

Folate Contents, Nutritional Quality and Consumer Acceptability of Yogurt Fortified with Red Beetroot Extract

*Nyamete, F.A. and R.J. Mongi

Department of Food Technology, Nutrition and Consumer Sciences,
Sokoine University of Agriculture, P.O. Box 3006, Morogoro Tanzania.

*Corresponding author: *nachep@yahoo.com*

Abstract

Folate is one of essential vitamin which plays an important role in human life. This vitamin cannot be synthesized by humans and must be obtained externally from foods to prevent folate deficiency. Folic acid, which is chemically synthesized form of folate, is often used for food fortification and supplementation, but higher intake can result into adverse effects such as vitamin B12 deficiency. Therefore, novel methods to increase concentrations of naturally occurring folate in foods have grabbed the interest of researchers. Bio-fortification of B vitamin folic acid in plain yogurt by using red beetroot as a novel strategy to increase "natural" folate levels was explored in this study. Yoghurt samples A (Control), B, C and D were produced at 0%, 5%, 10%, and 20% of milk substitutions with red beetroot powder. Proximate compositions, folate content and sensory analyses were determined to ascertain the quality attributes of the products. Folate content increased significantly ($p < 0.05$) by 84.3, 84.4 and 88.8% with blended red beetroot extract samples at 5, 10 and 20%. The pH of samples containing 20% red beetroot extract significantly ($p < 0.05$) decreased by a factor of 0.86; while titratable acidity was increased by a factor of 2.07 relative to the plain yoghurt. At 20% beetroot extract inclusion level, the protein content of the samples was significantly ($p < 0.05$) increased by 21.25% relative to the control while fat content shows no significant difference. On the other hand, carbohydrate content of the plain yoghurt was significantly ($p < 0.05$) reduced by 33.7 – 44.0% in the presence of red beetroot after the fermentation period. In sensory evaluation, there were no significant change of sensory attributes up to 10% additional with exception of texture. Overall, the study shows that fortification of yoghurt with red beetroot up to 10% enhances folate levels with minor physical and chemical changes.

Keywords: Folate, red beetroot, bio-fortification, yoghurt

Introduction

Folate, also known as vitamin B9, vitamin folacin and pteroyl-l-glutamate is among the water-soluble B-group vitamins found in a wide variety of foods. It is one of the essential vitamins, which play an important role in human life especially synthesis of nucleotides, vitamins and some amino acid (Quinlivan *et al.*, 2002). However, folate cannot be synthesized by humans and must be obtained externally from the diet to prevent folate deficiency which can cause neural tube defects (NTDs) and other related diseases. Folic acid, which is the chemically synthesized form of folate, is often used for food fortification and in some supplements. However, higher intake can lead

to adverse health effects such as vitamin B12 deficiency (Selhub and Rosenberg, 2016).

Folate deficiencies are common in many developing countries (Antony, 2017). Populations with the highest risk of folate deficiency include children, the elderly and pregnant women, whose diets should therefore provide adequate levels of folate. During pregnancy, for example, folate plays a critical role in fetus neural tube development and helps prevent birth defects in newborn babies (Chitayat *et al.*, 2016).

Beetroot is among the highest vegetable contributors of folic acid (Delchier *et al.*, 2016).

About 100 gram of raw red beetroot contains 109 µg of the B vitamin folic acid, which is higher than for amaranth leaves, a common vegetable consumed in many African household diets (Jastrebova *et al.*, 2003). Thus, incorporating beetroot into mainstream diets is a logical way of improving folic acid intake, especially in the rural poor.

Fermentation is a common process used in creating flavorful products for human consumption. Yoghurt, for example, is commercially produced using carefully selected bacterial cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) which impart pleasant taste and aroma to the milk. Current industrial technologies have contributed to the development of a variety of flavored yogurt products using a range of flavorings and colorings from natural sources, including fresh fruits, vegetables, real nuts and candies such as chocolate pieces that are appealing because of the various micronutrients and sensorial content they deliver (Cruz *et al.*, 2010). Some vegetables such as red beetroot is known for its high folic acid content and natural color that could provide a different dimension to yoghurt sensory experience, its use in enhancing the nutrition of fermented products have not been scientifically substantiated. Thus, this research investigated potential use of red beetroot in bio-fortification of folic acid in yoghurt in order to identify opportunities for improving human health through increased vegetable intake.

Materials and methodology

Materials

Fresh whole cow milk was purchased from Magadu unit at the Sokoine University of Agriculture. Starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was supplied by the Department of Animal and Range Science (originally purchased from Dalton Biotecnologie, Italy). Red beetroots were obtained from Morogoro Municipal market. Chicken pancreas powder were obtained from Pel-Freez biologicals, USA; while toluene and folic acid were purchased from Sigma-Aldrich, USA. Protease and α -amylase enzymes were from Thermo Scientific, USA, respectively.

