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Effect of Anthocyanins Extracted from Peanut Skins, Roselle Calyces and Outer Peels of Onions on Quality and Colour Stability of Yoghurt Beverages during Storage

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ABSTRACT

Anthocyanin have been used as a natural food colourant and as a source of natural antioxidants. Aqueous extracts of dried roselle calyces, outer peels of yellow onions, and peanut skins were used as sources of anthocyanin and other functional components such as phenolic and flavonoid compounds to fortify yoghurt beverages. Antioxidants, total phenolics, total flavonoids and anthocyanin were estimated in the plant extracts. Colour stability was evaluated in yoghurt beverages fortified with anthocyanin extracted during the storage period at $4\pm 1^\circ\text{C}$ for three weeks. The highest amount of total phenolic content was observed in peanut skin extract ($1.645\text{ mg}\cdot\text{ml}^{-1}$), followed by dry outer peels of onions ($1.5\text{ mg}\cdot\text{ml}^{-1}$) and roselle calyces ($1.3\text{ mg}\cdot\text{ml}^{-1}$). Dry outer peels of onions showed the highest flavonoid content compared with peanut skin and roselle calyces extract. The highest amount of total anthocyanin was observed in roselle calyces ($3.877\text{ mg } 100\text{ g}^{-1}$), followed by aqueous extract of onions skin ($0.635\text{ mg } 100\text{ g}^{-1}$). During the storage period at $4\pm 1^\circ\text{C}$ for three weeks, yoghurt beverages physicochemical and functional properties had been evaluated. The anthocyanin improved the colour, appearance, and overall acceptability of fortified yoghurt beverages. The overall results suggested that it is possible to produce high-quality yoghurt beverages fortified by adding 0.2% extracts of dry peanut skins, dry roselle calyces, and dry outer peels of yellow onions, which obtaining colours degree of red-orange, cheery red, and orange-yellow, respectively, colour stability during the storage period at $4\pm 1^\circ\text{C}$ for three weeks.

Keywords: Anthocyanin, Yoghurt Beverages, Phenolic components, Flavonoid, Sensory Evaluation, Antioxidants Activity

INTRODUCTION

Colour is the most prominent indicator that reflects the quality and freshness of foods. Colourants are commonly applied in the food industry to meet consumer demands. However, during food processing and storage, colour degradation takes place. Synthetic dyes can have adverse effects on human health. Studies involving the development of natural food colourants from plant tissues also have been expanded (Quan *et al.*, 2019). Anthocyanins are substances that give colour to fruits, vegetables, and plants that occur naturally. In addition to chlorophyll, they are possibly the most critical group of visible plant pigments. Apart from giving plants colour, anthocyanins also have numerous health benefits, as they can protect against a wide range of oxidants through several different mechanisms (Kong *et al.*, and Abdel-Shafi *et al.*, 2019). An annual shrub, roselle calyces (*Hibiscus sabdariffa* L.), is commonly used for making jellies, jams, and beverages. Its brilliant red colour and unique flavour make it a precious food product. Roselle petal has anthocyanin, suitable colourant, and a possibly good source of antioxidants. Some of the best possible future commercial sources of anthocyanins from an economic point of view are those from which the pigment is a metabolic end-product of some industrial processes that included other value-added products, such as onion peels and peanut skins.

For that reason, more attention has been given to other potential by-products of anthocyanin-rich waste. The most

widely cultivated agricultural crop in the world is the onions (*Allium cepa* L.) Onion solid waste consists of the non-edible part of the onion bulb, which is the dry and semi-dry outer layers and the apical trimmings. The external dry layers of onion bulb, the main waste of onion, are valuable source polyphenols like flavonoids and anthocyanins (Mourtzinou *et al.*, 2018). Peanut, (*Arachis hypogaea* L.), pea family legume (Fabaceae), cultivated for its edible seeds. The eatable components of peanuts are the kernel and defensive skin. The skin has a pink-red colour and pungent taste and is typically removed in desserts and snack foods before peanut consumption or participation. Peanut skin also is rich in phenolics and possibly other healthy substances (Yu *et al.*, 2005).

The increasing of competitive food market has encouraged the food industry to explore better products capable of attracting more consumers, particularly young people. For these subjects of this article, a crucial factor for their commercial success is the products' presence. The products like "funny drinks" and dairy products with unique and attractive colours like red, orange, and blue seem very important. Anthocyanin-derived pigments that display these unusual colours can achieve new ideas driven by new attractive colours (Jackman *et al.*, 1987 and Mateus *et al.*, 2008).

Yoghurt is one of the most common dairy products widely consumed, and its sensory characteristics have a significant effect on consumers' acceptability (Saint-Eve *et al.*, 2006). Yoghurt beverage capable, classified as stirred

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yoghurt with low viscosity, is an increasing area of concern based on its simplicity, portability, and ability to deliver all the health and nutritional benefits of stirred or establish yoghurt (Thompson *et al.*, 2007). Flavoured yoghurt beverages are usually available in multiple flavours in Egypt. The current study was aimed to identify anthocyanin's effects (extracted from dry peanut skins, dry roselle calyces, and dry outer peels of yellow onions) on model yoghurt beverages and to evaluate the colour stability, physicochemical and functional characteristics throughout storage at 4±1°C for three weeks.

MATERIALS AND METHODS

Anthocyanins Sources and Extraction:

Anthocyanin was extracted from dry rosella calyces (*Hibiscus subdariffa* L.), dry peanut skins (*Arachis hypogaea* L.), and dry outer peels of yellow onions (*Allium cepa* L.). They are obtained from the local market, farms, and storage silos at Zagazig, Egypt. Fresh bulk buffaloes' milk (83.9% moisture, 6% fat, 5.6% lactose, 3.7% protein, and 0.81% ash) was obtained from the dairy products unit at Food Science Department, Faculty of Agriculture, Zagazig University. Starter cultures FD-DVS ABY-3 Probio-Tec® containing *Bifidobacterium lactis* BB-12, *Lactobacillus acidophilus* LA-5, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* were purchased from Chr. Hansen S.A. Laboratories by Misr Food Additives (MIFAD), Egypt, Milwaukee, WI. Stabilizer and emulsifier containing guar gum E412, sodium carboxymethyl cellulose E466, and mono and diglyceride of fatty acid E471 (1:1:1) were obtained from Food Additives "EGY- DAIRY" (10th of Ramadan city, Egypt). Commercial grade sugar (sucrose) was obtained from a local market.

Distilled water (DW) was used as a safe solvent for extracting pigments from roselle calyces, dry outer peels of onions, or peanut skins. The extracted pigments were determined, according to Pouget, *et al.* (1990). Powders (10 g) of each plant material were added to 200 ml of DW and kept overnight at 4 ° C. The mixture was filtered by a filter paper (Whatman No. 1); the filtrates were then accumulated and lyophilized in a Freeze Dryer (Thermo-Electron Corporation-Heto power dry LL300 Freeze Dryer, Czech Repub).

Antioxidants Activity Estimation:

DPPH radical-scavenging activity:

The capacity of the electron donation of the pigments from roselle calyces, dry outer skins of onions or peanut skins was evaluated by removing the purple-coloured of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution according to the method reported by Ramadan, *et al.* (2008). Each pigment extract (50, 100, 200, 500, 1000 and 2000 µg·ml⁻¹) dissolved in methanol was added (100 µl) to 3 ml of 0.1 mmol DPPH. The absorbance was measured at room temperature against a control (DPPH only) in methanol at 517 nm after the incubation period (30 min). The percentage of free radical DPPH's antioxidant activity (percent inhibition) was calculated as follows:

$$\% \text{inhibition} = \frac{[\text{Absorbance of Control} - \text{Absorbance of Sample} / \text{Absorbance of Control}] \times 100}{}$$

The sample concentration that scavenges 50 percent of the DPPH radicals (SC₅₀) was measured by linear curve regression, demonstrating scavenging percentage versus concentration of the sample.

ABTS radical-scavenging activity

ABTS (2,2'-Azinobis-(3-Ethylbenzthiazolin-6Sulfonic Acid)) assay was conducted as described by Re, *et al.* (1999). The stock solutions were prepared by adding 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The two stock solutions were mixed in equal amounts in the dark at room temperature for 12 h to prepare the working solution. ABTS solution (1 ml) was diluted with 60 ml of methanol. Then 10 µl of each pigment extract (50, 100, 200, 500, 1000, and 2000 µg·ml⁻¹) were added to 5 ml of ABTS solution and incubation for 7 min. The absorbance was measured against a control (ABTS solution only) at 734 nm. The percentage of antioxidant activity (% inhibition) of free radical ABTS was calculated as follow:

$$\% \text{inhibition} = \frac{[\text{Absorbance of Control} - \text{Absorbance of Sample} / \text{Absorbance of Control}] \times 100}{}$$

The sample concentration scavenging 50 percent of the ABTS radicals (SC₅₀) was calculated by linear curve regression showing scavenging percentage versus sample concentration (Abdel-Hamid *et al.*, 2017).

