Determination of Glycaemic Responses of dairy yoghurt incorporated with spice oleoresins (*Cinnamomum zeylanicum*, *Curcuma longa*).

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Abstract*—Diabetes mellitus, which is characterized by high levels of blood glucose, is one of the most common metabolic disorders. Blood glucose level of individuals can be controlled by the incorporation of spices in the diet. The main objective of this study was to manufacture spice oleoresin (*Cinnamomum zeylanicum, Curcuma longa*) incorporated, high quality, novel dairy yoghurts with reduced glycaemic impact. The developed yoghurt products were compared with typical dairy yoghurt as the control, mainly for the glycaemic impact after consumption, using 16 healthy volunteers in a randomized crossover study. The blood glucose concentration of individuals was measured using a blood glucose monitoring system at fasting state and at 30, 45, 60, 90, 120 min following ingestion. Glucose response curves were plotted for individuals, and control was used as the standard. In addition, shelf life, compositional and physiochemical properties were determined for the prepared yoghurt products. The developed novel and control yoghurt products were within the acceptable standards and significant differences were not observed in compositional and physiochemical properties. However, significant ($p < 0.05$) reduction was observed in peak blood glucose concentration and area under the curve (AUC) of individuals for both novel yoghurt products compared to the control. Percentage peak glucose concentration reduction for cinnamon and turmeric yoghurt products were 9.61% and 9.26%, respectively compared to the control. Mean peak blood glucose concentration for control, cinnamon and turmeric yoghurt products were 113.38±6.39, 102.50±6.00, 102.88±5.38 mg/dL, respectively and mean AUC were 11951±523, 11012±611, 10941±530 ((mg/dL)min), respectively. Hence, incorporation of spice oleoresin *(Cinnamomum zeylanicum, Curcuma longa)* has significantly reduced the glycaemic impact of dairy yoghurt.

Keywords—Blood glucose responses, Cinnamon, Dairy yoghurt, Glycaemic impact, Turmeric.

I. INTRODUCTION

Diabetes mellitus is the most common metabolic disorder characterized by elevated levels of blood glucose and the prevalence of diabetes is increasing across the world [1]. One of the healthy ways of controlling diabetes is through the diet [2]. A variety of traditional herbs and spices used in Sri Lanka from ancient times have elicited beneficial effects on diabetes. In this study, two spices were selected – Ceylon cinnamon (*Cinnamomum zeylanicum*) and Turmeric (*Curcuma longa*) for the formulation of a novel dairy yoghurt. These two spices have proven potential benefits in diabetes management [2]. Already yoghurt is known as a low Glycaemic Index (GI) food which can reduce the risk of diabetes mellitus [3]. So incorporation of GI suppressing spices would enable people to consume these new products even after a starchy main meal. Therefore, the current study aims to explain the influence of cinnamon and turmeric along with yoghurt on glycaemic responses respectively, while providing best overall quality.

II. METHODOLOGY

A. Preparation of yoghurt

Yoghurt products were prepared according to the procedure shown in Figure 1. Control was also prepared by using the same procedure without the addition of any oleoresins of spices. The ingredients were used in following proportion: 86.0% milk, 11.1% sugar, 3.3% full cream milk powder, and 0.6% of gelatin by weight basis, and 3% (w/v) starter culture. Oleoresins were used in three different concentrations 50 ppm, 100 ppm, and 150 ppm separately for the production of yoghurt.

B. Sensory evaluation

Yoghurts with three different concentrations for each of the 2 types of oleoresins (cinnamon and turmeric), were given to members, who were asked to rank sensory properties (appearance, aroma, taste, mouth feeling, texture and overall acceptability) using a five point hedonic scale with the contribution of 30 semi trained panelists to select the best sample.

At last two selected yoghurt products made with cinnamon and turmeric were compared with a control for proximate compositions, physiochemical analysis, shelf life evaluation and glycaemic impact analysis.

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C. Analysis of proximate composition

The moisture (oven drying method), ash content (AOAC official method 923.03 - gravimetric method), crude protein (AOAC method 978.04 - micro Kjeldahl method), fat content (AOAC official method 2000.18 - Gerber method) and total carbohydrate content (AOAC Method 44.1.30 - phenol sulphuric method) of prepared yoghurt products were determined accordingly [4]. At last the fibre content was determined by subtracting the total of the above components from 100. Also, total solids (TS) and solid not fat (SNF) values were estimated using above obtained results.

