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Effects of Adding Cocoa (*Theobroma cacao* L.) Pulp Nectar to Stirred Yoghurts on Physicochemical and Sensory Properties

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Abstract: Cocoa (*Theobroma cacao* L.) comprise of a fruit 'pod' which contains seeds embedded in mucilaginous pulp. In this first study, the objectives were to investigate the effects of adding cocoa pulp nectar (0, 15 and 30% v/v) to stirred yoghurts on their physicochemical and sensory properties. The Total Soluble Solids (TSS) of cocoa pulp was increased from 5- 40°Brix with sucrose, processed at 80°C for 30 min, addition of 0.25% xanthan gum and further processed at 90°C for 3 min. Focus group guided product development in the selection of yoghurt with desirable level of cocoa pulp nectar. The addition of cocoa pulp nectar to yoghurt increased ($p<0.01$) TSS, lowered consistency ($p<0.05$) and Total Titratable Acidity (TTA) as % lactic acid ($p<0.01$). Yoghurt with 30% cocoa pulp nectar had 21°Brix, consistency 8.0 cm/30 sec, pH 4.38 and 0.17 g/100 g lactic acid. This yoghurt was liked very much to extremely for flavour and overall acceptability. On storage at 4°C for 4 weeks, the yoghurt became less viscous, more acidic, darker, less chromatic and more yellowish-green. A 225 g serving of yoghurt was fat-free, good source of protein, K and P and excellent Ca.

Key words: *Theobroma cacao* L., cocoa pulp nectar, stirred yoghurt, physicochemical, hedonic scores, nutritional analysis

INTRODUCTION

Cocoa is one of the export commodities that contribute to foreign exchange earnings in the agricultural sector of Trinidad and Tobago, West Indies (Maharaj *et al*., 2003). The production of cocoa in Trinidad and Tobago was 393.5 thousand kg, of which 296.9 thousand kg was for export and 26.6% in local sales (Central Bank of Trinidad and Tobago, 2005). There are potentially lucrative local markets for a wide range of value added products made from otherwise discarded primary cocoa processing by-products and lower grade fermented and dried cocoa beans (Sukha, 2003).

The cocoa fruit is grossly underutilized considering that the seeds which are the basis of commerce comprise about 10% of its weight (Lopez *et al*., 1984). Each dicotyledonous bean is enveloped in a sweet, white, mucilaginous pulp comprising approximately 40% of the seed fresh weight (Schwan and Wheals, 2003) depending upon cocoa variety, season and region as well as pod ripeness (Freire *et al*., 1999). The pulp surrounding the seed contains about 10-15% sugars, 1% pectin and 1.5% citric (Lopez *et al*., 1984). In the traditional curing of cocoa, the pulp has no other utility, but during the cocoa fermentation.

Yoghurt is produced worldwide by fermentation of milk with two species of lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus* (Oliveira *et al*., 2002), which are active in a symbiotic relationship (Vedamuthu *et al*., 1992). *S. thermophilus* produces some diacetyl, which gives yoghurt its creamy or buttery flavour, whereas *L. delbrueckii* sp. bulgaricus produces acetaldehyde, which helps to give yoghurt its characteristic sharp flavour. Recently, emphasis has been placed on developing new fermented milks containing microorganisms called probiotics (Micanel *et al*., 1997; Vinderola *et al*., 2000); of these, lactic acid bacteria are mainly employed in the dairy industry (Lee *et al*., 1999).

Therefore, the objectives of the study were to investigate the effects of adding varying levels of cocoa pulp nectar to stirred yoghurts on their physicochemical and sensory properties. Also, the changes in yoghurt on storage were analyzed at 4°C for 4 weeks.