Anthocyanin determination

Onion peels, peanut skins, and rosella total anthocyanin content were determined according to Du and Francis (1973), where 100 ml of the extracting solvent was added to a known volume of the filtered extract. The intensity of colour for water and acidified ethanol was determined at wavelengths of 520 and 535 nm using a spectrophotometer, respectively. The following formula determined the total content of anthocyanins attributed to cyanidin-3-glucoside:

$$\text{Total anthocyanin (mg / 100g)} = \frac{[\text{Absorbance} \times \text{Dilution Factor} / (\text{Sample weight} \times 5.99)] \times 100}{}$$

The total soluble solids

Total Soluble Solids (TSS) were determined, according to Horwitz and Latimer (2000).

Total phenolic contents (TPC) determination

The Folin-Ciocalteu assay method was used to estimate total phenolic content (Kähkönen *et al.*, 1999). Specimens (300 µL; triplicate (1.5 ml of Folin-Ciocalteu reagent (10 times dilution) were incorporated into the test tubes with 1.2 ml of sodium carbonate (7.5% w/v). The tubes were left for 30 min; then, the absorbance was measured at 765 nm. Total phenolic was demonstrated in mg 100 g⁻¹ of drying plant materials equivalent to the gallic acid. The calibration equation of the gallic acid was $y = 0.0009x + 0.214$ ($R^2 = 0.9679$), where y is absorbance, and x is the concentration in µg·ml⁻¹.

Total flavonoid contents (TFC) determination

According to Ordonez, *et al.* (2006), the total flavonoid contents were evaluated with some modifications. Two milliliter aliquot of 2% AlCl₃ ethanolic solution was added to 500 µL (1000 µg·ml⁻¹) of extract. The absorbance was measured at 420 nm after 1h. The total content of flavonoids expressed as quercetin equivalent (QE) was calculated using the following formula:

$$y = 0.0012x + 0.008 \quad (R^2 = 0.944).$$

If X is the absorbance, Y is the concentration (µg QE), and R^2 is the correlation coefficient.

Preparation of the sucrose solution:

Pure cane sugar was bought from the market for making 8% sucrose syrup, heated at 85 °C/15 min, cooled, and stored in a refrigerator (4±1°C). The sucrose syrup had been freshly prepared and used at within 24 h from preparation.

Preparation of yoghurt beverages:

Fresh buffaloes' milk was divided into four portions; the first one was served as control, without any addition (C); in portion two, three, and four added 0.1%, 0.2%, and 0.3 (w/w) from each extract of dry peanut skins, dry roselle calyces and dry outer peels of yellow onions, respectively. All milk portions, stabilizer was added stabilizer at ratio 0.5% (w/w) and homogenized at 60°C at 1000 Kpa and heated to 72°C for 15 sec. Rapidly cooled to 42°C. Starter culture (FD-DVS ABY-3 Probio-Tec®) was added at a rate of 0.05% (w/w), distributed in 75 ml in sterile glass bottles (200 ml). All treatments were incubated at 42°C until complete coagulation was reached, and then resultant yoghurt s were kept in a refrigerator (4±1°C) overnight, and then the yoghurts were mixed with added 75 ml sucrose solution. The yoghurt beverages of treatments were then shaken and stirred, then kept at 4±1°C for three weeks. Treatments were evaluated freshly and after one, two, and three weeks.

Composition analysis of yoghurt beverages:

Total solid, protein, fat contents, titratable acidity (as lactic acid %) and pH value were measured using a pH meter (Jenway 3505, Staffordshire, UK) of yoghurt beverage samples were examined according to AOAC (2007). Kosikowski and Mistry (1977) previously determined total volatile fatty acids (TVFA). Flavour compounds as acetaldehyde and diacetyl in yoghurt beverages were determined as described by Lees and Jago (1969). Acetaldehyde reacts with semi-carbazide to form semi-carbazone, which has an absorption value at a wavelength of 224 nm; meanwhile, diacetyl has an absorption value at a wavelength of 270 nm.

Proteolysis in yoghurt beverages by O-phthaldialdehyde (OPA) assay:

Church *et al.* (1983) drew up the OPA reagent as defined. The OPA solution was prepared by mixing the next materials and diluting to a final volume of 50 ml with dH₂O: 25 ml of 100 mM sodium tetraborate; 2.5 ml of 20% (w/w) sodium dodecyl sulfate (SDS); 40 mg of OPA (dissolved in a mixture of 1 ml methanol and 100 µL of β-mercaptoethanol. This reagent was freshly prepared and used within 2 h of preparation. A small aliquot of water yoghurt extract was added to the OPA reagent of 1 ml. By inversion, the solution was mixed and incubated at 25 °C for 2 min. The solution absorbance was measured at 340 nm by a spectrophotometer (a JENWAY 6705 UV / VIS spectrophotometer), and the peptide concentration was measured against tryptone standards.

Colour measurement:

Colour characteristics of yoghurt beverages (L*, a*, and b*) were managed according to Rao *et al.* (2011) colour analyzer Hunter Lab (Hunter Lab Colour Flex EZ, USA). The L* value (lightness index scale) varies from zero (black) to 100 (white), whereas a* value shows redness (+a) or greenness (-a*) and the b* value is related to yellowness (+b) or blueness (-b*). Samples were put in Petri dishes and loaded them up to the top. The petri dish was put directly on the detector to the colourimeter. Colour intensity (C), hue angle (h_{ab}) (0° or 360° = purplish-red, 90° = yellow, 180° = bluish-green, 270° = blue, (a*/b* proportion has been used as an indicator of the apparent redness index (RI), whiteness index (WI) and total colour difference (E) compared to untreated control, were estimated as:

$$C = (a^{*2} + b^{*2})^{0.5}$$

$$h_{ab} = \arctan (b^*/a^*)$$

$$RI = a^*/b^*$$

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{0.5}$$

Where L₀, a₀, and b₀ were the reference sample L, a, and b values, which are the fresh specimens throughout storage.

Sensory evaluation:

Sensory assessment of yoghurt beverages fortified with natural colour extracts throughout the storage period at 4±1°C for three weeks. The degustation panel contained staff recruited from the Department of Food Science, Faculty of Agriculture, Zagazig University, following the scheme described by Ranadheera, *et al.* (2012). The panelists evaluated the colour and appearance, aroma, body and texture, taste, and overall acceptability, based on a 9 point hedonic scale.

Statistical analysis

Data measurements were carried out by using SPSS (version 20) of the statistical software (SPSS Inc., Chicago, IL, US.). For all analyses, a p-value ≤ 0.05 was found to be statistically significant.

RESULTS AND DISCUSSION

Antioxidant activity of plant material:

The DPPH assay was used to determine the antioxidant activity of plant material. The concept of this assay is to donate hydrogen atoms to neutralize DPPH's purple radicals, so when the absorbance is measured, the DPPH's colour will be reduced and turned to pale yellow or colourless. The increased antioxidant activity indicates that samples are also highly capable of reducing free radicals (Inggrid and Santoso, 2016). Figure (1A) shows the inhibition percentage of anthocyanins' antioxidant activity at various concentrations (50, 100, 200, 500, 1000, and 2000 µg·ml⁻¹) extracted from rosella calyces, dry outer onion peels, or peanut skins using DPPH assay. The investigated anthocyanins of roselle calyces have a higher DPPH scavenging activity than dry outer peels of onions and peanut skins. The radical scavenging effect increased with increasing anthocyanin concentrations in all tested samples. The most increased DPPH radical scavenging activity of anthocyanin (96% ±3.9, 89% ±3.2, and 82% ±3.0, for roselle calyces, dry outer peels of onions and peanut skins, respectively) was obtained at 2000 µg·ml⁻¹. Antioxidant activity (inhibition %) for anthocyanins at different concentrations (50, 100, 200, 500, 1000, and 2000 µg·ml⁻¹) extracted from roselle calyces, dry outer peels of onions, or peanut skins using ABTS assay are presented in Figure (1B). The scavenging effects on the ABTS radical also showed that the radical scavenging effect increased with increasing anthocyanin concentrations in all tested samples. The highest ABTS radical scavenging activity of anthocyanin (90% ±2.8, 82% ±1.9, and 73% ±1.8 for roselle calyces, dry outer peels of onions, and peanut skins, respectively) was obtained at 2000 µg·ml⁻¹. The analyzed specimens' antioxidant activity may be associated with various factors such as the level of pigments, total soluble solids, total phenols, and flavonoid contents since these compounds present as free radical scavengers throughout oxidation reaction (Sim and Nyam, 2019). The current results are in keeping with the results (amount of pigments, total soluble solids, total phenolic, and total flavonoid compounds) obtained by Samir, *et al.* (2019). The SC₅₀ values, which refer to the minimum concentration of the antioxidants necessary for scavenges 50% of the radicals (DPPH or ABTS), were calculated, and the results are presented

in Table 1. The antioxidant activity expressed as SC_{50} ($\mu\text{g}\cdot\text{ml}^{-1}$) of anthocyanins isolated from the roselle calyces, dry outer peels of onions, or peanut skins. Low SC_{50} values mean high antioxidant activity (Osman *et al.*, 2019). The sequence for DPPH SC_{50} ($\mu\text{g}\cdot\text{ml}^{-1}$) is shown in Table 1: roselle calyces > dry outer peels of onions > peanut skins. In the ABTS assay, the antioxidant activities were in the similar order: roselle calyces > dry outer peels of onions > peanut skins.

