



## Research article

# Development of yoghurt incorporated with beetroot puree and its effect on the physicochemical properties and consumer acceptance

Mercia Lionel Adjei<sup>a,\*\*</sup>, Abena Boakye<sup>a,\*</sup>, Godwin Deku<sup>b</sup>, Nana Baah Pepra-Ameyaw<sup>a,d</sup>, Antoinette Simpah Anim Jnr<sup>c</sup>, Ibok Nsa Oduro<sup>a</sup>, William Otoo Ellis<sup>a</sup>

<sup>a</sup> Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

<sup>b</sup> Dairy/Beef Cattle Research Station, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

<sup>c</sup> Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

<sup>d</sup> Schmid College of Science and Technology, Chapman University, California, USA

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## ABSTRACT

**Background:** Yoghurt is one of the most popularly consumed fermented products which provides several nutritional benefits. Yoghurt products often include flavour and colour additives however, growing awareness of the risks associated with synthetic food additives has necessitated the need to explore more natural colour and flavour as food additives.

**Methods:** This study evaluated the effect of beetroot puree as flavouring and colourant in yoghurt production and quality. To develop the yoghurt product, incubation time and proportion of beetroot puree were optimized based on the resulting pH, titratable acidity, colour, and viscosity using response surface methods.

**Results:** Optimum yoghurt formulations were obtained in products containing 2 %, 2.03 % and 8 % beetroot puree following an incubation of 2.5h. Increasing beetroot puree did not affect the pH and titratable acidity of the yoghurt samples but slightly influenced the viscosity of the yoghurt. The colour of yoghurt was mainly affected by the puree concentration. A consumer acceptance test was conducted on the optimized products compared to a control sample without beetroot. Yoghurt incorporated with 8 % beetroot puree was the least preferred with a mean score of 6.08, whereas yoghurt incorporated with 2.03 % beetroot puree was the most preferred (7.42), with a higher acceptance than the control (7.28).

**Conclusion:** /Implications for industry: Findings from the study provide evidence for exploration of beetroot yoghurt as a natural product alternative to the use of synthetic flavour and colour additives in yoghurt.

## 1. Introduction

Yoghurt has become a major dairy product worldwide due to its savoury taste and nutritional benefits [1]. Production of yoghurt is based on the inoculation of live and active bacterial culture with different types of milk often with the addition of fruit flavours [2]. The colour and flavour of yoghurt products are essential to consumer appeal and are thus important factors in production [3,4]. Not

\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [meradak64@gmail.com](mailto:meradak64@gmail.com) (M.L. Adjei), [abenaboakye13@gmail.com](mailto:abenaboakye13@gmail.com) (A. Boakye).

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surprisingly, several synthetic additives have been explored to enhance the colour and flavour of yoghurt products [4,5].

Use of synthetic food colours is a common practice in the food industry largely because they are readily available, affordable, and easy to use in restoring novel food shades [6]. They are generally water-soluble chemical substances that can be used in food without further processing and are often azo dyes that are not found in nature [3]. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset Yellow FCF are examples of commonly used synthetic colours in food processing [7,8]. However, the safety of synthetic food colours has been a controversial query. Some authors have associated its use with incidents of liver, kidney and testes malfunction [9,10]. Following the increasing awareness of potential risks associated with synthetic additives, consumer interest in natural colours and their use have substantially increased [11]. This has necessitated the need to explore natural colours in the food industry, especially in the commercial yoghurt industry given its prominent role as a healthy dietary option.

Beetroot is well-known as an ancient vegetable high in carotenoids, flavonoids, nitrates, vitamins and minerals and its most copious red colourant, termed betanin/beetroot red [12,13]. The European Union has approved the use of betalains as food colourant and branded as E162 [14]. Several researchers have explored the use of beetroot juice and powder in yoghurt production, however few has experimented on the use of beetroot puree in yoghurt production [15–17]. The addition of beetroot syrup in yogurts, in addition to being an alternative to using artificial colouring in the industry has been reported to provide health benefits to consumers by forming bioactive compounds in a recent similar study [18].

In this study, we examine the utilization of beetroot puree as a natural color and flavor additive in yogurt production. Our primary objective is to assess the impact of incorporating beetroot puree on the physicochemical properties of the yogurt, including pH, titratable acidity, viscosity, and color. Additionally, we aim to determine consumer acceptance of the developed yogurt."

## 2. Materials and methods

### 2.1. Source of materials

Materials used in yoghurt preparation included spray-dried whole milk powder (TMC DAIRIES (N.I) Ltd, Northern Ireland, UK), synthetic flavour (Arome 3 Lions Essence, Senegal), starter culture (YC-381, Thermophilic Yoghurt Culture- YoFlex, CHR HANSEN, Denmark). Beetroot was obtained from the Central Market in Kumasi, Ghana.

### 2.2. Preparation of beetroot puree

Beetroot puree was prepared according to the method employed by Guldiken et al. [19]. The beetroot samples were washed with clean water, and the black spots were removed. The samples were then peeled and cut into smaller sizes averaging approximately 28 g. The cut beetroots pieces were wrapped with aluminium foil in batches of 112 g and baked in a preheated oven (Sharp electric oven, EO-42K-3, Japan) for 45 min at 200 C. The baked samples were allowed to cool at a room temperature after which they were added to 6 ml of water and pureed in a commercial blender (Kimatsu mixer grinder, Model – Spectra 750 W) for 3 min at 1-min intervals.

### 2.3. Experimental design

Preliminary tests were conducted to define the limits for puree substitution and incubation/fermentation time. For puree substitution, the lower and upper limits were set at 2 % and 8 %, and for fermentation periods; 2.5 h and 3 h. A response surface design (using Statsgraphics centurion software, version 21) was then used to generate the formulations based on the defined limits.

Ten different formulations were generated using the response surface design based on the incubation period and beetroot puree concentration and duplicated to get 20 formulations. In all, 20 formulations were used in the optimization. The formulations were then optimized based on their physicochemical properties including pH, titratable acidity, viscosity and colour. The top three formulations exhibiting optimal physicochemical properties with high composite desirability were yoghurt substituted with 2, 2.03 and 8 % beetroot and as such chosen for analysis.