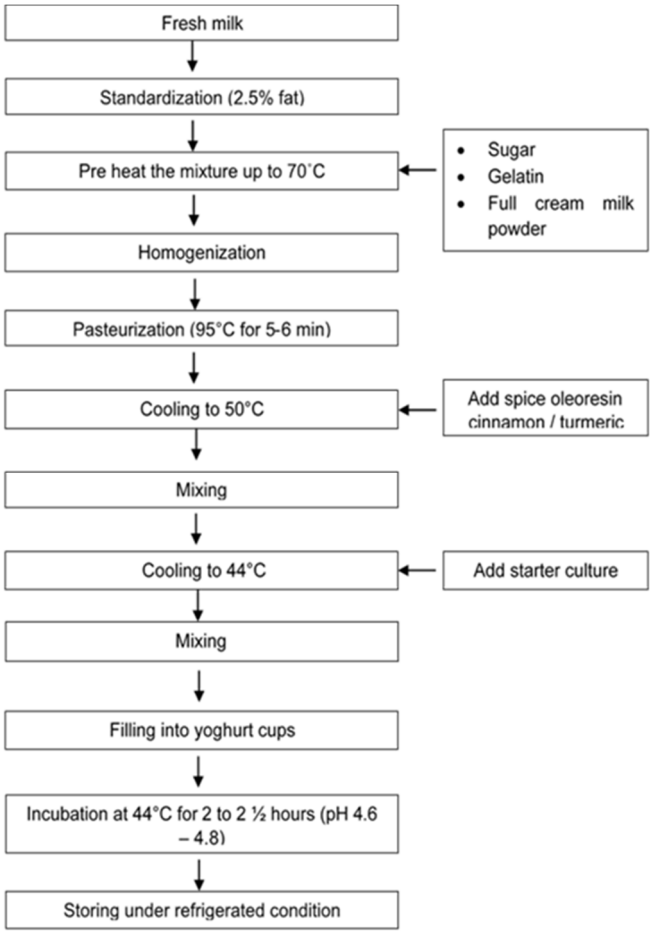

Fig. 1. Preparation of yoghurt using spice oleoresin

D. Analysis of physiochemical parameters

The pH, titratable acidity, and water holding capacity (WHC) of developed yoghurt products were measured on the 1st, 5th, 10th and 15th day of the storage period at temperature 4°C. Titratable acidity was expressed as percentage of lactic acid using the equation below. Also, apparent viscosity and texture profile analysis were measured on 7th day of storage period at temperature 4°C. The pH was measured using a digital pH meter (Eutech-510). Water holding capacity (WHC) was measured using a centrifuge (HERMLE Z306, Germany) and Whatman 1 filter paper – No.42 according to the method of Yao et al [5]. The viscosity of the samples was measured using a Digital viscometer (BDV – 9S) and samples were tested using spindle no.4 with a speed of 30rpm for a uniform time period of 5 min, and the viscosity was expressed in mPa·s. Texture profile (hardness, cohesiveness, adhesiveness, gumminess, springiness and chewiness) was analyzed [6] using a texture analyzer (Brookfield CT3 50K) - (Probe: TA4/1000 , test type: TPA, trigger load: 10 g, target: 10 mm, target type: distance, pre-test speed: 2 mm/s, test speed: 0.5 mm/s, post-test speed: 3 mm/s).

$$\text{Water holding capacity \%} \left(\frac{w}{w}\right) = \frac{\text{Weight of the precipitation}}{\text{Weight of the analyzed yoghurt}} \times 100\% \quad (1)$$

$$\text{Lactic acid \%} = \frac{\text{Vol of NaOH} \times \text{Normality of NaOH} \times \text{Molecular weight of lactic acid}}{\text{Weight of the yoghurt samples}} \times 100\% \quad (2)$$

E. Shelf life evaluation

Organoleptic properties - overall acceptability (sensory evaluation) and microbial analysis (count of coliform, yeast and mold) - of developed yoghurt products were tested on 1st, 5th, 10th and 15th day of the storage period at temperature 4°C.

Microbial analysis: The count of coliform (ISO 4831:2006) using the MPN method, and yeast and mold (ISO 6611:2004) were determined. The colonies were counted using a colony counter and results were expressed in terms of colony forming unit per ml (CFU/ml).