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MATERIALS AND METHODS

Processing of cocoa pulp nectar: Cocoa pods (accession Trinidad Selective Hybrid, TSH 919) of weight 648.29±41.91 g, length 20.27±0.72 cm and diameter 29.91±0.58 cm were thoroughly washed in a 500 ppm of sodium hypochlorite solution, wiped and air-dried. Figure 1 shows the processing steps for cocoa nectar and stirred cocoa pulp nectar yoghurt. The pulp and seeds from each pod were separated and weighed. The pulp was extracted by blending with a shielded blade in an Oster blender (model No. 889 16R, New Hartford, Connecticut, USA). The pulp was sieved using a 2 mm sieve (USA Standard Testing Sieve A.S.T.M.E-11 Spec 2 mm 0787 No. 10), the mass (g) of pulp recorded. The % of total pulp obtained before sieving based on mass (w w^{-1}) of total seeds and pulp weight was 92.3. On sieving, there was a reduction in mass of 14.1% (w w^{-1}). The pulp was pasteurized at 90°C for 30 min (Kottermann Labortechnik W-Germany D-3/62 hot water bath) and cooled. For cocoa nectar, the TSS of cocoa pulp was increased from 5- 40°Brix by addition of granulated sucrose, heat processed at 80°C for 5 min, cooled to 20-22°C, followed by addition of 0.25% w v^{-1} xanthan gum (Tastemakers Ltd., Port-of-Spain, Trinidad, West Indies) with further processing at 90°C for 3 min. The TTA (% citric acid) and pH of cocoa pulp were 0.04 and 4.02% and for cocoa pulp nectar were 0.064 and 4.19%, respectively.

Yoghurt processing: To Nestle UHT Full Cream Milk, 100% fresh cow's milk (Trinidad and Tobago Ltd., Churchill-Roosevelt Highway, Valsayn, 6% of Kerrygold Instant Skimmed Milk Powder (packaged by Multifoods Corporation Ltd. Diamond Vale Industrial Estate, Diego Martin, Trinidad W.I. for The Irish Dairy Board) was added to the full cream milk and homogenized with 0.25% or 2.50 g of yoghurt culture (YC-180Yo-flex freeze-dried lactic culture, Christian Hansen, Inc., Milwaukee, Wisconsin, USA). The mixture was incubated for fermentation in a water bath at 40°C for 16 h to pH 4.19. The TSS of yoghurt was increased from 10-13°Brix by addition of 3% of granulated sucrose w v^{-1} (Brechin Castle Ltd., Couva Trinidad).

Focus group evaluation of yoghurts: The focus group method was used to get an early assessment of the prototypes (ASTM, 1979). This research method is significant in gaining insight into consumer's preferences and defining critical attributes of a product (Galvez and Resurreccion, 1992; Resurreccion, 1998). Two focus groups of 9 panellists evaluated the yoghurt treatments