Validation of results generated from the response surface design was done by conducting laboratory tests on the physicochemical properties (pH, titratable acidity, viscosity and colour) of the optimized formulations to verify if they correspond to the results obtained from the response surface design.

### 2.4. Yoghurt production

Yoghurt was prepared in accordance with the method of Yadav et al. [17]. The milk was pasteurized at a temperature of  $85 \pm 0.1$  °C and cooled to  $44 \pm 0.1$  °C. The pasteurized milk was inoculated with 3 % of the starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Two sets of the mixture were incubated at  $43 \pm 1$  °C, one at 2.5 h and the other at 3 h respectively. The obtained yoghurt was stirred, and sugar and beetroot puree were added per the generated formulations. The yoghurt was then chilled at a refrigeration temperature of  $4 \pm 0.1$  °C. However, for the control sample, commercial synthetic colourant and flavouring were used instead of beetroot puree.

### 2.5. Analysis of yoghurt quality

pH: The pH was measured according to the procedure described by Kadam et al.[20]. Approximately 20 ml of yoghurt sample was

poured into a beaker and the pH determined using a digital pH-meter (HANNA-pH 210, Germany).

**Titratable acidity:** Titratable acidity was determined using the method described by Nejad et al. [21]. Exactly 1 ml of yoghurt sample was mixed with 9 ml of distilled water. Three drops of phenolphthalein were added to the mixture and titrated against 0.1 M NaOH. Titration end point was indicated by the appearance of a pink colour and titratable acids (lactic acid percentage equivalent) was calculated from the titre values.

$$\text{Titratable acidity} = \frac{(10 \times \text{volume of NaOH} \times 0.009 \times 0.1)}{W} \times 100\%$$

Where, 10 = Dilution factor; W = weight of sample for titration; V NaOH = Volume of NaOH used to neutralize the lactic acid; 0.1 = Normality of NaOH; 0.009 = equivalent of lactic acid normality.

**Viscosity:** Viscosity was determined following the method described by Fetahagić et al. [22]. Yoghurt viscosity was measured using a viscometer (Visco Basic Plus, Wagtech International). Approximately 100 ml of yoghurt sample was measured into a 100 ml beaker and viscosity determined at  $18 \pm 0.1$  °C, using spindle number 3 of the viscometer at 20 rpm.

**Colour:** The colour of the yoghurt samples was measured using a CR-400 Chroma Meter (Konica Minolta Chroma Co., Japan) according to the method used by Ghasempour et al. [23]. The yoghurt samples were placed in a Petri dish (width 5 cm and height 2 cm) and their International Commission on Illumination (CIE)  $L^*a^*b^*$  parameters were measured.  $L^*$  indicates the degree of lightness with a range between black (0) to white (100),  $a^*$  indicates the degree of redness (positive values) or greenness (negative values), and  $b^*$  represents the degree of yellowness (positive values) or blueness (negative values). All tests were carried out in triplicates.

## 2.6. Consumer acceptance of yoghurt samples

A consumer assessment of the organoleptic properties of the samples was conducted using a panel of 120 including university students and staff, familiar with yoghurt. The overall acceptability of the product was assessed on a nine-point hedonic scale as described by Chatterjee et al. [24] ranging from 1 = dislike extremely and 9 = like extremely. Following the method described by Gacula et al. [25], the just-about-right (JAR) scale was used to evaluate colour, taste, flavour, aroma, thickness and sugariness. Check-all-that-apply (CATA) test was also conducted on the yoghurt samples. Attributes assessed included pleasant taste, appealing pink colour, smooth, creamy, pleasant fermented milk smell, pleasant aroma, strawberry colour, not smooth, not creamy, unpleasant fermented milk smell, unpleasant taste and rooty smell. Cups containing the yoghurt samples were coded with three-digit random numbers and the samples were served to the panellists in a randomized order. Purified drinking water and cucumber were used as palate cleansers in between the assessment of samples. All other standard protocols for conducting sensory evaluation were observed.

## 2.7. Ethical consideration

All panellists signed an informed consent form and participation was voluntary. The study was certified by the Committee on Human Research, Publication and Ethics (CHRPE/AP/412/20). In accordance with CHRPE regulations, the data was anonymised for confidentiality.

## 2.8. Statistical analysis

Five (5) yoghurt formulations were generated out of which three (3) were selected as optimal formulations based on their composite desirability (Table 1). These optimal formulations included three yoghurt samples substituted with 2, 2.03 and 8 % beetroot puree.

Analysis of the data collected was performed using STATGRAPHICS centurion XV Software (version 15.2.11) for the instrumental tests and SPSS, and Xlstat Statistical Software (version 2014) for the consumer sensory data. Statistical significance was determined using analysis of variance (ANOVA) tested at  $p = 0.05$ .

**Table 1**  
Yoghurt formulations generated using response surface for product optimization.

Solution	Beetroot puree conc <sup>a</sup>	Incubation period	Titratable acidity	pH	Composite desirability
1	2.00	2.5	0.634636	4.4	0.913819
2	2.03	2.5	0.634697	4.4	0.913661
3	8.00	2.5	0.636068	4.4	0.910081
4	2.00	3.0	0.790705	4.2	0.000000
5	8.00	3.0	0.807136	4.2	0.000000

<sup>a</sup> Concentration.

### 3. Results and discussion

#### 3.1. Optimization of beetroot yoghurt formulations

Results from Fig. 1 showed that as the incubation time increased, the pH value for the yoghurt decreased. The highest incubation time of 3 h recorded the lowest pH value of 4.2, while the lower incubation time of 2 h 30 min recorded the highest pH value of 4.4 (Fig. 1). The decrease in pH during incubation is ascribed to the production of more lactic acid with time [26]. On the other hand, the puree concentration did not have an influence on the pH of the yoghurt.