F. Determination of glycaemic impact

The method of this study was adapted from Wolever et al [7]. Sixteen healthy volunteers including both sexes with normal fasting glucose level were selected. The control was used as the standard food. The novel test yoghurt products (cinnamon yoghurt and turmeric yoghurt) and the control were served to the same individual on separate occasions randomly. Following an overnight fast of 8 - 12 hours, blood glucose concentration was measured at fasting state and 30, 45, 60, 90, 120 min following the ingestion of 2 cups of yoghurt product (each cup 90 g) using the PRODIGY blood glucose monitoring system (USA, F.D.A. approved). Serum glucose concentrations were plotted against time for each individual. The area under curve (AUC) and peak reduction in the curve were calculated separately for each individual for each specific product. Ethical clearance (No. 56/19) for the study was obtained from the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura.

G. Statistical analyseis

IBM SPSS Statistics V21 software – Friedman test for sensory evaluation, Minitab 17 statistical software – One Way Analysis of Variance (one way - ANOVA) with 95% confidence interval for proximate analysis, physiochemical analysis and glycaemic impact analysis, and Excel 2013 - for the calculation of area under the curve - were used.

III. Results and Discussion

Sensory evaluation results were obtained from the Friedman test. According to the results (TABLE I) the 100 ppm cinnamon / turmeric oleoresin incorporated yoghurt product was selected as the best, because it has the highest mean rank for most of the sensory attributes and it has significant difference than the other two products ($p < 0.05$).

According to proximate analysis (TABLE II), significant variation was not observed ($p > 0.05$) within the samples in compositional properties. SLS requirements for a normal yoghurt are that it should contain 3.0% minimum fat, 8.0%

minimum nonfat milk solid, and 2.7% minimum protein [8]. Hence, the results reveal that all the developed yoghurt products were within the acceptable range of nutritional requirements.

The results of all physiochemical parameters for control, cinnamon yoghurt, and turmeric yoghurt indicate that pH and WHC have significant reduction and titratable acidity has significant increase throughout the storage period ($p < 0.05$) and also there was no significant difference ($p > 0.05$) among the above three samples in all physiochemical parameters (pH, titratable acidity, WHC, viscosity and texture profile analysis) (TABLE III). Lactic acid development within the storage period causes the reduction in pH value and increases titratable acidity value. According to the SLSI recommendation (SLS 824: Part 2: 1989) for yoghurt, pH values should not be less than 4.2 in all types of yoghurt and titratable acidity values should be in the range of 0.8 – 1.25% in all types of yoghurt. So the developed yoghurts were within the acceptable range of requirements.

Hence, it can be concluded that the developed yoghurt products with spice oleoresins have similar compositional and physiochemical characteristics to those of the control.

TABLE I Mean rank values and p- values for sensory properties

Sensory parameters	Cinnamon yoghurt				Turmeric yoghurt			
	50 ppm	100 ppm	150 ppm	p-value	50 ppm	100 ppm	150 ppm	p-value
Appearance	2.27	2.63	1.10	0.00	2.23	2.52	1.25	0.00
Aroma	2.13	2.42	1.45	0.00	1.88	2.35	1.77	0.03
Mouth feeling	1.77	2.38	1.85	0.01	1.78	2.33	1.88	0.03
Taste	2.05	2.95	1.00	0.00	2.18	2.75	1.07	0.00
Texture	2.07	2.27	1.72	0.07	2.02	2.17	1.82	0.32
Overall acceptability	1.98	2.55	1.47	0.00	2.07	2.50	1.43	0.00

TABLE II Mean values of nutritional parameters

Parameters	Control	Cinnamon added yoghurt	Turmeric added yoghurt
Moisture %	75.99 ± 0.09^a	76.04 ± 0.05^a	76.03 ± 0.15^a
Ash %	0.97 ± 0.01^a	1.00 ± 0.05^a	0.99 ± 0.02^a
Fat %	3.07 ± 0.03^a	3.03 ± 0.06^a	3.03 ± 0.06^a
Protein %	3.81 ± 0.10^a	3.81 ± 0.10^a	3.81 ± 0.10^a
Carbohydrate %	15.28 ± 0.06^a	15.26 ± 0.09^a	15.29 ± 0.06^a
Fiber %	0.88	0.86	0.85
TS %	24.01	23.96	23.97
SNF %	20.94	20.93	20.94

