream. Concentrated type is incubated in tanks, concentrated and cooled before being packed. This type is sometimes called strained yoghurt, sometimes labneh, labaneh[3].

Yogurt is a functional food which includes probiotics, prebiotics and symbiotic. Probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance"[4] while prebiotics is the "non-digestible food ingredient that beneficially affects the host by

selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". Symbiotic is a combination of probiotics and prebiotics that "beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastro-intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria" [5]. The probiotic yogurt, having probiotic effect is a fermented milk product with adjuvant microorganisms. A high population of probiotic organisms in the colon contributes to good intestinal health [6]. Consequently, consumption of products such as yoghurt containing viable probiotic organisms adds benefit to human gut health. Moreover, yoghurt is an excellent source of calcium, phosphorus, potassium and contains significant quantities of general vitamins. Yoghurt could be used for feeding, owing to its higher Ca/Na ratio [7]. Different ingredients, flavours and appearance are there in different yoghurt types. The quality and composition of yoghurt are affected by the applied bacterial cultures in the milk fermentation process. Between the two species of bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, a symbiotic relationship is there which causes to rapid acid development than in single strain culture[1]. The selection of starter culture combinations when manufacturing yoghurt, the desirable characteristics expected to be achieved are considered in order to provide wide range of benefits for the consumers. Usually, 2-4% of starter culture is added depending on its activity.

Yoghurt having high nutritional and therapeutic properties is being highly consumed and produced [8]. Therefore, the fortification of nutrients for yoghurt is abundant in current dairy industry. The current trend of dairy products is to fortify with fruits such as natural fruit juice, dry fruits and pulp [9]. Aesthetic value of new product can be increased by using fruit juice as a functional pigment in the fermented milk with an array of colors and flavor properties [10].

Two significant barriers as heat and acidity are provided by yoghurt against the growth of pathogens. A safe product is ensured by both of these barriers. Acidity alone has been questioned by recent outbreaks of food poisoning by E. coli O157:H7 that is acid-tolerant. E. coli O157:H7 is easily destroyed by pasteurization (heating). Therefore, it is essential to use either boiled milk or commercially pasteurized milk for manufacturing yoghurt [5].

The Food and Drug Administration standard of identity for yoghurt drinks specifies >8.25% milk solids-not-fat and fat levels to satisfy nonfat yoghurt (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients. Generally, yoghurt drinks are flavoured with natural or artificial fruit pieces or fruit juices according to the consumer preferences which is varied from country to country. Strawberry, raspberry, carrot, apple concentrates, pineapple, lemon or orange concentrates or essences are the fruit flavors used in yoghurt drinks [11]. Fruit additions have an increasing effect on yoghurt consumption[12].

MATERIALS AND METHODS

DEVELOPMENT OF DRINKING YOGHURT WITH CARROT PULP AND ORANGE JUICE

For the preparation of yoghurt base, standardized, homogenized, pasteurized, preheated cow milk was mixed with other ingredients (skimmed milk powder, sugar, gelatin, potassium sorbate) and pasteurized at 95°C for 5 minutes. Inoculation of yoghurt culture (*Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. bulgaricus*) was done followed by cooling to 44°C, then incubation was carried out at 44°C until the pH value of the mix reduces up to 4.5 and cooled to 4°C.For the preparation of carrot – orange mix, grated carrot was blended with water to 4:1 ratio for obtaining carrot pulp and mixed with extracted orange juice into three mixing ratios as 1:2, 1:1, 3:2, and pasteurized at 105°C for 1 minute. The prepared pasteurized carrot – orange mixes were mixed with yoghurt base into three formulae (carrot pulp, orange juice and yoghurt base) as formula A-1:2:17, formula B- 1:1:8, formula C-3:2:15 while stirring and filled into bottles; stored at 4°C.

PRELIMINARY SENSORY TRIAL

For identification of the best formula of carrot pulp, orange juice and yoghurt base, a sensory trial was conducted using the samples coded as 253 for formula A, 426 for formula B, 389 for formula C and 528 for the control sample with trained sensory panel and nine-point hedonic scale. The data collected from the preliminary sensory trial was analyzed by Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney test of Minitab 17 version.