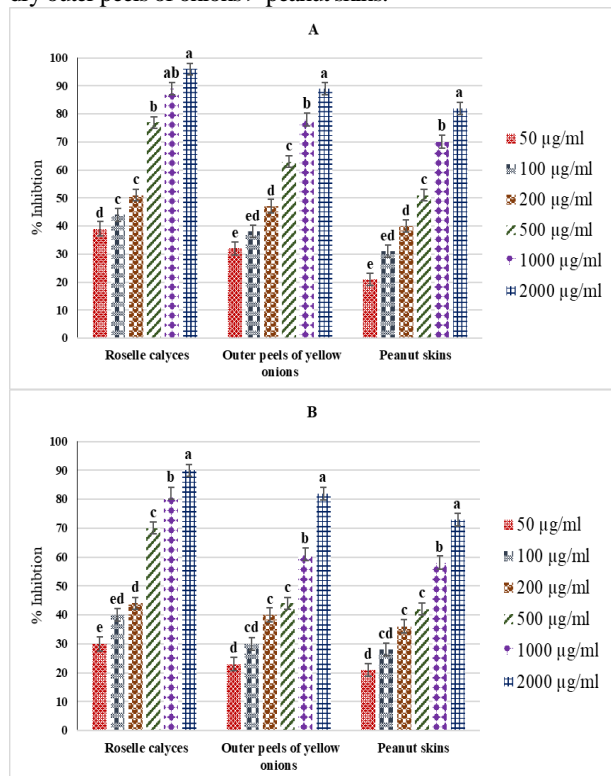


Fig. 1. Antioxidant activity (inhibition %) for anthocyanins at different concentrations (50, 100, 200, 500, 1000 and 2000 $\mu\text{g}\cdot\text{ml}^{-1}$) extracted from roselle calyces, dry outer peels of onions or peanut skins using, A: DPPH assay and B: ABTS assay.

Table 1. DPPH activity (SC_{50} ; $\mu\text{g}\cdot\text{ml}^{-1}$) and ABTS activity (SC_{50} ; $\mu\text{g}\cdot\text{ml}^{-1}$) of anthocyanins at different concentrations (50, 100, 200, 500, 1000 and 2000 $\mu\text{g}\cdot\text{ml}^{-1}$) extracted from roselle calyces, dry outer peels of onions or peanut skins

Sample	SC_{50} [$\mu\text{g}\cdot\text{ml}^{-1}$]	
	DPPH	ABTS
Roselle calyces	200	220
Dry outer peels of onions	350	440
Peanut skins	500	625

SC_{50} : The concentration of the sample that scavenges 50% of the radicals.

DPPH = 2,2-Diphenyl-1-picrylhydrazyl

ABTS = 2,2'-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid

Total phenolic and flavonoid contents in anthocyanin plant extract:

Many secondary metabolites like flavonoids, carotenoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, beta-carotene, and alpha tocopherols produced from plants, where the antioxidant activity used (McCall and Frei, 1999). Folin-Ciocalteu method was used to measure the total phenolic content. The phenolic content of the various anthocyanin pigment was already different (Fig. 2). The highest amount of total phenolic content was observed in peanut skin pigment ($1.645 \text{ mg}\cdot\text{ml}^{-1}$)

followed by dry outer peels of onions ($1.5 \text{ mg}\cdot\text{ml}^{-1}$), and roselle calyces pigment showed the lowest content of total phenolic ($1.3 \text{ mg}\cdot\text{ml}^{-1}$) (Fig. 2). The results are consistent with those gained by Caillet, *et al.* (2006). Dry outer peels of onions showed the highest amount of flavonoid compared with peanut skin and roselle calyces pigments (Fig. 2). Plant polyphenol extracts' overall phenolic content is positively correlated with the free-radical scavenging activity (Caillet, *et al.*, 2006 and Skrede *et al.*, 2004). The most significant polyphenol amounts in peanut skins have resulted in a more significant antioxidant activity measured by DPPH and ABTS assays in the current study (Table 1).

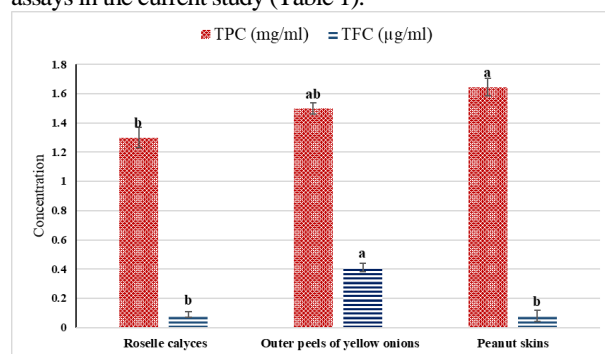


Fig. 2. Total phenolic and flavonoid contents in anthocyanin pigment extracted from roselle calyces, dry outer peels of onions and peanut skin.

Total anthocyanin and total soluble solids of plant material:

The anthocyanin pigments content and total soluble solids are shown in Figure (3). The highest amount of total anthocyanin was observed in roselle calyces ($3.877 \text{ mg } 100 \text{ g}^{-1}$), followed by aqueous extract of onion skin ($0.635 \text{ mg } 100 \text{ g}^{-1}$). Peanut skin showed the lowest amount of anthocyanin ($0.208 \text{ mg}/100\text{g}$) with distilled water. The same trend was observed with the total soluble solids. Due to their antioxidant activity, attractive colour, and stability in high acid foods, anthocyanins benefit the food industry (Wallace and Giusti, 2008).

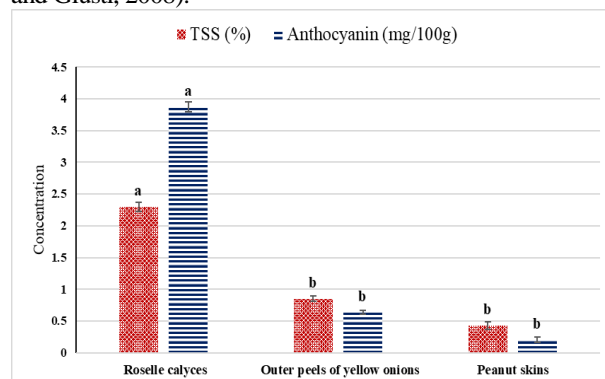


Fig. 3. Total anthocyanin content and total soluble solids extracted from roselle calyces, dry outer peels of onions and peanut skin, extracted by distilled water.

Chemical composition of yoghurt beverages:

Table 2 shows the gross chemical composition and physicochemical properties of yoghurt beverages fortified by anthocyanin extracted from dry peanut skins, dry roselle calyces, and dry outer peels of yellow onions as natural colour during the storage period at $4\pm 1^\circ\text{C}$. Total solids, protein, and fat contents among all treatments were not statistically significant. Changes in the nitrogen materials pattern are likely occurring throughout yoghurt storage, which can never be

identified by measuring the total protein content by using the Kjeldahl method. Serra, *et al.* (2009) showed an increase in the content of hydrophobic peptides and free amino acids throughout yoghurt storage, resulting from hydrolysis of caseins and fractions of whey proteins. After three weeks of storage, yoghurt beverages total fat content immediately after production did not change significantly from that of yoghurt beverages. These results are consistent with those reported previously (Ścibisz, *et al.*, 2019). During the storage period, the pH of yoghurt beverages indicated differences. In tandem with the growth of the culture medium and probiotic bacteria, the initial pH of milk was decreased during processing period. It is commonly recognized that the change in the milk of microorganisms resulted in a decrease in pH among all these microorganisms, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, *L. acidophilus* La5, and *Bifidobacterium lactis* BB12. In addition, total pH declines of all stored yoghurt beverages also were noted mostly during storage time. At the end of the experiment, there was a significant change in pH between control beverages and all yoghurt beverages. This decrease in pH may have been partly related to the lactic acid bacteria. Yoghurt beverages were made using a culture containing both *S. thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which accelerate acidification in yoghurt beverages after fermentation throughout storage related to that free from *Lactobacillus delbrueckii* subsp. *bulgaricus* (Kailasapathy *et al.*, 2008). It can be noted that yoghurt beverages control (C) had a greater decrease in pH values and an increase in titrable acidity values in comparison to yoghurt beverages fortified with natural colour extracted

from rosella (R1, R2, and R3), yellow onion (Y1, Y2, and Y3) and peanut skins (P1, P2 and P3), respectively during storage at 4±1°C. Lactic acid bacteria (LAB) generate β-galactosidase that cleaves the lactose present in the yoghurt and remains active, including under refrigeration, leading to accumulation of the acids produced by the starter microorganisms as metabolized by-products. The yoghurt beverages with added natural colour (anthocyanin) in their composition can be related to the light effect on the pH values and the titratable acidity when increased concentration of anthocyanin. The existence of exogenous buffer components in yoghurt such as proteins, citrates, phosphates, and lactates can lead to pH differences to be less prevalent than acidity changes (Tamime and Deeth, 1980).