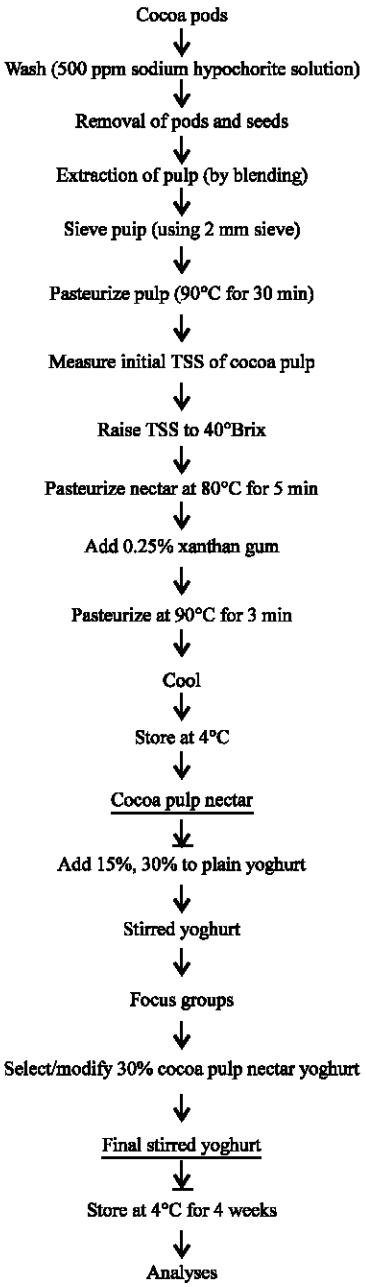

Fig. 1: Steps in the production of stirred cocoa pulp nectar yoghurts

(4 oz or ~120 mL) incorporating varying levels of cocoa nectar (0, 15 and 30% v v^{-1}). Panellists evaluated the sensory attributes of the yoghurts for appearance and colour, mouthfeel/consistency, flavour, acidity and overall acceptability. Panellists were asked for their comments for modified and more acceptable yoghurt.

Modified yoghurt: Based on consensus of the two focus groups, modified stirred yoghurt with 30% cocoa pulp

nectar v v^{-1} was produced. In this yoghurt, the TSS was reduced from 25-21°Brix and pH was increased from 4.20-4.38. This product was evaluated on physicochemical and microbiological quality on storage at 4°C for 4 weeks. The yoghurt was evaluated for sensory quality.

Hedonic testing: The 50 panellists (50% females; 50% males) who comprised of staff and students from the University of the West Indies, Trinidad, West Indies and Cocoa Research Unit rated the modified 30% cocoa pulp nectar yoghurt on appearance, consistency, aroma, flavour and overall acceptability based on the 9-point hedonic scale: 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely (Peryam and Pilgrim, 1957; Resurreccion, 1998). These panellists were (yrs) 16-35 (60%), 36-55 (34%) and over 55 (6%).Additionally, in the questionnaire, the panellists were asked to indicate whether the yoghurt had a cocoa pulp flavour (yes or no), whether they would purchase (would purchase and would not purchase) this yoghurt if available on the market, how often they consume yoghurt (regularly, sometimes and irregularly) and for their comments.

Physicochemical analyses: The pH of cocoa pulp, plain yoghurt and stirred cocoa pulp nectar yoghurt was measured tested on an Orion pH meter (model 520, Orion Research Inc., Boston, USA). The Total Soluble Solids (TSS) as °Brix was measured using a hand-held refractometer (Leica Model, Atago E Type Series, Leica Inc., Buffalo, N.Y.) Consistency was measured as the flow rate of cocoa nectar yoghurt on a Bostwick consistometer (Cenco Brand, Redman Scientific Company, San Francisco, USA). The sorrel yoghurt was poured into the holding compartment and levelled off. The gate was opened to release the trigger and the rate of flow as cm/30sec was recorded (Gould, 1978). Total Titratable Acidity (TTA) as % citric acid (cocoa pulp and cocoa pulp nectar) and lactic acid (cocoa pulp nectar yoghurt) was determined by titration as described by Gould (1978). The 10 mL samples were titrated with 0.1 N sodium hydroxide (NaOH) using 2 drops phenolphthalein indicator and the point of colour change to pink was recorded for each sample. The colour of cocoa pulp nectar and cocoa pulp nectar yoghurts was determined using the Minolta portable tristimulus colorimeter, (Model number: Cr-200b, manufactured by Minolta Co, Osaka Japan). The colorimeter was standardized using a white tile (Minolta Calibration plate CR-a43; L92.6 a 0.3137 b 0.3209) and tristimulus values of L a and b recorded. The respective implement was placed at the bottom of the glass beakers containing the samples and a reading was taken Chromaticity was measured in L, a, b coordinates where, L represents the value (lightness of the colours and is larger for lighter colour) a is negative for green and positive for red and bis negative for blue and positive for yellow. Chroma (C) and Hue angle (H°) were calculated from a and b (Francis, 1998; MacDougall, 2000). C_{ab} is a correlate visual for saturation. The H° of 0, 45, 90, 180 and 270 represented bluish red, orange, yellow, green and blue, respectively.