Methods

Research design

Complete randomized design was used in this study with blend type as the main factor. Its effect on proximate composition, folic acid and sensory attributes were assessed and compared. The mathematical expression is as shown in the equation below.

$$y_{ij} = \mu_i + \tau_i + \epsilon_{ij}$$

$$i = 1, 2, \dots, t, j = 1, 2, \dots, (i, t, j = 1, 2, \dots)$$

Where μ is the overall mean, τ_i is the *i*th beetroot treatment effect and ϵ_{ij} is the random effect due to *j*th replication receiving *i*th beetroot treatment.

Plain yogurt preparation

Whole milk was filtered to remove any physical contaminants, boiled at 85°C for 5 minutes and then cooled to 43°C (Harte *et al.*, 2003). The milk was inoculated using 2% *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starter cultures and then incubated at 43°C for approximately 4 hours until about a pH of 4 was reached. Yoghurt was then stored at a temperature of 8°C for 12 hours to allow equilibration of the product for sensory evaluation.

Preparation of beetroot powder

Fresh beet roots were carefully washed, peeled, sliced thinly (3mm) and dried in the solar dryer to 8% moisture on average. The dried beetroot slices were ground up into fine powder using a Laboratory grinder (RT-02, Mill Powder Tech, Taiwan). The powder was stored in an airtight container to keep it fresh prior to use in product development.

Preparation of beetroot juice-yogurt blends

The red beetroot powder was mixed with pasteurized milk at 30°C to make a 10% w/v beetroot extract in order to avoid adding water into the yoghurt samples. The mixture was properly stirred and left for 10 minutes to allow extraction then filtered by using a thin muslin cloth into a dry clean container for temporary storage.

Pasteurized milk was divided into four equal portions and each was mixed with the prepared beetroot extract at 5, 10 and 20% to make

beetroot extract and milk blends. The control did not have the beetroot extract. The blends were then inoculated immediately with yoghurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) followed by incubation at 43°C until set at a pH of 4 (approx. 4 hrs.). Yogurt was then stored at a temperature of 8°C for 12 hours.

Determination of physical and chemical properties of yogurt and beetroot blends

Titrateable acidity

Titrateable acidity was determined using potentiometric titration procedure as described in AOAC method number 947.05 (AOAC, 1995). About 20 g of yoghurt sample was mixed with water (1:2 ratio w/v) and transferred into an Erlenmeyer flask into which 3 drops of phenolphthalein indicator were added and titrated with 0.1N NaOH to pH 8.1 using a glass electrode pH-meter (Model HI 9124, Hanna Instrument Inc, Romania) at 20°C. Titrateable acidity was reported as % lactic acid by weight.

$$\% \text{Lactic acid} = \frac{\text{volume NaOH} \times 0.009 \times 100}{\text{weight yoghurt}}$$

Acidity was then calculated using a formula above and results were expressed as moles of acid per 100g of the sample.

pH

The pH was determined by method described by (Nyamete *et al.*, 2016) using a glass electrode laboratory pH-meter (Model HI 9124, Hanna Instrument Inc., Romania). Triplicate analysis was done using 20 g samples mixed with 100 mL of distilled water.

Proximate analysis

Proximate composition of the plain and yoghurt samples blended with red beetroot extract were determined using AOAC (1995) methods. Moisture content (% MC) was determined by drying samples in an oven at 105°C for 24 hours. Crude protein percentage (% CP) was determined by Kjeldahl method (AOAC method no. 972.16) with the Kjeltac 8400 analyzer unit (FOSS, Denmark) and the percentage nitrogen obtained was used to calculate the % CP using

the relationship: % CP = % N × 6.25. Percent fat was determined using Soxhlet system HT extraction technique (AOAC method no. 905.02) and percentage ash was determined using method no. 945.46 by incinerating the samples in a muffle furnace at 550°C for four hours, then cooling the ash in a desiccator prior to weighing. Carbohydrate content was calculated by difference.

Folate determination

Preparation of standard folic acid solution

A stock standard was prepared by taking 2 mg of folic acid and dissolving it in 25% ethyl alcohol, then adjusting the pH to 7.0 with 0.1N NaOH prior to topping the volume up to 250 mL with 25% ethyl alcohol solution. The stock standard was then diluted to prepare the working standards.

To prepare a standard curve using spectrophotometry, a working buffer solution of ascorbic acid (pH 6.1) was sterilized by autoclaving at 121°C and 15 psi for 10 minutes. *Lactobacillus casei* ATCC 7469 pellets (Sigma Chemical Co., USA) were then inoculated and incubated in the solution maintained at 35-37°C for 18-24 hours, then finally boiled at 100°C to stop the growth of the bacteria. The bacterial growth was measured by a UV-Spectrophotometer (Thermo Fisher Scientific GENESYS 20 Spectrophotometer) at wavelength of 630 nm and results used in developing the standard curve.