The titratable acidity of the yogurt samples was significantly affected by the duration of incubation, with a longer incubation time resulting in an increase in the product's titratable acidity. Puree concentration did not affect the titratable acidity of the yoghurt samples (Fig. 2). Titratable acidity measures the total organic acid present in yoghurt and is an important parameter to be measured because the key mechanism during the fermentation period in yoghurt production is to produce acids [27]. Titratable acidity values of 0.62–0.80 % obtained in this study were also similar to findings by Okoye and Animalu, who reported values in the range of 0.6–0.86 % for yoghurt incorporated with sweet potato [28].

Viscosity of yoghurt describes the thickness of yoghurt. Consumers prefer yoghurt with higher viscosity because they have a nicer mouthfeel than yoghurt with low viscosity [29]. The viscosity of yoghurt is affected by several factors, and key among these is the incubation time. Incubation time influences the growth optimization of the starter culture, lactic acid bacteria (LAB) and its ability to produce yoghurt gel [30]. Moisture content of yoghurt thus decreases as a result of the increase in total solid caused by LAB cell proliferation. The results of the present study confirmed the above as the viscosity of the yoghurt samples increased with increasing incubation time and puree concentration (Fig. 3).

The yoghurt colour was generally affected by the concentration of the beetroot puree. As the concentration of the puree incorporated in the yoghurt increased, the lightness value ( $L^*$ ) of the yoghurt reduced as the yoghurt turned from white to pink (Fig. 4). This was due to the reddish colour of beetroot, which influenced the colour of the yoghurt. Incubation time did not affect the colour of the yoghurt. Januário et al. conducted a study using 10 % beetroot juice to flavour yoghurt which gave the yoghurt a pink colour,  $L^*$  value of 50 [31]. This value was lower than a range of 58.49–72.76 recorded in this study. This could be due to the higher concentration (10 %) of beetroot juice used as compared to the highest concentration of 8 % used in this present study. Shalaby and Hassenin[32], also reported an  $L^*$  value of 59.48 for yoghurt incorporated with 2 % beetroot powder which was higher than 58.49 recorded for yoghurt incorporated with 2 % beetroot puree in this study. Higher value for  $L^*$  indicates a lighter pink colour which implies that yoghurt incorporated with 2 % beetroot powder had a lighter colour as compared to yoghurt incorporated with 2 % beetroot puree in this study. This could be due to the moisture content in the puree as compared to the powder which contains no moisture.

#### 3.2. Physicochemical evaluation of the optimized samples and control

Physicochemical (pH, titratable acidity, viscosity and colour) and sensory analysis were conducted on the three samples and a control sample. The samples were labelled as T1, T2 and T3 while the control was labelled as T0. Results indicated no significant differences in the pH of all samples except for T0 and T3 ( $p < 0.05$ ). This shows that, even though the puree did not strongly affect the pH of the yoghurt, higher concentrations affected the pH hence increasing the pH value. Also, no significant differences ( $p > 0.05$ ) were recorded in the titratable acidity in all samples. Titratable acidity values recorded in the study for all the yoghurt samples met the recommended minimum value of 0.6 % set by the Codex Alimentarius for yoghurt and related products and the Polish Standard PN-A-86061:2002 (min. 0.6 % lactic acid) [33] (Table 2). These are the standards ascribed to in Ghana and many developing countries. Results reported in Table 2 corresponds to results obtained from the response surface design (Table 1). This shows the validity of the optimized values.

Puree concentration slightly influenced the viscosity of yoghurt. T1 and T2 were similar but significantly different ( $p > 0.05$ ) from the control (T0) and T3. T0 recorded the lowest viscosity of 0.5987, while T3 recorded the highest viscosity of 1.0063 (Table 2).

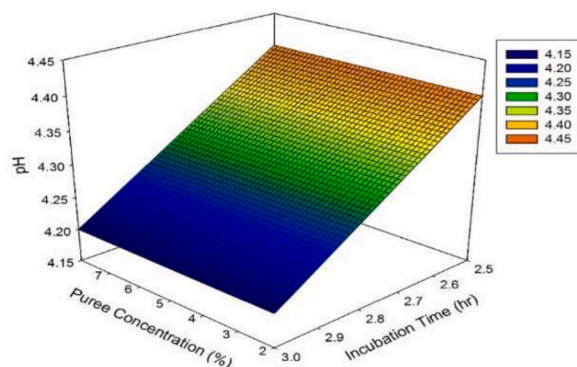


Fig. 1. Effect of incubation period and puree concentration on pH of yoghurt.

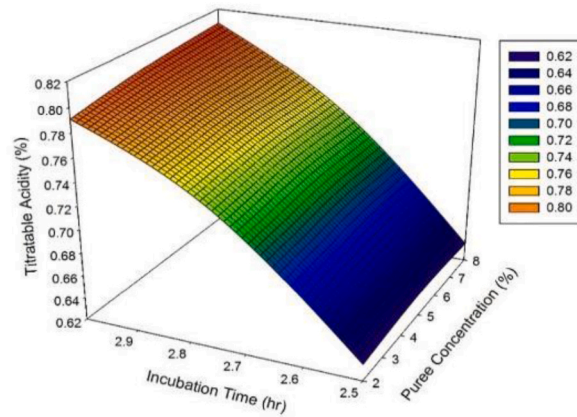


Fig. 2. Effect of incubation period and puree concentration on titratable acidity of yoghurt.

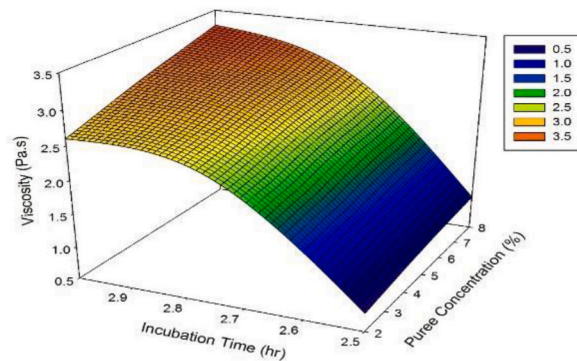


Fig. 3. Effect of incubation period and puree concentration on viscosity of yoghurt.