Microbiological analysis: The 30% cacao nectar yoghurt was evaluated for microbial changes every week on storage at 4°C for 4 weeks. Microbial analyses were performed according to Swanson *et al.* (1998). Microorganisms were enumerated on Potato Dextrose Agar (PDA, Oxoid Basingstoke, England), Tomato Juice Agar (TJA, Oxoid), Plate Count Agar (PCA, Oxoid), Eosin Methylene Blue Agar (EMBA, Oxoid), Mannitol Salt Agar (MSA, Oxoid) and Lee's Agar (LA., Laboratory made) for lactic acid bacteria-*Escherichia coli*, Stapylococci and *Streptococcus thermophilus*, yeasts and moulds and total mesophilic aerobic counts. PCA plates were incubated at 30°C for 24 h, TJA and PDA plates at 28°C for 24 h and LA, EMBA and MSA plates at 35-37°C for 48 h (Benson, 1985). LA plates were incubated in anaerobic jars. Plates with 30-300 colonies were counted and microbial counts expressed $\log_{10}$ cfu mL^{-1}.

Statistical analysis: Using Minitab 13 Released, 2000 (Minitab Inc., 3081 Enterprise Drive, State College Pa 16801-3008,USA), one-way Analysis of Variance investigated the effects adding cocoa pulp nectar (0, 15 and 30%) on physiochemical quality (TSS consistency, pH, titratable acidity and colour) of yoghurt samples and the effects of storage on physicochemical and microbiological quality of yoghurts. Significant means were separated by LSD at $p<0.05$ and correlations to determine contribution of sensory attributes to overall acceptability were done using Pearson correlations determined significant relationships between sensory overall acceptability to other sensory attributes.

Compositional analysis: Yoghurt with 30% cocoa pulp nectar was analysed for total solids, crude fat, crude protein, ash according to AOAC (1999). The samples were freeze dried (Martin Christ Alpha 1-4 Freeze dryer (Lyophilisateur, Christ Loc-1m Alpha 1-4 Bioblock Scientific BP111-67403/LLKIRCH Cedex Burkert, Germany) at -54°C and pressure at 0.070 mbar for 5 days to 2-3 g/100 g moisture. Corrective dry matter was determined by drying the samples at 105°C for 24 h to

determine corrective total solids. Fat was determined by ether extraction. The samples were refluxed for approximately 16 h by an automatic Soxhlet extraction apparatus, Soxtherm Automatic, Gerhardt. Crude protein was determined by the Kjeldahl distillation method. From ash samples, Ca, Mg and Fe were determined by Atomic Absorption Spectroscopy using a Unicam Spectrometer (Thermo Spectronic SP90 AA, Pye Unicam Ltd. Cambridge UK). K and Na were determined by Flame photometer (Cole- Parmer Instrument Company Illinois 60648 Model No. 2655-00 Digital Flame Analyzer). P was determined by UV Vis spectrometer (Cary 5G UV-Vis-NIR Spectrophotometer, Varian Australia).

RESULTS AND DISCUSSION

Physicochemical characteristics: The physicochemical characteristics of cocoa pulp nectar were: L 40.77- 41.10, chroma C 1.37-1.41, Hue° 84.57-87.56, TSS 40°Brix, consistency of 6.8-7.0 cm/30 sec, pH of 4.19 and TTA of 0.04-0.06% citric acid. Table 1 shows that adding cocoa pulp nectar to plain yoghurt resulted in significant changes in consistency, TSS, TTA, L and chroma On increasing the addition of cocoa pulp nectar, yoghurt was less viscous at 30% level ($p<0.05$), TSS increased ($p<0.01$) and % lactic acid decreased ($p<0.01$). Yoghurt with 15% cocoa nectar was most dark ($p<0.01$) and least chromatic.