Folate content determination in the yogurt samples

Folate contents in the samples were determined according to Rahman *et al.* (2015) method with minor modification. Approximately 1 mL of sample was homogenized in 100 mL ascorbic acid buffer (pH 6.1) and autoclaved (Pelton & Crane Delta 10 Autoclave, USA) at 121°C and 15 psi for 10 minutes, and then cooled to 37°C. These samples maintained at a pH of 7.2 were incubated with protease enzyme at 37°C for 3 hours, and then boiled in a 70°C water bath for 10 minutes to inactivate the enzyme before cooling to 37°C upon which they were incubated with α -amylase enzyme for additional

2 hours. The samples were then incubated with chicken pancreas powder and 0.5 mL toluene at 37°C for 16 hours and then autoclaved (Pelton & Crane Delta 10 Autoclave, USA) at 121°C and 15 psi for 10 minutes to inactivate the enzymes. After cooling the samples to 37°C, they were then filtered using a Whatman no.1 filter paper (Sigma Aldrich, USA). The filtrates were inoculated with *Lactobacillus casei* ATCC 7469 pellets (Sigma Chemical Co., USA) and incubated at 35-37°C for 18-24 hours before boiling at 100°C to stop the growth of bacteria. Finally, measurement of bacterial growth was recorded by taking the absorbance at 630 nm using UV Spectrophotometer (Thermo Fisher Scientific GENESYS 20Spectrophotometer).

Results and Discussion

pH and Titratable acidity

Table 1 shows that, pH decreased and acidity increased with increasing concentration of beetroot in the samples. The pH and titratable acidity of plain yoghurt samples were 4.09 and 0.73, respectively. Addition of red beetroot extract had noticeable impact on the pH and titratable acidity of the yoghurt samples. The most significant impact was observed in samples containing 20% red beetroot extract where the pH significantly ($p < 0.05$) decreased by a factor of 0.86; while titratable acidity significantly increased by a factor of 2.07 relative to the plain yoghurt.

Table 1: Chemical composition of plain and beetroot blended yogurt

Sample	pH	Titratable Acidity
0% Beetroot (Plain Yoghurt)	4.09 ± 0.05 ^c	0.73 ± 0.01 ^b
5% Beetroot blended yoghurt	3.70 ± 0.00 ^{ab}	0.92 ± 0.02 ^a
10% Beetroot blended yoghurt	3.61 ± 0.03 ^{bc}	0.80 ± 0.003 ^{ab}
20% Beetroot blended yoghurt	3.56 ± 0.08 ^a	1.513 ± 0.09 ^c

Data presented as arithmetic means ± SD (n=9)

Means within column with different superscript letters are significantly different ($p < 0.05$).

Sensory evaluation

The consumer acceptability test of the yoghurt samples was carried out at the Department of Food, Nutrition and Consumer Studies laboratory at Sokoine University of Agriculture. Forty semi-trained panelists aged 20 years and above tasted about 50 mL of each yoghurt sample and scored the products using 9-point hedonic scale (where 1 = dislike extremely and 9 = like extremely) as described by (Mongi *et al.*, 2013). They rated their degree for liking for color, aroma, taste, texture (mouth feel) and overall acceptability of each sample. Water was provided to clean their palates between tests.

Statistical analysis

The data were analyzed using MSTAT-C software for ANOVA to determine significant difference in different assessed parameters between the factor levels. Mean were separated using Duncan's Multiple Range Test at $p \leq 0.05$.

The decrease in pH and increase in titratable acidity in the presence of red beetroot extract could be due to the acidic nature of the beetroot extract material as well as increased culture growth attributable to the beetroot. Previous studies (Nuraeni *et al.* 2014) also found high culture growth and activity in probiotic beetroot juice fermented with yoghurt culture. The US Food and Drug Authority (2010) define low-acid foods as those into which acid(s) or acidic food(s) are added to produce a finished equilibrium pH of 4.6 or below. Yoghurt acidity, caused mainly by lactic acid bacteria, is an important component of yoghurt organoleptic quality (Etienne *et al.*, 2013). The results of Nuraeni *et al.* (2014) also suggest that beetroot extracts can significantly impact the pH and titratable acidity of food samples in the same manner as lactic acid bacteria. The titratable acidity values observed in the yoghurt samples blended with red beetroot extracts are still within International Dairy Guidelines (Nuraeni *et al.*, 2014).

Proximate composition

Moisture contents

Table 2 shows the moisture proximate contents of beetroot based yoghurt. Proximate composition parameter values differed significantly ($p < 0.05$) between samples. The moisture content of plain yoghurt was 74.22%. However, addition of red beetroot extract into the yoghurt samples at 5, 10 and 20% levels, significantly ($p < 0.05$) increased their moisture content by 15.8, 16.2 and 16.9%, respectively. Iwalokun and Shittu, 2007 also reported similar high moisture content in yoghurt samples blended with vegetable juices which they attributed to high moisture content of the added vegetable juices.

can increase the absorption of minerals such as calcium (Holt *et al.*, 2013) and promote lower blood pressure (Seppo *et al.*, 2003). On the other hand, the whey (high in branched-chain amino acids such as valine) may provide various health benefits, including weight loss and lower blood pressure (Pal and Ellis, 2010).