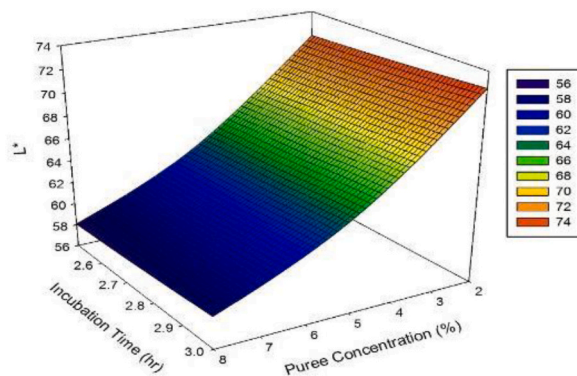


Fig. 4. Effect of incubation period and puree concentration on colour of yoghurt. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Viscosity values recorded were lower than those reported by Shalaby and Hassenin [32], for stirred yoghurt incorporated with red beet powder (1 % and 2 % concentration) which ranged between 3.2 and 3.7 Pa s at a temperature of 6 °C. Lower viscosity values recorded in this study could be due to the temperature ( $18 \pm 0.1$  °C) at which the viscosity was measured as compared to the temperature at which it was measured by Shalaby and Hassenin [32].

The difference in the colour of the yoghurt samples (T1, T2 and T3) was related to the different concentrations of the beetroot puree. The L\* values for the yoghurt samples were significantly different ( $p < 0.05$ ) and ranged between 55.40 and 75.09. As expected the higher L\* value was recorded for the control sample indicating that its whiteness and brightness is higher than the other samples. It was observed that yoghurt samples were pink in colour and T3 recorded the highest a\* value which is due to the higher concentration

**Table 2**  
Physicochemical properties of yoghurt samples with or without beetroot puree.

Samples	Parameters		
	pH	Titrateable acidity (%)	Viscosity (mPa.s)
T0	4.420 ± 0.000 <sup>a</sup>	0.63 ± 0.006 <sup>a</sup>	0.5987 ± 0.002 <sup>a</sup>
T1	4.423 ± 0.006 <sup>ab</sup>	0.64 ± 0.012 <sup>a</sup>	0.7877 ± 0.002 <sup>b</sup>
T2	4.427 ± 0.006 <sup>ab</sup>	0.64 ± 0.017 <sup>a</sup>	0.8170 ± 0.004 <sup>b</sup>
T3	4.433 ± 0.006 <sup>b</sup>	0.64 ± 0.015 <sup>a</sup>	1.0063 ± 0.024 <sup>c</sup>

Values are presented as Mean ± Standard Deviation. <sup>a-b</sup> values with same superscripts in the same column indicates no significant difference at  $p = 0.05$ .

T0 (control), T1 (2 % beetroot puree), T2 (2.03 % beetroot puree) and T3 (8 % beetroot puree).

(8 %) of the beetroot puree. The colour for the control sample and T2 were similar, which makes its concentration (2.03 %) the best substitute for the synthetic colourant in terms of colour. Positive values recorded for  $b^*$  indicates the presence of a yellow colour (Table 3). Beetroot is a good source of betalains (red and yellow pigments) which explains the positive  $b^*$  values obtained in the present study. However, the beetroot flavoured yoghurt samples recorded lower  $b^*$  values than the control. The data is not unusual as the synthetic colour used to prepare the control sample is reported to have higher concentration of tartrazine which is yellow colour [34].

### 3.3. Consumer acceptability test

#### 3.3.1. Overall acceptability of yoghurt samples

Fig. 5 shows the mean values for the overall acceptability of the samples [T0 (control), T1 (2 % beetroot puree), T2 (2.03 % beetroot puree) and T3 (8 % beetroot puree)] evaluated by the sensory panel. The mean values for T0, T1 and T2 were 7.28, 6.95 and 7.42, respectively, which represents *like moderately* on the hedonic scale. There was no significant differences ( $p > 0.05$ ) in the mean values however, there were differences in their numerical values with T2 (2.03 % beetroot puree) being the most preferred, followed by T0, the control sample, and T1 (2 % beetroot puree). T3 with a mean value of 6.08 was significantly different ( $p < 0.05$ ) from the three samples and was the least preferred. This indicates that a higher concentration of the beetroot puree negatively affected the overall acceptability of the product. This notwithstanding, the obtained mean acceptance of 6.08 (like slightly) was still appreciable for a consumer assessment of likeness [35] and shows potential for uptake especially if the yoghurt is to be promoted based on its health potential (Fig. 5).

#### 3.3.2. Attribute intensity profiles (just-about-right) of yoghurt samples

An evaluation of the intensity scores on key quality attributes of yogurt showed significant effect of favour, colour, consistency and sugar sweetness on consumers' overall acceptance of yoghurt. For the control sample (T0), mean drops for colour (high 1.42, low 0.37), flavour (high 1.42, low 0.10) and sugar sweetness (high 1.07, low 0.01) (Table 4) indicated that consumers strongly did not like the product when they considered the levels of these attributes to be low ( $p \leq 0.05$ ). Low colour, flavour and sugar sweetness of the control sample caused a significant drop in the overall liking of the product whereas higher levels did not affect the overall liking of the product. For T1 (2 % beetroot puree), there was a significant drop ( $p \leq 0.05$ ) in the overall liking for all the attributes when the levels were rated low which explains the lower mean liking scores recorded (Table 5). There was no significant mean drop ( $p \geq 0.05$ ) for colour, flavour, consistency and sugar sweetness when it was rated to be high hence no significant effect on the overall liking of the product. For high levels of flavour and sugar sweetness, the mean drops were negative because even though few panel members (25.21 % and 17.65 %, respectively) rated it to be high, there was higher acceptance (mean liking score of 7.77 and 7.24 for flavour and colour, respectively). For sample T2 (2.03 % beetroot puree) it was observed that, when colour, flavour, consistency and sugar sweetness were rated to be high, their mean liking score of 8.10, 7.93, 7.72 and 7.72 respectively was higher than when it was rated to be just-about-right (JAR) (mean liking score of 7.46, 7.41, 7.48 and 7.47) which made their mean drops negative (Table 6). For T3 (8 % beetroot puree), majority (60.50 %) of the panel members rated the colour to be high which gave lower acceptance (mean liking