Focus group response: The control yoghurt (0% cocoa pulp nectar; plain yoghurt) was the least liked in sensory attributes compared to 15 and 30% cocoa nectar yoghurts. Panellists indicated that the control yoghurt was too acidic, not sweet enough, undesirable in colour and not flavourful. The 15% cocoa pulp nectar yoghurt was slightly too acidic, had slight cocoa flavour, not sweet enough, unattractive in colour (L 71.4±0.02; C 9.0±0.09; H° 97.7±0.01, Table 1) too fluid (8.5 cm/30 sec, Table 1) but had an appealing unique aroma. Yoghurt with 30% cocoa pulp nectar was most preferred of all yoghurts. This yoghurt had a fruity flavour, unique aroma and good consistency (8.0 cm/30 sec, Table 1). However, it was slightly too acidic and too sweet (25.0° Brix; Table 1). Table 2 shows the physicochemical composition of 30% cocoa pulp nectar yoghurt which was analysed during storage.

Hedonic testing: Yoghurt with 30% cocoa pulp nectar was like moderately to very much for appearance (7.30±0.21), consistency (7.04±0.22) and aroma (7.04±0.21) and liked very much to extremely for flavour (8.70±0.18) and overall acceptability (8.80±0.18). Overall acceptability was positively correlated ($p<0.01$) to flavour ($r = 0.79$), consistency ($r = 0.65$), appearance ($r = 0.54$) and aroma ($r = 0.47$).

Generally, 44% of panellists consumed yoghurt regularly on a weekly or daily basis, 42% consume on a monthly basis and 14% irregularly. Most (76%) tasted the cocoa pulp flavour in yoghurts. cocoa flavour. Others (24%) felt either the cocoa flavour was not intense enough or they had never tasted cocoa pulp before. Most panellists (80%) would purchase this yoghurt if available on the market. Panellists commented favourably on the smooth consistency and unique flavour but indicated that colour could be enhanced by addition of artificial colouring.

Table 1: Effects of adding cocoa pulp nectar on physicochemical quality of yoghurt

	Cocoa pulp nectar (%)				
Quality attributes	0	15	30	LSD	p-value
Consistency (cm/30 sec)	8.50±0.0	8.50±0.0	8.00±0.0	0.10	*
TSS, Brix°	13.00±0.0	17.00±0.0	25.00±0.1	0.10	**
pH	4.20±0.0	4.24±0.0	4.20±0.0	0.05	n.s
TTA (% lactic acid)	0.19±0.1	0.17±0.1	0.14±0.1	0.02	**
L	74.30±0.4	71.40±0.2	73.40±0.6	1.42	**
C	9.70±0.04	9.00±0.09	10.10±0.1	0.22	**
H°	85.90±7.7	97.70±0.1	94.40±0.4	-	n.s

n.s-not significant; *significant at $p<0.05$; ** significant at $p<0.01$

Table 2: Physiochemical changes of 30% stirred cocoa pulp nectar yoghurt on storage

	Storage, weeks					
Properties	0	2	3	4	LSD	p
Consistency (cm/30 sec)	8.00±0.0	8.50±0.0	9.70±0.0	13.000±0.1	0.60	**
TSS, Brix°	21.00±0.1	20.00±0.1	20.00±0.1	20.000±0.1	1.50	n.s
pH	4.38±0.0	4.38±0.0	4.34±0.0	4.300±0.0	0.03	*
TTA, % lactic acid	0.17±0.1	0.18±0.1	0.20±0.1	0.200±0.1	0.15	*
L	76.50±0.30	72.03±0.23	72.03±0.23	69.230±0.25	2.52	**
C	11.00±0.20	9.51±0.20	9.51±0.20	9.170±0.12	2.46	**
H°	99.07±0.43	92.420±0.57	92.41±0.57	95.630±0.07	2.82	**