Fat

The fat content in the plain yoghurt samples used in this study was 0.59%. Addition of red beetroot extract to the yoghurt samples didn't have any significant impact to the fat content of the samples studied, a trend similarly reported by Damunupola *et al.*, (2014). Most

Table 2: Chemical composition of plain and beetroot blended yogurt (%)

Sample	Moisture	Protein	Fat	Ash	Carbohydrate
0% Beetroot (Plain Yoghurt)	74.22 ± 0.21b	3.67 ± 0.21b	0.59±0.13b	0.10 ± 0.01a	21.42 ± 0.6c
5% Beetroot blended yoghurt	85.95 ± 0.35a	3.84 ± 0.16b	0.65 ± 0.21ab	0.13 ± 0.02a	9.45 ± 0.42b
10% Beetroot blended yoghurt	86.28 ± 0.21a	3.63 ± 0.19ab	1.15 ± 0.09b	0.13 ± 0.01a	8.81 ± 0.53b
20% Beetroot blended yoghurt	86.79 ± 0.35a	4.45 ± 0.09a	1.38 ± 0.18a	0.16 ± 0.04a	7.23 ± 0.48a

Data presented as arithmetic means ± SD (n=9)

Means within column with different superscript letters are significantly different ($p < 0.05$).

Protein

The protein content of plain (control) yoghurt used in this study was 3.67%. Addition of red beetroot extracts up to 10% level did not significantly increase protein content of the yoghurt samples; however, at 20% beetroot extract inclusion level, the protein content of the samples was significantly ($p < 0.05$) increased by 21.25% relative to control. The protein content of beetroot varies with variety and processing methods used, and reportedly ranges from 9.60 to 12.87% (Damunupola *et al.*, (2014). However, Yadav (2016) reported even lower levels (4.30% protein) in the beetroot material they studied. Even so, these protein levels reported in beetroot were high enough to significantly increase the protein content of the yoghurt samples in this study, especially at 20% inclusion level. Yoghurt is a great source of high-quality protein, including both casein (80%) and whey (20%). Casein (predominantly alpha-casein in yoghurt)

commercially available yoghurts are also low-fat or fat-free. Unlike beetroot fat, milk fat has a unique diversity of fatty acids, most of which are important in human metabolism (Lindmark, 2008). In fact, beetroot is generally a low-fat vegetable. Regular consumption of beetroot has been associated with a lower risk of cardiovascular diseases, cancer, stroke and Alzheimer's disease (Rad *et al.*, 2016). Therefore, incorporating red beetroot into low-fat yoghurt may not cause any harmful metabolic consequences typically associated with high-fat diets.

Carbohydrate

The total carbohydrate content of the plain yoghurt used in this study was 21.42%. However, the addition of red beetroot extract significantly ($p < 0.05$) decreased the total carbohydrate content of the yoghurt samples compared to control. The most significant ($p < 0.05$) decrease

was reported on the yoghurt sample blended with 20% red beetroot extract, which showed a 66.25% decrease relative to control. Similar studies by Elizabeth and Onyinyechi (2011) also reported lower carbohydrate levels in fruit flavored yoghurt samples. The increase in titratable acidity in all the yoghurt samples blended with red beetroot extract and subsequent decrease in total carbohydrates shows that most of the available lactose in yoghurt is converted to lactic acid by the bacterial cultures, which is expected to impact its taste and aroma.

Folic acid content

In this study, we observed a 535 – 789% increase in folic acid content relative to plain yoghurt, when

the yoghurt samples were blended with various amounts of red beetroot extracts (Figure 1A), which also corresponded to the total folic acid content reported (Figure 1B).

The level of folate content at 20% red beetroot inclusion rate into yoghurt (4.27 $\mu\text{g}/100\text{g}$ of dry matter) compared to that at 5% (3.05 $\mu\text{g}/100\text{g}$ of dry matter) could suggest bio-fortification is dose dependent. Similar trend was also reported by (Shohag *et al.*, 2011) and (Jägerstad *et al.*, 2005) in yoghurt samples blended with various amounts of root tuber foods and beetroot juice. In this study, we found that at 20% red beetroot inclusion level, the amount of folate in the yoghurt samples was within the range of