**Table 3**  
Colour coordinates ( $L^*$ ,  $a^*$  and  $b^*$ ) of yoghurt samples with or without beetroot puree.

Samples	Parameters		
	$L^*$	$a^*$	$b^*$
T0	75.09 ± 0.030 <sup>a</sup>	22.13 ± 0.065 <sup>a</sup>	8.46 ± 0.031 <sup>a</sup>
T1	69.85 ± 0.057 <sup>b</sup>	21.58 ± 0.041 <sup>b</sup>	3.06 ± 0.012 <sup>b</sup>
T2	68.97 ± 0.035 <sup>c</sup>	21.96 ± 0.150 <sup>a</sup>	3.10 ± 0.010 <sup>b</sup>
T3	55.40 ± 0.051 <sup>d</sup>	26.91 ± 0.209 <sup>c</sup>	3.28 ± 0.035 <sup>c</sup>

Values are presented as Mean ± Standard Deviation. <sup>a-b</sup> values with same superscripts in the same column indicates no significant difference at  $p = 0.05$ .

T0 (control), T1 (2 % beetroot puree), T2 (2.03 % beetroot puree) and T3 (8 % beetroot puree).

$L^*$  ranging from 0 (black) to 100 (white),  $a^*$  ranging from red (+ $a^*$ ) to green (- $a^*$ ),  $b^*$  ranging from yellow (+ $b^*$ ) to blue (- $b^*$ ).

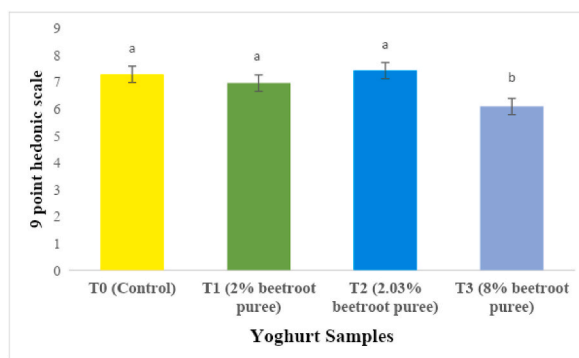


Fig. 5. Overall consumer acceptability of the control sample and yoghurt incorporated with beetroot puree.

Table 4

Intensity of product attributes on overall acceptability of T0 (control sample).

Attributes	Level	Frequency %	Liking scores	Mean drops	p-value
Colour	Low	31.93 %	6.39	1.42	<0.0001
	JAR	45.38 %	7.81		
	High	22.69 %	7.44	0.37	0.3084
Flavour	Low	21.85 %	6.19	1.42	<0.0001
	JAR	50.42 %	7.62		
	High	27.73 %	7.52	0.10	0.9275
Consistency	Low	19.33 %	6.30	1.15	
	JAR	53.78 %	7.45		
	High	26.89 %	7.63	-0.17	0.5441
Sugar sweetness	Low	26.89 %	6.50	1.07	0.0028
	JAR	50.42 %	7.57		
	High	22.69 %	7.56	0.01	0.9991

Table 5

Intensity of product attributes on overall acceptability of T1 (2 % beetroot puree).

Variable	Level	Frequency %	Liking scores	Mean drops	p-value
Colour	Low	29.41 %	6.03	1.34	<0.0001
	JAR	50.42 %	7.37		
	High	20.17 %	7.25	0.12	0.9246
Flavour	Low	23.53 %	5.50	1.71	<0.0001
	JAR	51.26 %	7.21		
	High	25.21 %	7.77	-0.55	0.0663
Consistency	Low	17.65 %	5.14	2.28	
	JAR	51.26 %	7.43		
	High	31.09 %	7.20	0.24	0.3134
Sugar sweetness	Low	23.53 %	6.25	0.89	0.0098
	JAR	58.82 %	7.14		
	High	17.65 %	7.24	-0.10	

score of 6.01) (Table 7). There was a significant mean drop for the overall liking of sugar sweetness and flavour when it was rated to be high ( $p < 0.05$ ). For colour and consistency, even though the overall liking decreased because it was rated to be high, the drop was not significant ( $p > 0.05$ ). To develop this product, levels for all the attributes must be taken into consideration as high levels of colour, flavour, consistency and sugar sweetness of the product largely affected the overall acceptance of the product.

### 3.3.3. Check-all-that-apply evaluation of the attributes that influenced consumer liking scores of samples

An evaluation of the relationship between the sample attributes of T0 (Control), T1 (2 % beetroot puree), T2 (2.03 % beetroot puree) and T3 (8 % beetroot puree) and their mean liking scores showed that, positive attributes that influenced the liking score were pleasant aroma, strawberry colour, appealing pink colour and pleasant fermented smell. Even though smooth and creamy were positive attributes, they were far away from the overall liking which implies that they did not influence the overall liking of the yoghurt samples. The negative attributes from the chart were, 'not smooth, not creamy, rooty smell, unpleasant taste, unpleasant fermented smell and unappealing pink colour'. Fig. 6 presents the relationship between the CATA (Check-All-That-Apply) descriptors and overall liking (hedonic) scores for each product.