(Different letters a-c in the same row indicate significant difference, $p < 0.05$)

When considering shelf life evaluation, a significant difference was not observed ($p > 0.05$) in overall acceptability throughout the storage period for all types of yoghurt products. But in microbiological analysis, spice- oleoresin added yoghurt products showed better stability than the control, probably due to antimicrobial properties of spices. The coliform test has shown negative results for the presence of coliforms / *E. coli* during the storage period for all three yoghurts. SLS 516 Part 12: 2013 (ISO 7251: 2005) states the *E. coli* maximum limit in yoghurt products to be 0.3 MPN/g. According to the SLS 516 Part 2: Sec 1: 2013 (ISO 21 527-1,2: 2008), specified by Sri Lanka Standards Institute, the yeast and mold count limit in yoghurt should be less than 10 CFU/g throughout the storage period. In both spice-added yoghurts, no yeast and mold counts were recorded during the shelf life. But in the control yoghurt two yeast and mold colonies were observed on the 15th day because of the absence of any preservatives. So it can be concluded that, cinnamon/turmeric oleoresin added yoghurts were safer for consumption within 15 days at 4°C storage conditions. Shelf life was estimated as 15 days for the two novel yoghurt products.

Glycaemic impact analysis

According to the plotted glucose response curve (Figure 2), significant peak reduction ($p < 0.05$) in mean blood glucose concentration (TABLE IV) was observed for both cinnamon and turmeric oleoresin added yoghurt products, but the peak time is almost similar (30 min) in all individuals. The percentage of peak reduction for cinnamon and turmeric added products were approximately 10% compared to control (cinnamon = 9.61%, turmeric = 9.26%). Peak glucose concentration was calculated using the equation below.

$$\%\ \text{of peak serum glucose concentration reduction} = \frac{(\text{Control yoghurt peak} - \text{Spices added yoghurt peak})}{\text{Control yoghurt peak}} \times 100\% \quad (3)$$

The mean area under the curve of the two novel products added with cinnamon and turmeric (TABLE IV), were significantly reduced compared to the control ($p < 0.05$). No significant difference ($p > 0.05$) in AUC and peak reductions between cinnamon and turmeric added yoghurt products were observed, indicating their similar effectiveness in reducing post prandial blood glucose levels. Altogether, results reveals that, both selected spices are effective in controlling diabetes mellitus.