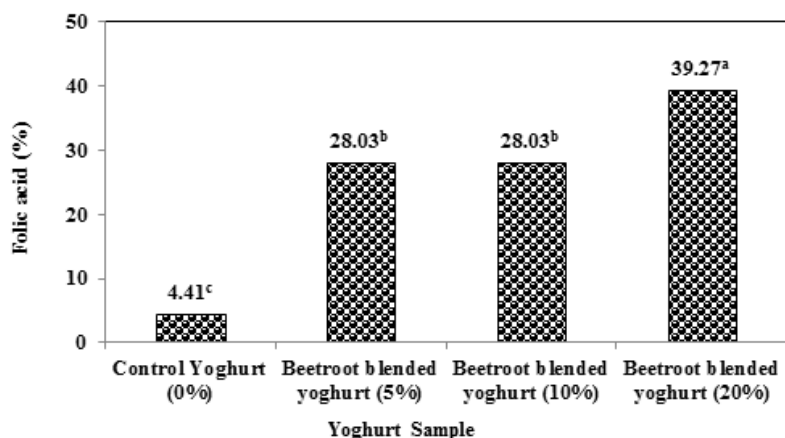
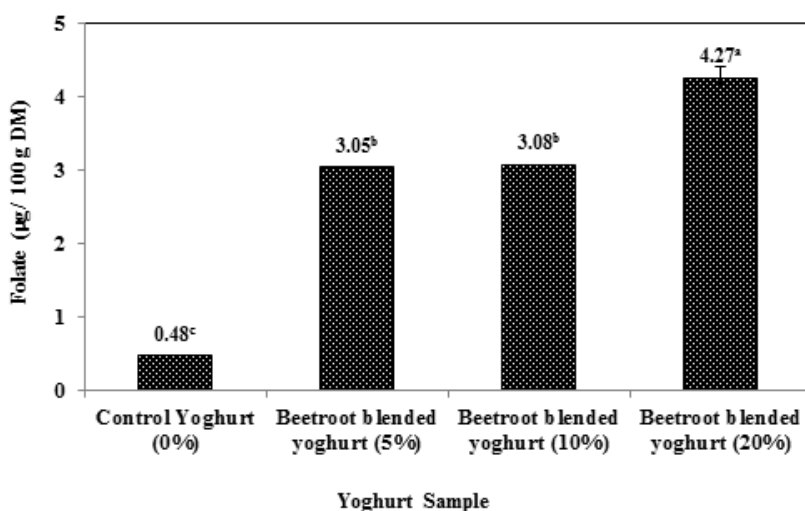


Figure A: Folic acid content ($\mu\text{g}/100\text{ g DM}$) and Figure B: percentage (%) in yoghurt samples.

those reported by Rad *et al.*, (2016), suggesting red beetroot can be a good substitute for other additives used in enhancing folate content of yoghurt.

According to USDA National Nutrient Database for Standard Reference, 48 different food items under the general description of yogurt contain an average of 62 and 10.08 µg/100 g sample of folic acid and folate respectively. This folic acid average value corresponds to about 15.5% of the recommended dietary allowance (RDA) in the diet, based on a 400 µg RDA level for a mature adult. The folate level is significantly higher than those reported in our study and mostly included added folic acid averaging as high as 30.54 µg per 100g sample. In commercial scale, fortification with folic acid helps compensate for losses during processing. This study shows that an 800 g of yoghurt sample blended with 5% red beetroot extract will be enough to achieve 12 µg of folate daily recommended value required by lactating and pregnant mothers. Additionally, this study also recommends that consumption of 800:600 blend of the yoghurt samples containing 5, 10 and 20% of the red beetroot extract, respectively, is most likely to deliver the daily recommended folate intake value of 600-800 µg/day to pregnant and lactating mothers. This may help prevent neural tube defect (NTDs) in infants and children (Hobbs *et al.* 2012).

Consumer acceptability

Color

In this study, yoghurt samples containing red beetroot extracts were evaluated to articulate

appearance attributes that could align with consumer expectations. The yoghurt sample with 20% red beetroot extract was perceived as less appealing to the panelists with no statistical significant difference compared to the control. No significant (p<0.05) difference was observed in color for control samples and those blended with 5 and 10% red beetroot extract. The color of food products is an important sensory attribute for consumer acceptance and choice. In yoghurt, plain and vanilla and strawberry flavored yoghurt are the most dominant varieties available in the market and have the appearance with the most resonance and appeal with consumers.

Aroma

The popularity of yoghurt depends mainly on its sensory characteristics, of which aroma and taste are most important. Aroma is a sensation occurring because of the interaction of the volatile food components with the olfactory receptors, whose stimulus can be orthonasal (perception through smell) or retronasal (perception through the oral cavity during mastication) (Reineccius 2006). The typical aroma of yoghurt is characterized chiefly by acetaldehyde (Cheng, 2010). Since several other complex compounds and factors affect the overall yoghurt aroma, the panelists in this study relied on both orthonasal and retronasal stimuli to evaluate the aroma of the samples.