ANALYSIS OF CHEMICAL COMPOSITION

The product developed followed by the formula selected from sensory trial was subjected to chemical composition analysis including moisture content, total solid content, total ash content according to AOAC methods, crude protein contentaccording toKjeldahl method, fat contentby Gerber method, total carbohydrate content using Dubois method [13],beta carotene content by UV-Visible spectrometric method [14].

SHELF LIFE EVALUATION

In order to detect the shelf life of developed drinking yoghurt, microbial properties, physiochemical properties and sensory properties were analyzed for 35 days with intervals of 7days with $4^{o}C$ storage condition.

MICROBIAL PROPERTIES

Using ISO standardized microbiological methods of evaluating shelf life of cultured product, the coliform counts withViolet Red Bile Agar medium and yeast & mold count with Yeast Dextrose Chloramphenicol Agar medium were detected.

PHYSIOCHEMICAL PROPERTIES

pH value

The pH value of sample was determined using pH meter (Mettler Toledo seven compact pH ion meter).

Water Holding Capacity (WHC)

The water holding capacity of developed drinking yoghurt was determined according to a method described in[15]. Briefly, 20g of drinking yoghurt sample was weighed and centrifuged at 1250 x g for 10 minutes. The whey expelled was filtered and weighed. The procedure was triplicated. Water holding capacity was calculated using following equation.

$$\text{Water holding capacity } (gkg^{-1}) = \frac{\text{Weight of drinking yoghurt sample(g)} - \text{Weight of whey expelled(g)}}{\text{Weight of drinking yoghurt sample(g)}} \times 1000$$

Titratable Acidity (TA)

For the determination of titratable acidity of the sample a titrimetric method described by [16] was used. Initially 1g of the homogenized sample was measured to the nearest 0.1g into a porcelain dish. Then 100ml of distilled water was added to obtain white colour solution since light orange color of drinking yoghurt sample disturbed the identification of end point. Then 2-3 drops of phenolphthalein indicator were added and mixed well. It was titrated with 0.1N NaOH while agitating until a permanent pink color was appeared. The acidity of the sample was reported as % of lactic acid by weight. The procedure was triplicated.

Brix value
The brix value was measured using brix meter(Atago brix meter).
Apparent Viscosity

The apparent viscosity was measured using viscometer (Brookfield). The spindle (No: 5) was fixed to the rotational viscometer after entering to the drinking yoghurt sample (20°C) which was filled into the beaker. It should be careful to enter the spindle to the sample as not forming air bubbles. Viscosity was measured at a shear rate of 20 rpm as per the procedure described in the manual. The dial reading was taken after 2 minutes of rotation. The viscosity was calculated using the following equation.
Viscosity in Centipoise (mPa·s) = Dial reading x Factor
Here, the factor was taken according to the speed and the number of spindle used.

SENSORY PROPERTIES

In order to confirm the consumer acceptability in the aspects of sensory attributes of drinking yoghurt throughout the period of shelf life, sensory evaluation was conducted 1st, 7th,14th, 21st, 28th and 35th days (initial, week 1, week 2, week 3, week 4, week 5) of shelf life. Sensory evaluation was carried out by 15 trained panelists with 9-point hedonic scale with respect to the sensory attributes; appearance, aroma, taste, texture/mouth feel and overall acceptability. The data gathered from sensory trials were analyzed using Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney test of Minitab 17 version.

RESULTS

Preliminary Sensory Analysis

Null hypothesis	H_0 : All medians are equal (there is no significant difference between two samples under the tested attributes)
Alternative hypothesis	H_1 : At least one median is different (there is a significant difference between two samples under the tested attributes)

Table 1: H_{cal} values for each sensory attribute in preliminary sensory trial

Attribute	H_{cal} value
Appearance	32.65
Aroma	19.38
Taste	26.76
Texture/Mouth feel	32.28
Overall Acceptability	35.77

Degree of freedom of the test samples; 3 – 1 = 2
Chi-square value for 5% significance level = 5.991

The obtained H_{cal} values from Kruskal Wallis test are greater than the relevant chi square value with respect to each sensory attribute at 5% confidence level which indicates the rejection of H_o concluding the significant difference of the four samples in aspects of sensory attributes.