Proteolysis in yoghurt beverages by OPA assay:

The OPA assay was used to assess milk protein proteolysis in yoghurt beverages, as shown in Table 2. The OPA values of control yoghurt beverages during the storage period showed an increase compared with all samples (p≤ 0.05), followed by roselle (R1, R2, and R3), yellow onion (Y1, Y2, and Y3), and peanut skins (P1, P2, and P3). When natural colour extracts were also present in the yoghurt beverages, the OPA values were slightly lower (p ≤ 0.05). The absorbance that forms the basis for the OPA values is related to the generated α-amino groups resulting from milk protein proteolysis. The results have been widely used as a suitable measurement of the proteolytic activity of yoghurt and probiotic bacteria (Shihata and Shah, 2000). Enzymes with proteolytic inactivity in yoghurt beverages are stabilized by natural colour extracts related to the reduction of OPA values.

Table 2. Gross chemical composition of yoghurt beverages fortified with dry peanut skins, dry roselle calyces and dry outer peels of yellow onions extracts as natural colour during storage period at 4±1°C for 3 weeks.

Property/ Storage period (week)	Treatment										
	Control	Peanut skins			Roselle calyces			Outer peels of yellow onions			
		P1	P2	P3	R1	R2	R3	Y1	Y2	Y3	
Total solids [%]	13.12±0.19 ^a	13.01±0.11 ^a	13.17±0.25 ^a	13.08±0.27 ^a	13.16±0.48 ^a	13.14±0.38 ^a	13.09±0.10 ^a	13.11±0.16 ^a	13.13±0.28 ^a	13.12±0.15 ^a	
Fat [%]	3.20±0.10 ^a	3.22±0.18 ^a	3.20±0.10 ^a	3.23±0.23 ^a	3.23±0.17 ^a	3.20±0.10 ^a	3.24±0.16 ^a	3.20±0.10 ^a	3.22±0.08 ^a	3.20±0.20 ^a	
Protein [%]	2.04±0.29 ^a	2.07±0.07 ^a	2.11±0.13 ^a	2.09±0.07 ^a	2.06±0.05 ^a	2.05±0.06 ^a	2.01±0.19 ^a	2.04±0.26 ^a	2.08±0.08 ^a	2.05±0.08 ^a	
Titratable acidity (%) lactic acid)	Fresh	0.73±0.04 ^a	0.70±0.05 ^a	0.69±0.02 ^a	0.69±0.03 ^a	0.70±0.02 ^a	0.70±0.04 ^a	0.70±0.01 ^a	0.71±0.04 ^a	0.70±0.05 ^a	0.69±0.03 ^a
	1	0.75±0.06 ^a	0.71±0.04 ^a	0.70±0.03 ^a	0.69±0.02 ^a	0.74±0.01 ^a	0.72±0.03 ^a	0.70±0.06 ^a	0.72±0.03 ^a	0.71±0.01 ^a	0.70±0.04 ^a
	2	0.78±0.05 ^a	0.75±0.01 ^{ab}	0.71±0.02 ^b	0.70±0.06 ^b	0.76±0.02 ^{ab}	0.74±0.01 ^{ab}	0.72±0.03 ^{ab}	0.75±0.04 ^{ab}	0.74±0.01 ^{ab}	0.73±0.03 ^{ab}
	3	0.83±0.02 ^a	0.79±0.03 ^{abc}	0.75±0.01 ^{bcd}	0.73±0.03 ^d	0.82±0.02 ^{abcd}	0.76±0.03 ^{bcd}	0.75±0.02 ^{bcd}	0.80±0.05 ^{ab}	0.76±0.04 ^{bcd}	0.74±0.02 ^{cd}
pH values	Fresh	4.86±0.13 ^a	4.97±0.3 ^a	4.96±0.6 ^a	5.01±0.4 ^a	4.95±0.35 ^a	4.96±0.4 ^a	4.97±0.13 ^a	4.95±0.35 ^a	4.96±0.24 ^a	4.99±0.31 ^a
	1	4.67±0.10 ^b	4.75±0.11 ^a	4.76±0.08 ^a	4.79±0.03 ^a	4.74±0.04 ^a	4.74±0.06 ^a	4.76±0.01 ^a	4.76±0.05 ^a	4.77±0.05 ^a	4.78±0.10 ^a
	2	4.58±0.08 ^b	4.71±0.10 ^a	4.74±0.09 ^a	4.77±0.04 ^a	4.70±0.18 ^a	4.72±0.01 ^a	4.75±0.7 ^a	4.72±0.12 ^a	4.74±0.03 ^a	4.76±0.05 ^a
	3	4.52±0.32 ^b	4.68±0.10 ^{ab}	4.70±0.2 ^{ab}	4.72±0.18 ^a	4.68±0.06 ^{ab}	4.70±0.08 ^{ab}	4.71±0.12 ^{ab}	4.68±0.08 ^a	4.70±0.04 ^{ab}	4.72±0.14 ^a
protein proteolysis by OPA assay [mg·g ⁻¹]	Fresh	98.15±4.17 ^a	97.21±5.79 ^a	96.69±4.31 ^a	96.05±5.95 ^a	98.84±4.16 ^a	97.06±5.94 ^a	96.73±4.27 ^a	98.12±5.88 ^a	97.06±2.94 ^a	97.21±5.17 ^a
	1	113.21±3.79 ^a	108.42±5.18 ^a	107.35±2.65 ^a	106.75±5.25 ^a	110.04±5.95 ^a	110.85±4.15 ^a	109.45±6.05 ^a	109.08±5.17 ^a	108.24±4.24 ^a	107.51±2.51 ^a
	2	152.48±8.52 ^a	144.36±4.36 ^a	142.37±0.36 ^a	140.67±3.33 ^a	145.81±3.19 ^{ab}	143.21±4.79 ^a	142.31±5.11 ^a	145.34±0.66 ^{ab}	142.54±1.46 ^a	141.87±1.13 ^a
	3	171.69±6.31 ^a	162.85±8.15 ^b	159.07±2.93 ^b	158.89±4.11 ^b	164.19±0.81 ^b	161.28±1.72 ^b	159.83±2.17 ^b	163.72±3.28 ^b	160.25±2.75 ^b	159.27±1.73 ^b
Acetaldehyde [µg·100 g ⁻¹]	Fresh	238.21±5.79 ^{bc}	240.24±2.76 ^a	236.01±1.04 ^{abc}	231.68±1.32 ^c	239.07±5.93 ^b	235.41±4.41 ^{abc}	232.13±2.87 ^{bc}	240.58±2.42 ^a	238.94±2.06 ^b	232.44±4.56 ^{bc}
	1	276.58±3.42 ^a	258.85±4.15 ^b	250.08±4.92 ^b	245.54±3.46 ^c	260.01±2.99 ^b	252.67±2.33 ^{bc}	248.74±2.26 ^{bc}	259.63±1.37 ^{bc}	253.14±1.86 ^{bcd}	249.27±4.73 ^{bc}
	2	297.63±3.37 ^a	263.23±2.77 ^b	261.85±5.15 ^{bc}	249.13±1.87 ^d	269.15±1.85 ^b	267.46±4.54 ^b	255.93±5.07 ^{cd}	267.85±3.15 ^b	265.77±5.23 ^b	250.09±4.91 ^d
	3	173.47±5.53 ^a	166.25±4.75 ^{bc}	149.33±2.67 ^d	140.17±3.83 ^c	172.11±4.89 ^b	159.78±2.23 ^c	147.73±4.29 ^d	165.69±3.69 ^{bc}	150.83±4.17 ^d	137.02±3.98 ^e
Diacetyl [µg·100 g ⁻¹]	Fresh	47.64±3.36 ^a	42.15±1.85 ^{bc}	40.00±4.60 ^b	37.37±3.63 ^b	43.47±3.53 ^{ab}	41.30±1.70 ^b	38.69±5.31 ^b	42.96±2.96 ^{ab}	41.20±0.80 ^b	38.10±1.90 ^b
	1	55.32±6.68 ^a	45.41±0.59 ^b	42.39±1.61 ^b	39.60±3.40 ^b	47.27±6.27 ^b	44.33±4.33 ^b	41.46±5.54 ^b	46.36±3.36 ^b	43.64±4.36 ^b	40.86±1.14 ^b
	2	59.53±5.53 ^a	46.18±1.82 ^{bc}	44.38±2.62 ^{bc}	40.18±1.82 ^c	48.94±4.06 ^b	46.92±1.08 ^{bc}	42.66±2.34 ^{bc}	47.83±2.17 ^b	45.82±3.18 ^{bc}	41.00±4.02 ^c
	3	34.69±2.31 ^a	29.17±3.83 ^{ab}	25.31±8.69 ^b	22.61±6.39 ^b	31.29±6.29 ^b	28.03±2.97 ^b	24.62±5.38 ^b	29.59±2.41 ^{ab}	26.01±2.99 ^{ab}	22.46±2.54 ^b
TVFA [0.1 N of NaOH· 100 g ⁻¹]	Fresh	5.53±0.69 ^a	5.15±0.75 ^a	5.07±0.47 ^a	5.02±0.98 ^a	5.35±0.46 ^a	5.19±0.61 ^a	5.11±1.09 ^a	5.12±0.14 ^a	5.05±0.26 ^a	5.03±0.84 ^a
	1	8.17±0.84 ^a	7.60±0.52 ^{abc}	6.77±0.88 ^c	6.63±0.61 ^c	8.01±0.56 ^{ab}	7.23±0.88 ^{abc}	7.08±0.60 ^{abc}	7.78±0.34 ^{abc}	6.99±0.25 ^{abc}	6.91±0.44 ^{bc}
	2	9.32±0.98 ^a	8.51±0.73 ^{abc}	7.59±0.65 ^{cd}	7.10±0.55 ^d	8.92±0.19 ^{ab}	7.97±0.37 ^{abcd}	7.52±0.43 ^{cd}	8.63±0.65 ^{abc}	7.84±0.41 ^{bcd}	7.35±0.63 ^d
	3	10.23±0.80 ^a	9.39±0.86 ^{abc}	9.00±0.35 ^{abf}	8.41±0.56 ^c	9.81±0.54 ^{ab}	9.42±0.69 ^{abc}	8.83±0.74 ^{bc}	9.59±0.48 ^{abc}	9.08±0.77 ^{abc}	8.61±0.62 ^{bc}