Overall, there was a significant (p<0.05) aroma differentiation perceived between the yoghurt sample containing 20% red beetroot extract and

Table 3: Sensory evaluation results for plain and yoghurt samples blended with red beetroot extract.

Sample	Color	Aroma	Taste	Texture	Acceptability
0% Beetroot (Plain Yoghurt)	7.60 ± 1.49a	7.80 ± 1.16b	8.10 ± 1.16b	7.80 ± 1.40b	8.23 ± 1.01b
5% Beetroot yoghurt	7.33 ± 0.73a	7.23 ± 1.11ab	7.70 ± 1.12ab	6.93 ± 1.51ab	7.67 ± 1.09ab
10% Beetroot yoghurt	7.57 ± 1.42a	7.57 ± 1.46ab	6.90 ± 1.77a	7.27 ± 1.34ab	7.13 ± 1.53a
20% Beetroot yoghurt	6.90 ± 1.49a	6.87 ± 1.63a	6.93 ± 1.78a	6.70 ± 1.93a	6.83 ± 1.84a

Data presented as arithmetic means ± SD (n=9)

Means within column with different superscript letters are significantly different (p<0.05).

the control yoghurt used in this study (Table 3). In fact, even at 5% inclusion level, the red beetroot extract did provide unique, differentiated aroma experience with the panelists and thus did not show any remarkable positive effect compared to the control.

Using red beetroot as a nutritional additive in this study led to significantly higher folate content relative to plain yoghurt samples (Table 2). However, the results obtained from sensory evaluation indicated that the yoghurt samples into which red beetroot extract had been added "compared favorably" amongst themselves in terms of aroma, and there was no significant difference. The effect of red beetroot on starter culture fermentation rates, survivability, organic acid concentrations, and degree of proteolysis is unknown and further work is required to explain its effect on sensory properties of yoghurt.

Taste

The yoghurt samples blended with 10 and 20% red beetroot extracts were perceived as significantly ($p < 0.05$) different than the control (Table 3). The presence of red beetroot seemed to reduce consumer expectations regarding sensory qualities. Indeed, yoghurt flavor is characterized by numerous volatile and non-volatile bacterial metabolites as well as carbonyl compounds, some of which are by-products of lactic acid fermentation (Cheng, 2010). There are many factors that affect overall flavor formation, release and perception, and include the effect of added flavor compounds and other minor inclusions (e.g. pieces of fruits, vegetables, nuts and candy). Significant amounts of red beetroot in yoghurt can contribute to the release of compounds and other flavor constituents that can add to the complexity of the overall aroma and taste. This implies that in order to effectively fortify yoghurt with red beetroot, product development efforts should focus of delivering the ultimate taste.

Texture

The panelists perceived the yoghurt sample containing 20% red beetroot extract as significantly ($p < 0.05$) different than that of the plain yoghurt, with the test sample scoring

below expectation. The same panelists found no significant ($p > 0.05$) differences amongst the three samples that contained different amounts of red beetroot (Table 3). The apparent texture effects perceived on the yoghurt samples blended with red beetroot used in this study is suspected to be due to both sensory and physicochemical interactions between perceptions, especially with the observed decrease in percent total solids which could have affected its rheological properties and hence mouth feel (Guichard, 2002) as levels of red beetroot extract increased (Table 1). Previous research by Salwa *et al* (2004) also reported a decrease in texture scores in yoghurt prepared with other vegetables.

Most yoghurt contains a considerable amount of fat and proteins which also impact their textural properties and aroma perception. In this study, we reported an increase in total protein content of yoghurt in the presence of red beetroot extract compared to plain yoghurt (Table 1) that we believe could interact with the yoghurt matrix and affect product texture. On the other hand, fats act as structuring materials as well as an excellent solvent for flavor compounds, which are mostly hydrophobic. Fats also influence the structure, texture, and flavor perception of fermented milk products (Routray and Mishra, 2011).

Overall acceptability

To understand consumer acceptability of the yoghurt samples studied in relation to overall perception and acceptability, a PCA bi-plot was used to map out the various attributes. 5% beetroot yoghurt had higher general acceptability scores compared to 10% and 20% beetroot yoghurts, however there were no significant difference ($p < 0.05$) observed between beetroot blended yoghurt. Plain yoghurt had significantly ($p < 0.05$) higher general acceptability compared to 10% and 20% beetroot blended yoghurt; however there was no significant difference in general acceptability between plain and 5% beetroot blended yoghurt. The high overall acceptability of control sample than blended samples could probably be attributed to its high hedonic scores of all sensory attributes especially taste as indicated by the PCA bi-plot. In addition

to nutritional quality, good performance of sensory attributes especially appearance, color and flavor is of greater importance towards consumer acceptability. Since beetroot blended yoghurt is a new product and consumers are not familiar with beet flavor in yoghurt, product development skills would be required to first create prototypes with organoleptic properties that would be more appealing and acceptable to consumers.