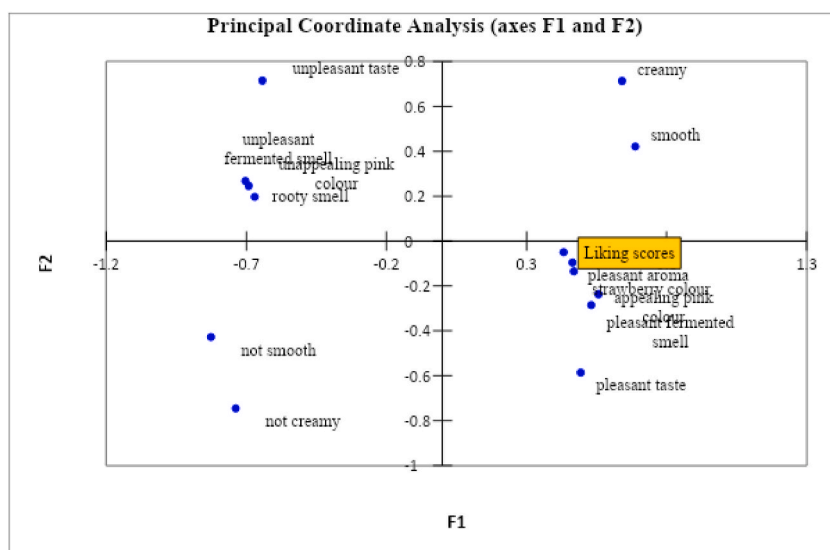
The quality of the analysis is good as the PCA accounted for 99.17 % of the correlation.

**Table 6**  
Intensity of product attributes on overall acceptability of T2 (2.03 % beetroot puree).

Variable	Level	Frequency %	Liking scores	Mean drops	p-value
Colour	Low	26.05 %	6.87	0.59	0.0107
	JAR	56.30 %	7.46		
	High	17.65 %	8.10	-0.63	
Flavour	Low	23.53 %	6.89	0.52	0.0652
	JAR	51.26 %	7.41		
	High	25.21 %	7.93	-0.52	
Consistency	Low	19.33 %	6.78	0.71	0.0348
	JAR	50.42 %	7.48		
	High	30.25 %	7.72	-0.24	
Sugar sweetness	Low	23.53 %	7.11	0.36	0.1306
	JAR	61.34 %	7.47		
	High	15.13 %	7.72	-0.26	

**Table 7**  
Intensity of product attributes on overall acceptability of T3 (8 % beetroot puree).

Variable	Level	Frequency %	Mean (Liking scores)	Mean drops	p-value
Colour	Low	13.45 %	5.56	0.9536	0.2356
	JAR	26.05 %	6.52		
	High	60.50 %	6.01	0.5022	
Flavour	Low	16.81 %	5.40	1.2667	0.0144
	JAR	45.38 %	6.67		
	High	37.82 %	5.69	0.9778	
Consistency	Low	17.65 %	6.29	-0.0130	0.2921
	JAR	36.97 %	6.27		
	High	45.38 %	5.85	0.4209	
Sugar sweetness	Low	29.41 %	5.17	1.6321	<0.0001
	JAR	47.06 %	6.80		
	High	23.53 %	5.7857	1.0179	



**Fig. 6.** Impact of attributes on the liking of the product.

From Fig. 7, T0 (control sample), T1 (2 % beetroot puree) and T2 (2.03 % beetroot puree) were closely related and associated to the positive attributes (creamy, smooth, pleasant taste, pleasant fermented smell, pleasant aroma, strawberry colour and appealing pink colour). However, these attributes were more closely related to T1 than T0 and T2 which implies why it was most the most preferred sample (Fig. 5). T3 (8 % beetroot puree) was far away from T0, T1 and T2 because the consumer panel perceived the unpleasant taste which affected its overall liking. This indicates that higher concentration of the beetroot puree affected the taste of the product.

It can be deduced that, when the product is not smooth, not creamy, has an unpleasant fermented smell and taste as well as an unappealing pink colour, it will not be liked by consumers as these attributes were not associated with the ideal product.



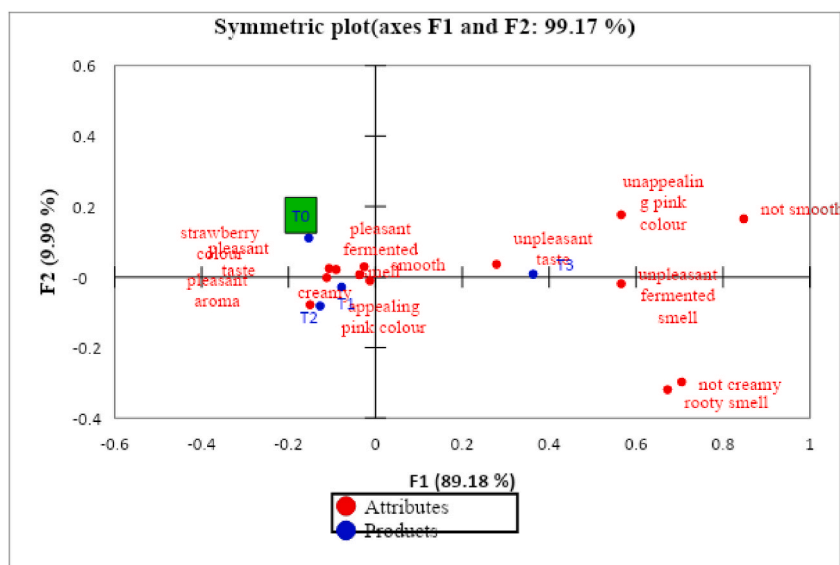


Fig. 7. Recommended sensory attributes per the identified attributes of the ideal product.

Fig. 7 presents the relationship between the sample attributes of T1 (2 % beetroot puree), T2 (2.03 % beetroot puree) and T3 (8 % beetroot puree) with that of the control (T0).

#### 4. Conclusion

In conclusion, our study successfully demonstrated the development of yogurt enriched with beetroot puree as a viable alternative to synthetic colouring agents. The manipulation of incubation periods revealed a direct influence on the yogurt's physicochemical properties, including a reduction in pH and an increase in titratable acidity with prolonged incubation. Moreover, viscosity exhibited a positive correlation with the extension of the incubation period.