Results presented as mean ± SE. Values in the same row having different superscripts letters are significantly different (p ≤ 0.05). C, control yoghurt beverages (without additives); P1, P2 and P3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry peanut skins, respectively; R1, R2 and R3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry roselle calyces, respectively; Y1, Y2 and Y3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry outer peels of yellow onions, respectively.

Flavour compounds of yoghurt beverages:

Acetaldehyde is identified as an essential flavour element in yoghurt, and the existence of lactobacilli in the starting culture can affect the total acetaldehyde component of the end product (Ekinici and Gurel, 2008). Table 2 shows the flavour compounds of yoghurt beverages fortified with anthocyanin extracted from dry peanut skins, dry roselle calyces, and natural colour during the storage period at $4\pm 1^\circ\text{C}$. Acetaldehyde's content significantly increased throughout fermentation, while the remaining volatile compounds showed a moderate increase during storage. The amounts of volatile compounds significantly increased after fermentation up to the second week of cold storage, possibly due to the starter's residual activity, and decreases gradually after that. These results in agreement with the previous results of Gueimonde *et al.*, (2003). By contrast, the development of several volatile compounds in yoghurt beverages, fortified by natural colour extracted of peanut skins (P1, P2, and P3), yellow onion (Y1, Y2, and Y3), and roselle (R1, R2, and R3), compared with control samples during storage at $4\pm 1^\circ\text{C}$, was decreased. These results suggested that the increase in acetaldehyde production in yoghurt. These results agree with the results reported previously by Ruas-Madiedo *et al.*, (1998).

On the other hand, it was found decreasing in acetaldehyde values during the storage period. Diacetyl values increased up to two weeks and then reduced until the end of the storage period. This could be related to the ability of lactic organisms to hydrolysis acetaldehyde and diacetyl. Joung, *et al.* (2016) found that the supplementation of yoghurt with plant leaves extract (*Diospyros kaki*, and *Nelumbo nucifera*) and the storage period hugely affected acetaldehyde production. The yoghurt odors vary depending on the relationship between several volatile compounds; an acetaldehyde-to-acetone ratio of 2:8, for example, is considered satisfactory (Accolas *et al.*, 1980). Total volatile fatty acids (TVFA) content (Table 2) increased in all yoghurt beverages treatments during storage periods. The control yoghurt beverages (C) had more increase TVFA content compared with yoghurt beverages fortified by natural colour extracted of roselle (R1, R2, and R3), yellow onion (Y1, Y2, and Y3), and peanut skins (P1, P2, and P3), respectively during storage at $4\pm 1^\circ\text{C}$. It might be related to starter cultures' proteolytic and lipolytic activity throughout their production and storage (Casaburi *et al.*, 2008).

Colour properties:

The colour scheme of yoghurt beverages has a considerable effect on consumer affirmation. It is also an indicator of an increase in the concentration of pigments that take place throughout storage. Based on the results obtained for the significant colour parameters (Table 3), changes were evaluated. A reduction in lightness (L^* , ranging from 0 to 100, from black to white) was found to increase with increases in natural colour concentration extracted from roselle (R1, R2, and R3), peanut skins (P1, P2, and P3), and yellow onion (Y1, Y2, and Y3), respectively, during storage at $4\pm 1^\circ\text{C}$. This behavior is associated with the increase in the red colour of natural-coloured yoghurt beverages, which reduces the remarkable ability to visualize white in the control yoghurt (C). Even for this variable, no significant difference ($p \leq 0.05$) was noted. The parameter red (a^*) increased the amount of natural colour collected for roselle (R1, R2, and R3), peanut

skins (P1, P2, and P3), and yellow onion (Y1, Y2, and Y3), respectively. The control yoghurt beverages (C) showed negative a^* values as it has no natural colour added, trying to verify with the above-mentioned L^* tendency. The RI (R1) and C^* (R2 and R3) decreased while ΔE increased for the yoghurt beverage samples, but RI increased for R2 and R3 during storage at $4\pm 1^\circ\text{C}$. The decrease of RI for R1, P1, P2, P3, Y1, Y2, and Y3 reflected a colour change toward yellowness, diminishing redness. The whiteness of RI for R1, P1, P2, P3, Y1, Y2, and Y3 increased with the increase in storage period, and similar patterns for L^* were also noted throughout the storage periods. Then lightness was recommended as the big contributing factor to whiteness. To compare the decolouration effect of storage period, we defined decolouration efficiency (DCE) as the change in ΔE during the storage period. A high DCE was observed in P1, R1, and Y1 at the end of the storage period. The DCE decreased observed for R2, R3, P2, P3, Y2, and Y3. The adjustments in instrumental colour parameters for yoghurt beverage samples are shown in Table 2. During the storage period, a particular increase in L^* content was measured; a reduction in L^* was found to increase with increased natural colour concentration collected to roselle (R2 and R3). Hassani and Sharifi (2012) confirmed that barberry yoghurt's lightness was enhanced during storage. The anthocyanin-fortified yoghurt beverages derived from dry peanut skins, dry roselle calyces, and natural colour throughout storage at $4\pm 1^\circ\text{C}$ demonstrated the decline of redness throughout storage illustrated by a change in the angle of colour. Treatments of yoghurt beverages reported a marked increase in this parameter, suggesting that the colour changed from red to orange during storage, most likely due to anthocyanin degradation or/and the development of yellow and brown polymerization compounds. This change was more pronounced in roselle samples (R2 and R3). The extent of hue angle light, on the other hand, increased almost constantly during RI storage for R1, P1, P2, P3, Y1, Y2, and Y3, gradually decreasing in yoghurt beverage samples (R2 and R3). In all yoghurt beverage samples, chroma (C^*) slightly decreased during storage, indicating that yoghurt beverage colour was much less intense over time. Variability in the yoghurt beverage colour parameters can be related to low anthocyanin content. Ścibisz *et al.*, (2019) confirmed that the colour differences between yoghurt beverages seem very less significant than those found in the concentration of anthocyanin extracts, based on the fact that the existence of other substances in yoghurt also had a significant colour effect. The anthocyanins can be easily degradable and influenced by pH, storage temperature, enzymes, and microbial activity, and can exhibit colourless or brown-coloured compounds according to Karaaslan, *et al.*, (2011) and Ścibisz *et al.*, (2019). It was confirmed that, particularly for the first two weeks, the anthocyanin content in yoghurt s showed a significant reduction during storage. The considerably higher decline of anthocyanins in blended yoghurt was also achievable due to a higher oxygen content absorbed during the stirring process to promote anthocyanin degradation. In addition, the variance may be attributed to the higher degradation of anthocyanin at higher pH (Wallace and Giusti, 2008). Certain potential explanations of the measured rapid rate of anthocyanin degradation in yoghurt blending seem to be that the lactic acid bacteria can generate hydrogen peroxide,