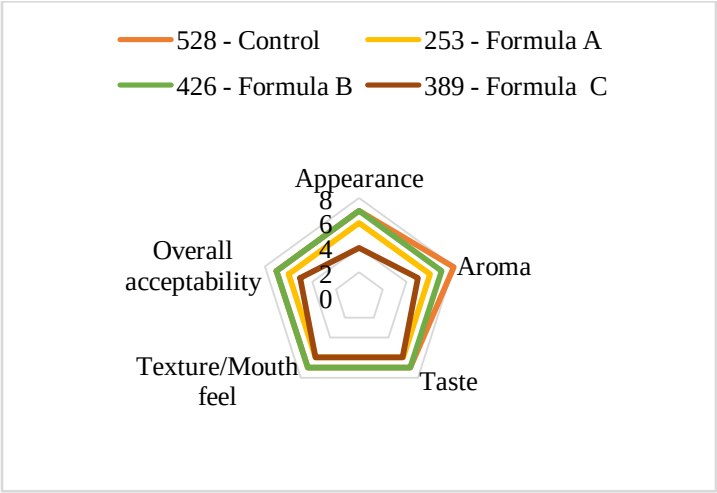

Figure 1: Web diagram for each sensory properties in preliminary sensory trial

In formula B, the obtained mean values for each sensory attributes except aroma are same as for control while other formulae are having lower mean values which leaded to select formula B as the best with respect to sensory properties and for further developments and analysis.

ANALYSIS OF CHEMICAL COMPOSITION

Table 2: Chemical composition of developed drinking yoghurt and plain yoghurt

Component	Developed drinking yoghurt	Plain drinking yoghurt
Moisture	81.73 ± 0.07%	83.43±0.05%
Total solid	18.27 ± 0.07%	16.57±0.05%
Total ash	0.58 ± 0.05%	0.67±0.00%
Crude protein	3.16 ± 0.10%	3.69±0.05%
Fat	2.2 ± 0.1%	4.05±0.05%
Total carbohydrate	4.59 ± 0.18%	4.7 ± 0.1%
Beta carotene	4.978± 0.001µg/g	0.428 ±0.001µg/g

SHELF LIFE EVALUATION

MICROBIAL PROPERTIES

Table 3: Variation of Coliform counts with storage at 4°C

Time period of storage (at 4°C)	Developed drinking yoghurt sample	Control
Initial	Negative	Negative
Week 1	Negative	Negative
Week 2	Negative	Negative
Week 3	Negative	Negative
Week 4	Negative	Negative
Week 5	Negative	Negative

Table 4: Variation of yeast & mold counts with storage at 4°C

Time period of storage (at 4°C)	Developed drinking yoghurt sample	Control
Initial	0	0
Week 1	0	0
Week 2	0	0
Week 3	0	0
Week 4	0	0
Week 5	0	0

PHYSIOCHEMICAL PROPERTIES

Table 5: Variation of physiochemical properties with storage at 4°C

Parameter	Initial	7th day	14th day	21st day	28th day	35th day
pH	4.42	4.26	4.26	4.26	4.26	4.20
WHC (%)	47.8	47.8	47.7	47.7	47.7	47.7
TA (%)	0.63	0.62	0.62	0.61	0.60	0.58
Brix (%)	15.2	15.2	15.2	15.2	15.2	15.2
Viscosity(mPa's)	300	300	300	300	300	300

SENSORY PROPERTIES

Null hypothesis H_0 : All medians are equal (there is no significant difference between two samples under the tested attributes)

Alternative hypothesis H_1 : At least one median is different (there is a significant difference between two samples under the tested attributes)

Table 6: H_{cal} values of sensory attribute with the shelf life

Attribute	H_{cal}
Appearance	1.37
Aroma	3.78
Taste	4.84
Texture/Mouth feel	1.59
Overall Acceptability	2.47

Degree of freedom of the test samples; 6 – 1 = 5
Chi-square (Appendix) value for 5% significance level = 11.070

The obtained Hcal values from Kruskal Wallis test are lower than the relevant chi square value with respect to each sensory attribute at 5% confidence level which indicates the acceptance of H_o concluding the no significant difference of the sample throughout 35 days in aspects of sensory attributes.