The addition of beetroot puree did not significantly impact the pH and titratable acidity of the yogurt samples, but it did exert a slight influence on viscosity. Notably, the concentration of puree substituted was a critical factor affecting the overall colour of the yogurt. The top-performing samples, namely T1 (2 % beetroot puree), T2 (2.03 % beetroot puree), and T3 (8 % beetroot puree), were identified as optimal based on our comprehensive analysis.

The most preferred yoghurt sample was T2 (2.03 % beetroot puree), followed by the control sample (T0), and then T1 (2 % beetroot puree). Panel members predominantly rated the colour and flavour of T1 and T2 as 'just-about-right,' indicating a preference for lower concentrations (2 % and 2.03 %) of beetroot.

The results obtained from physicochemical tests aligned with those derived from the response surface design, validating the reliability of the optimized values. Notably, yogurt incorporated with 2.03 % beetroot puree emerged as the most preferred among panellists, with over 50 % assessing its sugar sweetness, colour, flavour, and consistency as 'Just-about-right.'

Although T3 (8 % beetroot puree) was the least preferred in this study, its mean overall acceptance rating of 6.08 (representing like slightly), underscores its potential for consumer acceptance, particularly when promoted for its potential nutritional benefits. This study not only presents a promising natural alternative for yogurt colouring but also highlights the nuanced interplay between ingredient proportions and sensory preferences, laying a foundation for future investigations and product development.

Future studies should consider a thorough comparative analysis between beetroot puree and synthetic additives, incorporating factors like stability, shelf life, and regulatory compliance to provide a holistic assessment of their suitability in food applications. Also, subsequent research should assess the economic feasibility of using beetroot puree in yogurt production, conducting cost-benefit analyses to inform industry stakeholders.

#### Declarations

**Ethics Statement:** This study was reviewed and approved by the Committee on Human Research, Publication and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology with the approval number: CHRPE/AP/412/20. All participants provided informed consent to participate in the study and for the publication of their anonymised data.

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## Data availability statement

The data has not been submitted to any public repository. All relevant data is included in the article. Where necessary the raw data is available upon request to [abenaboakye13@gmail.com](mailto:abenaboakye13@gmail.com)/[meradak64@gmail.com](mailto:meradak64@gmail.com).

## CRediT authorship contribution statement

**Mercia Lionel Adjei:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abena Boakye:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Godwin Deku:** Writing – review & editing, Resources, Methodology, Investigation. **Nana Baah Pepra-Ameyaw:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Antoinette Simpah Anim Jnr:** Writing – review & editing, Conceptualization. **Ibok Nsa Oduro:** Writing – review & editing, Supervision, Conceptualization. **William Otoo Ellis:** Supervision, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mercia Lionel Adjei reports equipment, drugs, or supplies were provided by Kwame Nkrumah University of Science and Technology. Mercia Lionel Adjei also owns a small-scale yoghurt producing company.

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## References

- [1] W.A.D.V. Weerathilake, D.M.D. Rasika, J.K.U. Ruwanmali, M.A.D.D. Munasinghe, The evolution, processing, varieties and health benefits of yoghurt, *International Journal of Scientific and Research Publications* 4 (1) (2014) 2250–3153.
- [2] H. Chawla, M. Parle, K. Sharma, M. Yadav, Beetroot: a health promoting functional food, *Nutraceuticals* 1 (2016) 976–3872.
- [3] A. Aberoumand, T.R. Patel, A.M.M. An, Refrences of ANC food coloring : the natural Way A review Article on edible pigments properties and sources as natural Biocolorants in Foodstuff and food industry, *Journal of Dairy & Food Sciences* 4 (2) (2014) 511–523.
- [4] N.M. Meybodi, A.M. Mortazavian, M. Arab, A. Nematollahi, Probiotic viability in yoghurt: a review of influential factors, *International Dairy Journal, Elsevier Ltd* 109 (2020) 104793.
- [5] G.V. de Oliveira, M. Morgado, A.P. Pierucci, T.S. Alvares, A single dose of a beetroot-based nutritional gel improves endothelial function in the elderly with cardiovascular risk factors, *Journal of Functional Foods, Elsevier Ltd* 26 (2016) 301–308.
- [6] N. Saleem, Z. Nasreen Umar, S. Ismat khan, Survey on the use of synthetic Food Colors in Food Samples procured from different educational institutes of Karachi city, *Journal of Tropical Life Science* 3 (1) (2013) 1–7.
- [7] M. Oplatowska-Stachowiak, C.T. Elliott, Food colors: Existing and emerging food safety concerns, *Crit. Rev. Food Sci. Nutr.* 57 (3) (2017) 524–548.
- [8] M. Tripathi, S.K. Khanna, M. Das, Surveillance on use of synthetic colours in eatables vis a vis Prevention of Food Adulteration Act of India, *Food Control* 18 (3) (2007) 211–219.
- [9] A.A. Dafallah, A.M. Abdellah, E.A.A. Rahim, S.H. Ahmed, Physiological effects of Some artificial and natural food coloring on Young Male Albino Rats, *J. Food Technol. Res.* 2 (2) (2015) 21–32.
- [10] N.H. Mahmoud, W.E. Org, Toxic effects of the synthetic food dye Brilliant blue on liver, kidney and testes Functions in Rats, *J. Egypt. Soc. Toxicol* 34 (2006) 77–84.
- [11] A. Shamina, K.N. Shiva, V.A. Parthasarathy, Food colours of plant origin, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2 (87) (2007) available at:.
- [12] A. Callebaut, N. Terahara, M. De Haan, M. Declaire, Stability of anthocyanin composition in *Ajuga reptans* callus and cell suspension cultures, *Plant Cell Tissue Organ Cult.* 50 (3) (1997) 195–201.
- [13] B.H. Chen, H.Y. Peng, H.E. Chen, Changes of carotenoids, color, and vitamin A contents during processing of carrot juice, *J. Agric. Food Chem.* 43 (7) (1995) 1912–1918.
- [14] T. Clifford, G. Howatson, D.J. West, E.J. Stevenson, The potential benefits of red beetroot supplementation in health and disease, *Nutrients* 7 (4) (2015) 2801–2822.
- [15] Mbaeyi Nwaoha, Ifeoma Elizabeth, Nwachukwu Godstar Onyinyechi, Production and Evaluaton of yoghurt flavoured with beetroot (*Beta vulgaris* L.), *Journal of Food Science and Engineering* 2 (10) (2012) 583–592.
- [16] D. Kumar, D. Ramasamy, Studies on development of yoghurt flavoured with beetroot juice (*Beta vulgaris* L.), *International Journal of Advance Research in Biological Sciences* 3 (2) (2016) 108–117.
- [17] M. Yadav, D. Masih, C. Sonkar, Development and quality evaluation of beetroot powder incorporated yoghurt, *International Journal of Science, Engineering and Technology* 4 (4) (2016) 582–586.
- [18] M.E.M. Soutelino, D.B. da Silva, R. da Silva Rocha, B.C.R. de Oliveira, E.A. Esmerino, A.G. da Cruz, A.C.D.O. Silva, Yogurt added with beetroot extract: physicochemical parameters, biological activities and sensory evaluation by check-all-that-apply method, *International Journal of Food Science & Technology* 58 (6) (2023) 3303–3309.
- [19] B. Guldiken, G. Toydemir, K. Nur Memis, S. Okur, D. Boyacioglu, E. Capanoglu, Home-processed red beetroot (*Beta vulgaris* L.) products: Changes in antioxidant properties and bioaccessibility, *Int. J. Mol. Sci.* 17 (6) (2016) available at:.
- [20] P.S. Kadam, R.V. Kale, S. Imran Hash, Effect of different varieties of date Palm Paste incorporation on quality Characteristics of yoghurt, *Research Journal of Dairy Sciences, Medwell Publications* 4 (2) (2010) 12–17.
- [21] J.H. Nejad, A.M. Sani, M. Hojjatoleslami, Sensory acceptability and quality of flavored yoghurt enriched with *Spinacia oleracea* extract, *Nutrition and Food Science, Emerald Group Publishing Ltd* 44 (3) (2014) 182–192.