which will inhibit unacceptable organisms' growth and enhance anthocyanin destruction. Even though lactic acid bacteria are catalase-negative, hydrogen peroxide can build exposure to greater growth levels in medium (Jaroni and Brashears, 2000). Likewise, in anthocyanins with aromatic acids, sugar moiety's acylation reduces polarity and gives greater stability during storage and processing than most other natural pigments. Anthocyanins with acylating substitutes also demonstrate considerable stability throughout milk products

(Wallace and Giusti, 2008 & Giusti and Wrolstad, 2003). Concerning measurement taken in yoghurt beverages, it was noticed that the anthocyanin content in yoghurt beverages with various levels from rosella extracts, peanut skins and yellow onion demonstrating degree of cheery red, red orange and orange yellow when introduced to yoghurt beverages strengthened by natural colour derived from rosella calyces (R1, R2 and R3), peanut skin (P1, P2 and P3) and yellow onions (Y1, Y2, and Y3), respectively.

Table 3. Colour properties of yoghurt beverages fortified by dry peanut skins, dry roselle calyces and dry outer peels of yellow onions extracts as natural colour during storage period at 4±1°C for 3 weeks.

Property/ Storage period (week)	Control	Treatment									
		Peanut skins			Roselle calyces			Outer peels of yellow onions			
		P1	P2	P3	R1	R2	R3	Y1	Y2	Y3	
L*	Fresh	83.78±0.11 ^a	65.24±0.27 ^d	63.13±0.09 ^c	56.71±0.2 ^e	58.14±0.24 ^f	51.34±0.13 ^h	45.17±0.48 ^g	72.11±0.22 ^b	69.35±0.11 ^c	65.22±0.13 ^d
	1	83.95±0.07 ^a	65.83±0.12 ^d	63.25±0.08 ^c	56.75±0.13 ^e	58.51±0.40 ^f	51.23±0.12 ^h	45.02±0.26 ^g	72.73±0.21 ^b	69.48±0.06 ^c	65.57±0.24 ^d
	2	83.11±0.34 ^a	66.11±0.17 ^d	63.89±0.02 ^f	56.93±0.06 ^h	58.63±0.11 ^g	51.11±0.21 ⁱ	44.83±0.12 ^g	72.91±0.24 ^b	69.11±0.21 ^c	65.33±0.10 ^f
	3	84.44±0.34 ^a	66.54±0.31 ^d	64.45±0.14 ^f	57.24±0.18 ^h	58.97±0.28 ^g	50.67±0.54 ⁱ	44.56±0.50 ^g	73.12±0.53 ^b	70.28±0.72 ^c	65.51±0.55 ^e
a*	Fresh	-2.55±0.36 ^c	3.18±0.46 ^{bc}	3.32±0.84 ^{bc}	3.59±0.56 ^b	4.43±0.16 ^d	4.61±0.56 ^d	4.83±0.16 ^d	2.02±0.04 ^d	2.96±0.82 ^c	3.27±0.30 ^{bc}
	1	-2.59±0.16 ^c	3.10±0.02 ^{de}	3.30±0.50 ^{cd}	3.55±0.28 ^c	4.21±0.20 ^e	4.57±0.32 ^e	4.81±0.92 ^e	2.00±0.13 ^f	2.92±0.30 ^c	3.21±0.26 ^{ab}
	2	-2.60±0.36 ^f	2.99±0.24 ^d	3.18±0.14 ^{cd}	3.51±0.08 ^c	4.03±0.16 ^b	4.53±0.53 ^e	4.72±0.42 ^e	1.96±0.28 ^c	2.81±1.48 ^d	3.08±0.26 ^d
	3	-1.78±0.20 ^f	2.72±0.06 ^d	3.02±0.24 ^d	3.44±0.38 ^c	3.93±1.04 ^b	4.46±0.16 ^d	4.51±0.58 ^e	1.79±0.42 ^c	2.59±0.56 ^d	2.94±0.44 ^d
b*	Fresh	8.37±0.28 ^c	5.06±0.40 ^d	4.65±0.16 ^{bc}	4.33±0.24 ^{bc}	4.29±0.48 ^{bc}	3.81±0.50 ^{bc}	3.29±0.34 ^c	12.89±4.23 ^a	11.17±1.26 ^b	10.81±0.08 ^b
	1	8.45±0.42 ^d	5.01±0.44 ^c	4.62±0.82 ^{ef}	4.31±0.40 ^{bc}	4.17±0.24 ^{bc}	3.55±0.42 ^b	3.03±0.25 ^c	12.64±0.66 ^a	11.03±0.56 ^b	10.26±0.38 ^b
	2	8.49±0.90 ^d	4.91±0.60 ^c	4.51±0.41 ^{ef}	4.22±1.04 ^{ef}	4.01±0.54 ^f	3.29±0.24 ^{bc}	2.87±0.48 ^{bc}	12.48±0.98 ^a	10.94±0.16 ^b	10.13±1.70 ^f
	3	9.51±0.10 ^d	4.83±0.16 ^c	4.36±0.30 ^f	4.13±0.06 ^f	3.94±0.74 ^f	3.17±0.50 ^{bc}	2.62±0.26 ^b	12.27±0.14 ^b	10.81±0.44 ^b	10.01±1.06 ^f
C*	Fresh	8.75±0.22 ^c	5.98±0.82 ^d	5.71±0.58 ^c	5.62±0.22 ^c	6.17±0.28 ^{bc}	5.98±0.30 ^{bc}	5.84±0.22 ^{bc}	13.05±0.40 ^a	11.56±0.30 ^b	11.29±0.51 ^b
	1	8.84±0.14 ^d	5.89±0.30 ^c	5.68±0.12 ^c	5.58±0.34 ^c	5.93±0.24 ^c	5.79±0.82 ^c	5.68±0.46 ^c	12.80±0.70 ^a	11.41±0.61 ^b	10.75±0.86 ^f
	2	8.88±0.92 ^d	5.75±0.16 ^c	5.52±0.22 ^c	5.49±0.06 ^c	5.69±0.30 ^c	5.60±0.26 ^c	5.52±0.18 ^c	12.63±0.20 ^a	11.30±0.62 ^b	10.59±0.26 ^f
	3	9.68±0.26 ^d	5.54±0.28 ^c	5.30±0.42 ^{bc}	5.37±0.12 ^{bc}	5.56±0.10 ^c	5.47±0.40 ^c	5.22±0.22 ^{bc}	12.40±0.06 ^a	11.12±0.12 ^b	10.43±0.24 ^f
h _{ab}	Fresh	-73.06±0.96 ^f	57.85±0.12 ^d	54.47±0.32 ^c	50.34±0.54 ^f	44.08±0.11 ^g	39.57±0.30 ^h	34.26±0.70 ⁱ	81.09±0.44 ^a	75.16±0.30 ^b	73.17±0.36 ^c
	1	-72.96±0.90 ^f	58.25±0.18 ^d	54.46±0.14 ^c	50.52±0.30 ^f	44.73±0.16 ^g	37.84±0.16 ^h	32.21±0.52 ⁱ	81.01±0.48 ^a	75.17±0.34 ^b	72.63±0.58 ^c
	2	-72.97±0.72 ^f	58.66±0.32 ^d	54.81±0.24 ^c	50.25±0.18 ^f	44.86±0.12 ^g	35.99±1.04 ^h	31.30±0.36 ⁱ	81.07±0.08 ^a	75.59±0.24 ^b	73.09±0.44 ^c
	3	-79.40±0.66 ^f	60.61±0.24 ^d	55.29±0.34 ^c	50.21±0.50 ^f	45.07±0.34 ^g	35.40±0.22 ^h	30.15±0.45 ⁱ	81.70±0.47 ^a	76.53±0.16 ^b	73.63±0.38 ^c
RI	Fresh	-0.30±0.06 ^b	0.63±0.10 ^f	0.71±0.04 ^{bc}	0.83±0.06 ^d	1.03±0.16 ^e	1.21±0.26 ^f	1.47±0.30 ^g	0.16±0.08 ^g	0.26±0.11 ^g	0.30±0.13 ^f
	1	-0.31±0.08 ^f	0.62±0.06 ^{ab}	0.71±0.04 ^{ab}	0.82±0.06 ^{cd}	1.01±1.81 ^{bc}	1.29±0.36 ^{bc}	1.59±0.32 ^a	0.16±0.04 ^{ef}	0.26±0.02 ^c	0.31±0.05 ^{bc}
	2	-0.31±0.04 ^d	0.61±0.07 ^e	0.71±0.04 ^c	0.83±0.06 ^d	1.00±0.14 ^c	1.38±0.01 ^b	1.64±0.06 ^a	0.16±0.07 ^h	0.26±0.01 ^g	0.30±0.12 ^f
	3	-0.19±0.08 ^f	0.56±0.10 ^{ab}	0.69±0.06 ^{ab}	0.83±0.10 ^d	1.00±0.79 ^{bc}	1.41±0.16 ^{bc}	1.72±0.54 ^d	0.15±0.04 ^{ef}	0.24±0.14 ^{ef}	0.29±0.08 ^{bc}
ΔE	Fresh	-	-	-	-	-	-	-	-	-	-
	1	0.19±0.04 ^c	0.60±0.04 ^b	0.13±0.02 ^f	0.06±0.02 ^e	0.45±0.08 ^c	0.29±0.06 ^d	0.30±0.02 ^d	0.66±0.07 ^a	0.20±0.02 ^c	0.65±0.12 ^{ab}
	2	0.68±0.08 ^c	0.90±0.16 ^a	0.79±0.04 ^b	0.26±0.10 ^f	0.69±0.06 ^c	0.57±0.03 ^d	0.55±0.16 ^d	0.90±0.02 ^a	0.36±0.03 ^c	0.71±0.04 ^c
	3	1.53±0.02 ^a	1.40±0.18 ^b	1.38±0.10 ^b	0.59±0.06 ^e	1.03±0.12 ^{bc}	0.94±0.06 ^{ef}	0.96±0.07 ^{ef}	1.21±0.14 ^c	1.06±0.10 ^d	0.91±0.12 ^f
WI*	Fresh	81.57±0.22 ^a	64.73±0.26 ^d	62.69±0.16 ^f	56.35±0.72 ^h	57.69±0.24 ^g	50.97±0.2 ^{ai}	44.86±0.58 ⁱ	69.21±0.32 ^b	67.24±0.35 ^c	63.43±0.28 ^c
	1	81.68±0.40 ^a	65.33±3.58 ^d	62.81±0.72 ^f	56.39±0.36 ^h	58.09±0.30 ^g	50.89±0.3 ^{ai}	44.73±0.18 ⁱ	69.87±0.16 ^b	67.42±0.02 ^c	63.93±0.84 ^c
	2	80.92±0.26 ^a	65.63±0.72 ^d	63.47±0.52 ^c	56.58±0.10 ^g	58.24±0.26 ^f	50.79±0.11 ^h	44.55±0.36 ⁱ	70.11±0.46 ^b	67.11±0.52 ^c	63.75±0.32 ^c
	3	81.68±0.48 ^a	66.08±0.98 ^d	64.06±0.50 ^c	56.90±0.86 ^g	58.59±0.46 ^f	50.37±0.28 ^h	44.32±0.58 ⁱ	70.40±0.34 ^b	68.27±0.68 ^c	63.97±1.00 ^c