Table 7: Mean separation of sensory attribute with the shelf life

Attribute	P Value (Initial -Week 1)	P Value (Initial-Week 2)	P Value (Initial-Week 3)	P Value (Initial-Week 4)	P Value (Initial-Week 5)
Appearance	0.3646	0.3646	0.3646	0.6699	0.4619
Aroma	0.7985	0.7985	0.1777	0.8910	0.6845
Taste	0.7679	0.2825	0.5684	0.4164	0.6903
Mouth feel	0.3961	0.6026	0.3961	1.0000	0.7623
Overall Acceptability	0.3520	0.6464	0.5529	0.6299	0.9083

According to the results of Mann-Whitney test at 95% confidence level, the five sensory attributes; appearance, aroma, taste, texture/mouth feel and overall acceptability are not significantly different (P Value ≥ 0.05) with throughout the period of shelf life.

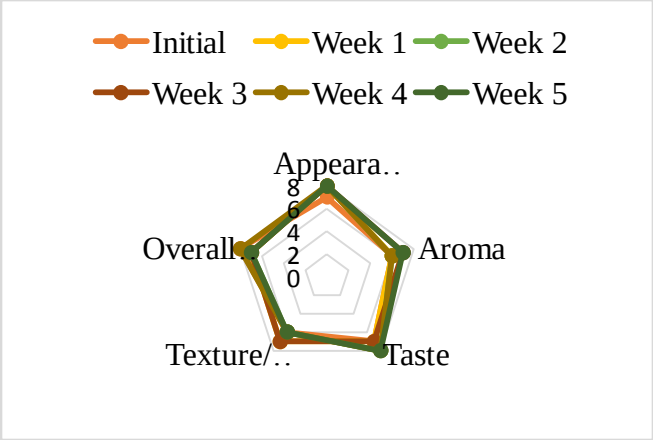

Figure 2: Web diagram of sensory attribute with the shelf life

According to the above web diagram, it can be identified that, there is not much variation of the sensory attributes with the period of shelf life as in the statistical analysis. The consumer preference with respect to sensory parameters has not changed with the storage time of the product.

DISCUSSION

Milk is a highly nutritious food which contains substances provide both energy and the building materials necessary for growth. Water, fat, protein and lactose are the four quantitatively dominant components of milk while the minor components are minerals, enzymes, vitamins, and dissolved gases. It satisfies the consumer demand for high quality innovative dairy products. Fermentation is a value added process of milk which adds more taste, better texture and enhanced shelf life for the product. Yoghurt is also a fermented product preferred by the worldwide consumers. However, there are two main types of yoghurt, set and stirred, based on the method of production and on the physical structure of the coagulum. With the innovations of dairy industry, nutritional substances such as probiotic cultures, minerals, natural and artificial fruit and vegetable flavors are incorporated to yoghurts. Drinking yoghurt which is under the category of stirred yoghurt, is now a highly consumable product in both national and international markets. With the developments of the dairy industry, more innovative types of drinking yoghurt are coming to the market and are in research level based on the fortification of essential nutrients for the human body. Such developments will enhance the nutritional value and also sensory properties of the plain yoghurt drink.

The study was carried out to develop a drinking yoghurt by incorporating carrot (*Daucuscarata L.*) pulp and orange (*Citrus sinensis*) juice. Carrot is mostly used vegetable in human nutrition. Carrot juice increases total antioxidant status and decreases lipid peroxidation in adults [17]. It is rich in beta carotene, ascorbic acid, tocopherol and classified as vitaminized food [18]. Therefore, carrot was used as the main beta carotene source and orange was added to enhance the sensory properties of the product and also as a beta carotene source. Orange is a rich source of vitamin C, flavonoids, phenolic compounds and pectins. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin[19]**.**

Beta carotene pigments are naturally occurring antioxidants in plant materials. According to recent studies, a diet high in carotenoids may reduce the risk of heart attack and assist in cancer prevention [20]. Fortification is one of the best methods to deliver the benefits of natural antioxidants for humans [21].

CONCLUSION

For the development of drinking yoghurt, the selected best formula of carrot pulp, orange juice and yoghurt base was 1:1:8 with respect to sensory attributes. By evaluating physiochemical, microbial and sensory properties for 35 days with the storage conditions of 4°C, the detected shelf life of developed drinking yoghurt was 35 days (5 weeks) which was containing beta carotene, 11.63 times higher than plain yoghurt.

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