n.s-not significant; * significant at $p<0.05$; ** significant at $p<0.01$

Table 3: Nutritional composition of 30% stirred cocoa pulp nectar yoghurt

Components	g/100 g or mg g^{-1} dry wt	g/100g or mg g^{-1} fresh wt	Actual serving size 225 g	Serving size on rounding	DRV/RDI	Daily value (%)
Total Fat	0.11	0.02	0.04	0	65 g	0
Protein	15.266	2.35	5.29	5	50 g	10
Calcium	466.64	93.33	209.99	210	1000 mg	21
Magnesium	56.94	11.39	25.62	26	400 mg	7
Potassium	882.06	176.41	396.93	397	3500 mg	11
Iron	1.20	0.24	0.54	1	18 mg	6
Sodium	253.93	50.79	114.27	114	2400 mg	5
Phosphorus	409.98	81.99	184.49	184	1000 mg	18

Source of RDI and DRV values: Nielsen (1998) Food Analysis

Effects of storage on physicochemical quality: Table 2 shows there were significant changes in some physicochemical parameters (consistency, pH, TTA, L C and H°) by week 2 of storage. On storage, cocoa pulp nectar yoghurt became less viscous, more acidic, darker, less chromatic and more yellowish-green. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus* are responsible for the typical yoghurt flavour and texture. Lactic acid bacteria ferment the lactose into lactic acid and flavour compounds such as acetaldehyde, carbon dioxide and diacetyl (Marshall, 1986). The yoghurt mixture coagulates during fermentation due to the drop in pH. The *streptococci* are responsible for the initial pH drop of the yogurt mix to approximately 5.0. The *lactobacilli* are responsible for a further decrease to pH 4.0 (Goff, 2005).

Effects of storage on microbiological quality: There was no presence of coliforms and staphylococci and no significant changes ($p>0.05$) in total aerobic microorganisms (4.74-6.78 $\log_{10}$ cfu mL^{-1}) during storage of yoghurt. By week 2 of storage, the number of lactobacilli was $\log_{10}$ 6.35±0.30 cfu mL^{-1} on Lee's agar and with further changes onwards. An increase in lactic acid may be used as an indirect measure of yoghurt bacterial growth (Vedamuthu *et al*., 1992). The number of lactobacilli on TJA did not vary significantly during storage from $\log_{10}$ 4.39±0.0 cfu mL^{-1} on week 4 of storage to $\log_{10}$ 5.24±0.4 cfu mL^{-1}on week 2. There was a significant ($p<0.01$) increase in yeasts and moulds on acidified PDA agar by week 2 of storage from no growth on week 0 to $\log_{10}$ 3.89±0.33 cfu mL^{-1}. This number of yeasts and moulds did not vary ($p>0.05$) further on storage.

Nutritional composition: Table 3 shows the nutritional composition of 30% cocoa nectar stirred yoghurt. This yoghurt could be classified as fat free because its content was less than 0.50 g according to the Food and Drug Administration (NLA, 1997). The yoghurt was low in Na (below 140 mg per reference amount). A serving of yoghurt was good in protein, K and P and excellent in calcium (Nielsen, 1998). Good source contains 10% or more of daily value, high or rich in or excellent source contains more than 20% or more of the Daily Value (NLA, 1997).

CONCLUSION

The utilization of the discarded and underutilized cocoa pulp as nectars in stirred yoghurts could provide an option for the diversification the cocoa industry. The microbiological quality indicated it was safe for consumption throughout the 4 week storage period at 4°C. There were significant changes ($p<0.01$) in some physicochemical quality parameters by week 2 of storage. Overall acceptance indicated that the 30% cocoa pulp nectar yoghurt was liked very much to extremely. A serving of 225 g yoghurt was fat free and good in protein, K and P and high (350-665 mg) in Ca. Most panellists identified the cocoa pulp flavour (76%) in yoghurts and would purchase the product if available on the market (80%).

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