Results presented as mean ± SE. Values in the same row having different superscripts letters are significantly different (p ≤ 0.05). C, control yoghurt beverages (without additives); P1, P2 and P3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry peanut skins, respectively; R1, R2 and R3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry roselle calyces, respectively; Y1, Y2 and Y3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry outer peels of yellow onions, respectively.

Sensory evaluation:

The results measured for colour and appearance, flavour, body and texture, taste, and general acceptability showed that adding natural colour extracts (Anthocyanin) had a beneficial impact on particular sensory properties (Table 4). Only specific treatment methods panelists managed to score yoghurt beverages fortification by natural colour extracts greater than control yoghurt beverages on average. The colour and appearance of the yoghurt beverage specimens were strongly scored for all the formulations; on the other hand, the addition of natural colour among the different preparations resulted in the most outstanding grades for total sensory characteristics. Once adding natural colour extracts to the yoghurt beverages, the best colours were acquired from roselle, peanut skins, and yellow onion was cheery red, red, and orange-yellow, respectively.

Furthermore, yoghurt beverages with rosella extracts had the best colour in R1 for fresh up to the first week, R2

for two weeks and R3 for three weeks throughout storage, yoghurt beverages with peanut skins in P2 for fresh up to first week, and P3 for two and three weeks, and yoghurt beverages with yellow onion in Y3 for fresh up to final storage. Although yoghurt beverages demonstrated, high acidity levels were able to maintain higher consumer acceptability. Overall sensory scores for these products remained low with the progress storage period, while the treatments with natural colour gained the highest scores for general sensory attributes. Amongst those sensory properties evaluated, taste reached the best scores for all preparations up to the first week and subsequently declined to the period of final storage. Even so, these results indicate that yoghurt beverages might become more appropriate and attractive by improving sensory attributes with a natural colour derived from roselle, peanut skins, and yellow onion (cheery red, red-orange, and orange-yellow, respectively).

Table 4. Sensory evaluation of yoghurt beverages fortified by dry peanut skins, dry roselle calyces and dry outer peels of yellow onions extracts as natural colour during storage period at 4±1°C for 3 weeks.