According to this study, however, addition of red beetroot to yoghurt led to varying sensory effects. The overall acceptability decreased with increasing levels of red beetroot extract. As observed by the consumer panel, unique vegetable flavor notes that dominated over the fermented yoghurt flavors might have caused different flavor (aroma and taste) perceptions that changed the overall acceptability of the yoghurt samples with red beetroot.

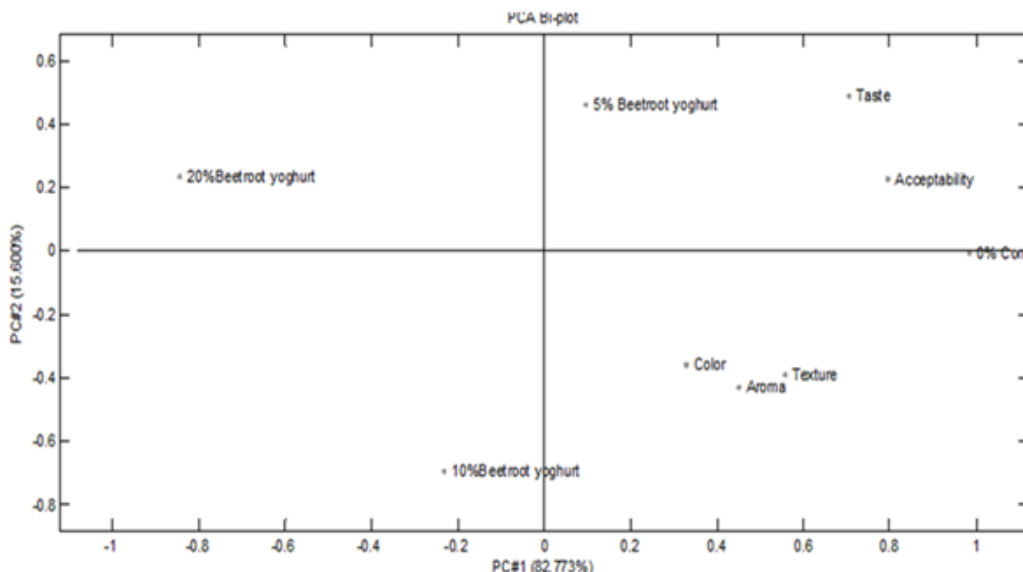


Figure 2: PCA bi-plot to map out the various sensory attributes for yogurt samples

Conclusion and recommendation

The study has shown that there was significant improvements in nutrient composition of yoghurt samples blended with beetroot extracts especially the vitamin B9 (folate). The improvement is believed to be contributed by beetroot extract nutrients and properties. This study observed that 5% beetroot blended had significantly improvement of folate compared to a plain yoghurt, and its acceptability is comparable to that of plain yoghurt. Moreover, yoghurt fortified with red beetroot had relatively high levels of protein and fat. An advantage of using red beetroot as the vehicle for supplementing yoghurt with folate is that the vegetable appeal might provide additional product possibilities for consumers and may enhance its acceptance based on the added plant-based protein and fat.

Beetroot blended yoghurt can be used in folate diet intervention programs especially to pregnant and lactating mothers; However, much work is still required to modify and balance the flavor components in order to meet ever-changing consumer expectations.

References

Quinlivan, E.P., McPartlin, J., McNulty, H., Ward, M., Strain, J.J., Weir, D.G., and Scott, J.M. (2002). Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *The Lancet*, 359(9302): 227-228.

Selhub, J., and Rosenberg, I.H. (2016). Excessive folic acid intake and relation to adverse health outcome. *Biochimie*, 126: 71-78.