- [22] S. Fetahagić, J. Denin-Djurdjević, S. Jovanović, O. Maćej, Influence of selected factors on the viscosity of set Style yogurt and acid Casein gel at Constant Speed of spindle Rotation, *J. Agric. Sci.* 49 (2) (2004) 233–250.
- [23] Z. Ghasempour, N. Javanmard, A. Mojaddar Langroodi, M. Alizadeh-Sani, A. Ehsani, E. Moghaddas Kia, Development of probiotic yogurt containing red beet extract and basil seed gum; techno-functional, microbial and sensorial characterization, *Biocatalysis and Agricultural Biotechnology*, Elsevier Ltd 29 (2020) available at.
- [24] S. Chatterjee, S. Chatterjee, B.P. Chatterjee, A.K. Guha, Clarification of fruit juice with chitosan, *Process Biochemistry* 39 (12) (2004).
- [25] M. Gacula, S. Rutenbeck, L. Pollack, A.V.A. Resurreccion, H.R. Moskowitz, The just-about-right intensity scale: functional analyses and relation to hedonics, *Journal of Sensory Studies*, John Wiley & Sons, Ltd 22 (2) (2007) 194–211.
- [26] Y. Peng, D.S. Horne, J.A. Lucey, Impact of preacidification of milk and fermentation time on the properties of yoghurt, *J. Dairy Sci.* 92 (7) (2009) 2977–2990.
- [27] A.G. De Brabandere, J.G. De Baerdemaeker, Effects of process conditions on the pH development during yogurt fermentation, *Journal of Food Engineering*, Elsevier Science Ltd 41 (3) (1999) 221–227.
- [28] J.I. Okoye, Animalu, Evaluation of Physico-chemical and microbiological properties of stirred yoghurt Stabilized with sweet potato (*Ipomoea Batatas*) Starch, *Continental J. Microbiology* 3 (2009).
- [29] L.C. Allgeyer, M.J. Miller, S.Y. Lee, Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics, *Journal of Dairy Science*, Elsevier 93 (10) (2010) 4471–4479.
- [30] A.T. Krisnaningsih, L.E. Radiati, Evuanarini H. Purwadi, D. Rosyidi, The effect of incubation time to the physicochemical and microbial properties of yoghurt with Local Taro (*Colocasia Esculenta*(L.) Schott) Starch as Stabilizer, *Curr. Res. Nutr. Food Sci.* 7 (2) (2019), <https://doi.org/10.12944/CRNFSJ.7.2.23>.
- [31] J.G.B. Januário, I.C.F. da Silva, A.S. de Oliveira, J.F. de Oliveira, J.N. Dionísio, S.J. Klososki, T.C. Pimentel, Probiotic yoghurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: physicochemical and texture evaluation, probiotic viability and acceptance, *Int. Food Res. J.* 24 (1) (2017) 359–366.
- [32] H.S. Shalaby, A.S.H. Hassenin, Effects of Fortification stirred yoghurt with red beet powder (RBP) on Hypercholesterolemia Rats, *European Journal of Agriculture and Food Sciences* 2 (5) (2020).
- [33] F.A.O. Who, “Codex Standard for Fermented Milks - CODEX STAN-243-2003”, *Codex Alimentarius*, 2011, pp. 56–58.
- [34] Petruta Oancea, Viorica Meltzer, Photo-Fenton process for the degradation of Tartrazine (E102) in aqueous medium, *J. Taiwan Inst. Chem. Eng.* 44 (6) (2013) 990–994, <https://doi.org/10.1016/j.jtice.2013.03.014>.
- [35] P. García-Segovia, M. Igual, J. Martínez-Monzó, Physicochemical properties and consumer acceptance of Bread enriched with alternative Proteins, *Foods* 9 (7) (2020) 933, 2020, Vol. 9, Page 933, Multidisciplinary Digital Publishing Institute.