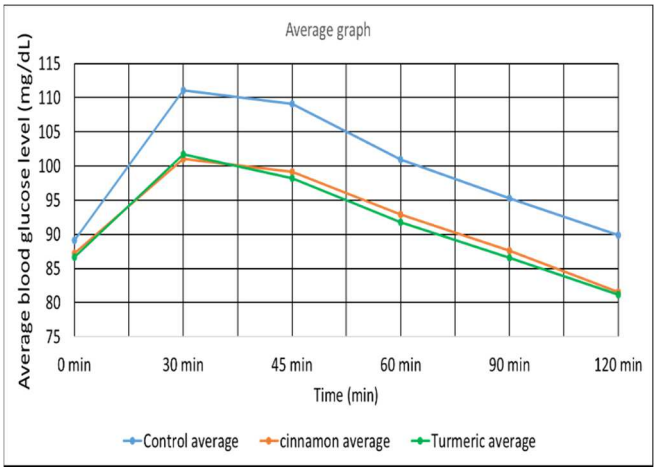

Fig.2. Average glucose response curve

TABLE III Mean values of physiochemical properties

	Control Yoghurt				Cinnamon Yoghurt				Turmeric yoghurt			
Storage period	1st day	5th day	10th day	15th day	1st day	5th day	10th day	15th day	1st day	5th day	10th day	15th day
pH	4.76 ± 0.01[a]	4.42 ± 0.01[a]	4.30 ± 0.01[a]	4.25 ± 0.01[a]	4.79 ± 0.01[a]	4.46 ± 0.01[a]	4.34 ± 0.01[a]	4.27 ± 0.01[a]	4.78 ± 0.01[a]	4.44 ± 0.01[a]	4.33 ± 0.01[a]	4.26 ± 0.01[a]
Titratable acidity %	0.75 ± 0.03[a]	0.85 ± 0.03[a]	0.92 ± 0.03[a]	0.97 ± 0.03[a]	0.80 ± 0.04[a]	0.88 ± 0.03[a]	0.95 ± 0.03[a]	1.02 ± 0.03[a]	0.78 ± 0.03[a]	0.87 ± 0.03[a]	0.94 ± 0.03[a]	1.00 ± 0.03[a]
Water Holding Capacity (WHC) %	93.22 ± 0.49[a]	90.46 ± 0.49[a]	87.16 ± 0.85[a]	84.68 ± 0.41[a]	92.33 ± 0.81[a]	88.26 ± 0.48[a]	85.88 ± 0.61[a]	83.12 ± 0.24[a]	91.75 ± 0.74[a]	87.86 ± 0.25[a]	85.14 ± 0.31[a]	82.97 ± 0.51[a]
Storage period	7th day				7th day				7th day			
Apparent viscosity (mPa.s)	3520.50±22.90[a]				3511.77±4.62[a]				3512.50±4.18[a]			
Texture parameters ▪ Hardness (mJ) ▪ Adhesiveness (mJ) ▪ Gumminess (g) ▪ Springiness (mm) ▪ Chewiness (mJ) ▪ Cohesiveness	20.07±0.72[a] 3.50±0.27[a] 133.68±0.93[a] 55.12±0.39[a] 77.36±0.35[a] 0.40±0.01[a]				18.97±0.24[a] 3.49±0.27[a] 132.34±1.07[a] 54.85±0.55[a] 76.79±0.34[a] 0.40±0.01[a]				19.86±0.31[a] 3.46±0.20[a] 132.94±0.72[a] 54.65±0.58[a] 77.33±0.59[a] 0.38±0.01[a]			

(Mean values of n = 3 ± Standard Deviation, Different letters a-c in same row indicate significant difference, $p < 0.05$ - Tukey's pairwise test)

TABLE IV Peak serum glucose concentrations, peaking times and area under the curves of individuals

T: Peaking time (min) **C**: Peak serum glucose concentration (mg/dL)

	Peak serum glucose concentrations and peaking times						**Area under the glucose response curve ((mg/dL)min)**		
Volunteer no.	**Control yoghurt**		**Cinnamon yoghurt**		**Turmeric yoghurt**		**Control yoghurt**	**Cinnamon yoghurt**	**Turmeric yoghurt**
	T (min.)	**C (mg/dL)**	**T (min.)**	**C (mg/dL)**	**T (min.)**	**C (mg/dL)**			
1	30	105	45	101	30	101	11070.0	10365.0	10687.5
2	45	115	30	98	30	106	11655.0	10687.5	11325.0
3	30	112	30	102	45	101	11992.5	11107.5	10560.0
4	30	131	30	118	30	120	13065.0	12075.0	11925.0
5	30	113	30	105	30	101	11557.5	10852.5	10650.0
6	45	111	30	105	30	102	12127.5	11280.0	11160.0
7	45	106	45	102	45	103	11827.5	11287.5	11415.0
8	45	113	45	104	30	100	11955.0	11422.5	10965.0
9	30	114	30	107	30	101	12105.0	11265.0	11145.0
10	45	112	45	109	30	107	12600.0	12352.5	11482.5
11	30	112	30	95	30	93	11340.0	10215.0	9705.0
12	30	124	45	104	45	107	12832.5	11077.5	11370.0
13	30	110	30	101	30	105	11625.0	11002.5	11032.5
14	30	112	30	93	30	102	11520.0	9990.0	10830.0
15	30	108	30	100	30	99	11670.0	10830.0	10117.5
16	45	116	30	96	30	98	12270.0	10387.5	10680.0
Mode	**30**		**30**		**30**				
Mean with S.D		**113.38 ± 6.39[a]**		**102.50 ± 6.00[b]**		**102.88 ± 5.78[b]**	**11950.8 ± 523[a]**	**11012.3 ± 611[b]**	**10940.6 ± 530[b]**

(Mean values of n = 16 ± Standard Deviation, Different letters a-c in same row indicate significant difference, $p < 0.05$ - Tukey's pairwise test)

IV. CONCLUSION

Incorporation of cinnamon and turmeric oleoresin have significantly reduced glycaemic impact in dairy yoghurts, without affecting their overall sensory and quality profiles.

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