- Antony, A.C., (2017). Evidence for potential underestimation of clinical folate deficiency in resource-limited countries using blood tests. *Nutrition reviews*, 75(8): 600-615.
- Chitayat, D., Matsui, D., Amitai, Y., Kennedy, D., Vohra, S., Rieder, M., and Koren, G. (2016). Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. *The Journal of Clinical Pharmacology*, 56(2): 170-175.
- Delchier, N., Herbig, A.L., Rychlik, M., & Renard, C.M. (2016). Foliates in fruits and vegetables: Contents, processing, and stability. *Comprehensive Reviews in Food Science and Food Safety*, 15(3): 506-528.
- Jastrebova, J., Witthöft, C., Grahn, A., Svensson, U., and Jägerstad, M. (2003). HPLC determination of folates in raw and processed beetroots. *Food Chemistry*, 80(4): 579-588.
- Cruz, A.G., Faria, J.A.F., Walter, E.H.M., Andrade, R.R., Cavalcanti, R.N., Oliveira, C.A.F., and Granato, D. (2010). Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology. *Journal of Dairy Science*, 93(11): 5059-5068.
- Harte, F., Luedecke, L., Swanson, B., and Barbosa-Canovas, G.V. (2003). Low-fat set yogurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing. *Journal of dairy science*, 86(4): 1074-1082.
- AOAC. (1995). Official Analytical Chemists, Official Methods of Analysis. Edited by AOAC. 16th ed. Vol. 2. Washington DC: AOAC
- Nyamete, F.A., Bennink, M., & Mugula, J.K. (2016). Potential of lactic acid fermentation in reducing aflatoxin B1 in Tanzania maize-based gruel. *African Journal of Food, Agriculture, Nutrition and Development*, 16(3): 11139-11151.
- Rahman, T., Chowdhury, M.M.H., Islam, M.T., and Akhtaruzzaman, M. (2015). Microbiological Assay of Folic Acid Content in Some Selected Bangladeshi Food Stuffs. *International Journal of Biology*, 7(2): 35.
- Mongi, R.J., Bernadette, N., Chove, B., and Wicklund, T. (2013). Descriptive sensory analysis, consumer liking and preference mapping for solar dried mango cv Dodo. *Food Sci. Qual. Manag*, 16: 16-23.
- Nuraeni, E., Arief, I.I., and Soenarno, M.S. (2014). Characteristics of Probiotic Koumiss From Goat Milk with Addition of Roselle Extract (*Hibiscus Sabdariffa* Linn). *Journal of the Indonesian Tropical Animal Agriculture*, 39(2): 117-125.
- Etienne, A., Génard, M., Lobit, P., Mbéguié-A-Mbéguié, D. and Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *Journal of Experimental Botany*, 64(6): 1451-1469.
- Iwalokun, B.A., and Shittu, M.O. (2007). Effect of *Hibiscus sabdariffa* (calyce) extract on biochemical and organoleptic properties of yogurt. *Pakistan Journal of Nutrition*, 6(2): 172-182.
- Damunupola, D.A.P.R., Weerathilake, W.A. D.V., and Sumanasekara, G.S. (2014). Evaluation of quality characteristics of goat milk yogurt incorporated with beetroot juice. *International Journal of Scientific and Research Publications*, 4(10).
- Yadav, P. (2016). Evaluation of Ensiled Sugar Beet Pulp (*Beta vulgaris*) As An Alternate Roughage For Dairy Cows (Doctoral dissertation, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana).
- Holt, C., Carver, J. A., Ecroyd, H. and Thorn, D. C. (2013). Invited review: Caseins and the casein micelle: Their biological functions, structures, and behavior in foods. *Journal of Dairy Science*, 96(10): 6127-6146.
- Seppo, L., Jauhiainen, T., Poussa, T., and Korpela, R. (2003). A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *The American Journal of Clinical Nutrition*, 77(2): 326-330.
- Pal, S. and Ellis, V. (2010). The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. *Obesity*, 18(7): 1354-1359.
- Lindmark Månsson, H. (2008). Fatty acids in bovine milk fat. *Food and Nutrition*

- Research*, 52(1): 1821.
- Rad, A.H., Khosroushahi, A.Y., Khalili, M., and Jafarzadeh, S. (2016). Folate bio-fortification of yoghurt and fermented milk: a review. *Dairy Science and Technology*, 96(4): 427-441.
- Elizabeth, I., and Onyinyechi, N.G. (2012). Production and evaluation of yoghurt flavoured with beetroot (*beta vulgaris L.*). *Journal of Food Science and Engineering*, 2(10): 583.
- Shohag, M.J.I., Wei, Y.Y., Yu, N., Zhang, J., Wang, K., Patring, J., ... and Yang, X.E. (2011). Natural variation of folate content and composition in spinach (*Spinacia oleracea*) germplasm. *Journal of agricultural and food chemistry*, 59(23): 12520-12526.
- Jägerstad, M., Piironen, V., Walker, C., Ros, G., Carnovale, E., Holasova, M., and Nau, H. (2005). Increasing natural food folates through bioprocessing and biotechnology. *Trends in food science and technology*, 16(6-7): 298-306.
- Hobbs, D.A., Kaffa, N., George, T.W., Methven, L., and Lovegrove, J.A. (2012). Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. *British Journal of Nutrition*, 108(11): 2066-2074.
- Reineccius, G. (2006). Choosing the correct analytical technique in aroma analysis. *Flavour in Food*, 2006: 81-97.
- Cheng, H. (2010). Volatile flavor compounds in yogurt: a review. *Critical reviews in food science and nutrition*, 50(10): 938-950.
- Guichard, E., (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, 18(1): 49-70.
- Salwa, A.A., Galal, E.A., & Neimat, A.E. (2004). Carrot yoghurt: Sensory, chemical, microbiological properties and consumer acceptance. *Pakistan Journal of Nutrition*, 3(6): 322-330.
- Routray, W., and Mishra, H.N. (2011). Scientific and technical aspects of yogurt aroma and taste: a review. *Comprehensive Reviews in Food Science and Food Safety*, 10(4), 208-220.