Properties/ Storage period (week)		Treatment									
		Control	Peanut skins			Roselle calyces			Outer peels of yellow onions		
			P1	P2	P3	R1	R2	R3	Y1	Y2	Y3
Colour and appearance (9)	Fresh	8.19±0.02 ^c	8.15±0.20 ^f	8.81±0.21 ^a	8.55±0.26 ^b	8.83±0.22 ^a	8.51±0.34 ^b	8.12±0.22 ^c	8.21±0.42 ^c	8.64±0.33 ^{ab}	8.85±0.30 ^a
	1	7.52±0.56 ^c	8.08±1.24 ^b	8.50±0.28 ^{ab}	8.43±0.32 ^{ab}	8.57±0.20 ^{ab}	8.43±0.32 ^{ab}	8.38±0.54 ^{ab}	8.38±0.26 ^b	8.52±0.30 ^{ab}	8.63±0.18 ^a
	2	7.15±0.30 ^d	7.53±0.38 ^e	8.11±0.44 ^b	8.44±0.64 ^{ab}	8.21±0.32 ^{ab}	8.54±0.24 ^a	8.43±0.22 ^{ab}	8.13±0.26 ^b	8.24±0.52 ^{ab}	8.51±0.38 ^a
	3	6.52±0.26 ^f	7.18±0.12 ^e	7.75±0.50 ^{bc}	8.31±0.08 ^{abc}	7.65±1.74 ^{de}	8.15±0.70 ^{abcd}	8.58±0.56 ^a	7.68±0.64 ^{abc}	8.15±0.30 ^{abcd}	8.35±0.10 ^{ab}
	Mean	7.35±1.29 ^c	7.74±1.01 ^d	8.29±0.89 ^{bc}	8.43±0.37 ^{ab}	8.32±1.19 ^b	8.41±0.49 ^{ab}	8.38±0.49 ^{ab}	8.10±0.65 ^c	8.39±0.52 ^{ab}	8.59±0.44 ^a
Aroma (9)	Fresh	8.55±0.10 ^a	8.50±0.40 ^a	8.41±0.18 ^a	8.25±0.70 ^a	8.51±0.78 ^a	8.35±0.30 ^a	8.27±0.26 ^a	8.57±0.29 ^a	8.41±0.29 ^a	8.36±0.49 ^a
	1	8.58±0.30 ^a	8.45±0.32 ^a	8.39±0.22 ^a	8.45±0.64 ^a	8.43±0.18 ^a	8.41±0.36 ^a	8.34±0.52 ^a	8.48±0.24 ^a	8.37±0.66 ^a	8.21±0.26 ^a
	2	8.31±0.32 ^a	8.11±0.48 ^a	8.07±0.66 ^a	8.01±0.58 ^a	8.12±0.56 ^a	8.12±0.22 ^a	8.07±0.08 ^a	8.21±0.18 ^a	8.15±0.34 ^a	8.16±0.36 ^a
	3	8.26±0.28 ^a	8.11±0.48 ^a	8.05±0.44 ^a	8.01±0.40 ^a	8.14±0.34 ^a	8.09±0.52 ^a	8.13±0.34 ^a	8.17±0.46 ^a	8.12±0.50 ^a	8.17±0.42 ^a
	Mean	8.47±0.35 ^a	8.29±0.52 ^{ab}	8.23±0.50 ^{ab}	8.18±0.63 ^b	8.30±0.56 ^{ab}	8.25±0.42 ^{ab}	8.20±0.37 ^b	8.36±0.44 ^{ab}	8.26±0.49 ^{ab}	8.23±0.37 ^{ab}
Body and texture (9)	Fresh	8.31±0.32 ^a	8.29±0.44 ^a	8.32±0.20 ^a	8.26±0.44 ^a	8.30±0.28 ^a	8.29±0.52 ^a	8.25±0.32 ^a	8.32±0.16 ^a	8.24±0.40 ^a	8.39±0.22 ^a
	1	8.24±0.52 ^a	8.20±0.60 ^a	8.40±0.18 ^a	8.17±0.44 ^a	8.26±0.36 ^a	8.22±0.39 ^a	8.13±0.24 ^a	8.19±0.22 ^a	8.16±0.28 ^a	8.23±0.24 ^a
	2	8.15±0.30 ^a	8.20±0.20 ^a	8.11±0.58 ^a	8.05±0.10 ^a	8.05±0.15 ^a	8.13±0.24 ^a	8.05±0.36 ^a	8.07±0.14 ^a	8.11±0.18 ^a	8.15±0.30 ^a
	3	7.75±0.30 ^a	7.71±0.58 ^a	7.68±0.34 ^a	7.72±0.24 ^a	7.75±0.60 ^a	7.58±0.32 ^a	7.70±0.64 ^a	7.65±0.28 ^a	7.64±0.52 ^a	7.73±0.44 ^a
	Mean	8.11±0.55 ^a	8.10±0.62 ^a	8.13±0.65 ^a	8.05±0.51 ^a	8.09±0.55 ^a	8.06±0.68 ^a	8.03±0.79 ^a	8.06±0.56 ^a	8.04±0.58 ^a	8.13±0.57 ^a
Taste (9)	Fresh	8.61±0.58 ^a	8.52±0.34 ^{abc}	8.39±0.63 ^{abc}	8.08±0.40 ^{bc}	8.44±0.20 ^{bc}	8.23±0.04 ^{abc}	8.11±0.78 ^{abc}	8.55±0.05 ^{ab}	8.21±0.58 ^{abc}	8.01±0.78 ^c
	1	8.75±0.30 ^a	8.27±0.26 ^b	8.04±0.52 ^b	7.83±1.08 ^b	8.24±0.80 ^b	8.12±0.24 ^b	7.86±0.48 ^b	8.32±0.24 ^b	8.04±0.16 ^b	7.91±0.22 ^b
	2	8.50±0.30 ^a	8.00±0.40 ^{bc}	7.80±0.61 ^{cd}	7.55±0.54 ^d	8.05±0.31 ^{bc}	7.76±0.28 ^{cd}	7.52±0.36 ^{cd}	8.17±0.06 ^b	7.80±0.20 ^{cd}	7.78±0.16 ^{cd}
	3	8.25±0.30 ^a	7.28±0.24 ^b	7.38±0.29 ^b	7.27±0.26 ^b	7.28±0.44 ^b	7.35±0.50 ^b	7.30±0.42 ^b	7.25±0.30 ^b	7.38±0.16 ^b	7.37±0.06 ^b
	Mean	8.53±0.51 ^a	8.02±1.02 ^{bc}	7.90±0.88 ^{bcd}	7.68±0.81 ^c	8.00±1.02 ^{bc}	7.87±0.76 ^{cd}	7.70±0.78 ^c	8.07±1.06 ^b	7.86±0.70 ^{cd}	7.77±0.61 ^{cd}
Overall acceptability (9)	Fresh	8.23±0.14 ^{de}	8.13±0.56 ^e	8.75±0.32 ^{ab}	8.58±0.60 ^{abcd}	8.32±0.16 ^{de}	8.83±0.64 ^a	8.51±0.28 ^{abcd}	8.41±0.22 ^{bcde}	8.75±0.30 ^{ab}	8.66±0.26 ^{abc}
	1	7.82±0.44 ^e	7.83±0.52 ^e	8.50±0.60 ^{ab}	8.13±0.26 ^{bcde}	7.85±0.22 ^{de}	8.53±0.38 ^a	8.22±0.24 ^{abcd}	7.91±0.18 ^{cd}	8.50±0.60 ^{ab}	8.28±0.36 ^{abc}
	2	39.43±1.36 ^b	7.75±0.50 ^d	7.75±0.50 ^d	8.25±0.31 ^a	7.83±0.46 ^{cd}	7.71±0.42 ^d	8.17±0.34 ^{bc}	7.77±0.26 ^d	7.85±0.62 ^{bcd}	8.19±0.30 ^{ab}
	3	6.55±0.50 ^d	7.24±0.72 ^e	8.11±0.52 ^a	7.55±0.62 ^c	7.37±0.33 ^c	8.09±0.48 ^{ab}	7.45±0.30 ^c	7.24±0.28 ^c	8.02±0.66 ^{ab}	7.65±0.32 ^{bc}
	Mean	7.48±1.34 ^f	7.74±0.83 ^e	8.40±0.64 ^a	8.02±0.90 ^{bc}	7.81±0.75 ^{de}	8.41±0.73 ^a	7.99±0.87 ^{cd}	7.85±0.92 ^{cd}	8.37±0.72 ^a	8.16±0.81 ^b
Total scores (45)	Fresh	42.09±0.50 ^{abc}	41.59±0.82 ^{cd}	42.68±1.14 ^a	41.72±0.40 ^{abcd}	42.40±1.6 ^{ab}	42.21±0.46 ^{abc}	41.26±0.24 ^d	42.06±0.38 ^{abc}	42.25±0.70 ^{abc}	42.27±0.74 ^{abc}
	1	40.88±0.28 ^a	40.83±0.76 ^a	41.83±1.88 ^a	41.01±0.32 ^a	41.35±0.54 ^a	41.71±0.98 ^a	40.93±1.18 ^a	41.28±1.29 ^a	41.59±2.58 ^a	41.26±1.32 ^a
	2	39.43±1.36 ^b	39.59±1.28 ^{ab}	40.34±0.54 ^{ab}	39.88±1.28 ^{ab}	40.14±2.72 ^{ab}	40.73±1.08 ^a	39.84±1.36 ^{ab}	40.43±0.66 ^{ab}	40.49±0.24 ^{ab}	40.63±0.70 ^{ab}
	3	37.33±2.42 ^c	37.52±0.80 ^c	38.97±1.40 ^b	38.86±1.53 ^{ab}	38.19±0.96 ^{abc}	39.26±1.16 ^a	39.16±1.92 ^{ab}	37.99±0.32 ^{bc}	39.31±0.52 ^a	39.27±0.12 ^a
	Mean	39.93±3.89 ^{de}	39.88±3.31 ^e	40.96±3.17 ^a	40.37±2.43 ^{bcde}	40.52±3.56 ^{abc}	40.98±2.48 ^a	40.30±2.08 ^{cd}	40.44±3.25 ^{abcd}	40.91±2.60 ^a	40.86±2.38 ^{ab}

Results presented as mean ± SE. Values in the same row having different superscripts letters are significantly different (p ≤ 0.05). C, control yoghurt beverages (without additives); P1, P2 and P3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry peanut skins, respectively; R1, R2 and R3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry roselle calyces, respectively; Y1, Y2 and Y3: yoghurt beverages with 0.1%, 0.2% and 0.3% anthocyanins extraction of dry outer peels of yellow onions, respectively.

CONCLUSION

From the foregoing results of the present work, it can be concluded that the anthocyanin extracted from dry peanut skins, dry roselle calyces, and dry outer peels of yellow onions as natural colour could be used in the production of yoghurt beverages. The beverages from different treatments had acceptable quality, colour stability, and composition compared with control during cold storage at 4±1°C for three weeks. The overall results showed that it is possible to produce good quality yoghurt beverages by adding 0.2% from each extract of dry peanut skins, dry roselle calyces, and dry outer peels of yellow onions.

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Declaration of Competing Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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تأثير الأنتوسيانين المستخلص من جلود الفول السوداني وبتلات الكركديه وقشور البصل الخارجية على جودة وثبات لون مشروبات الزبادي أثناء التخزين

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تم استخدام مستخلصات الأنتوسيانين كملون غذائي طبيعي وكمصدر لمضادات الأكسدة الطبيعية لمشروبات الزبادي، المستخلصات المائية من بتلات الكركديه الجافة، والقشور الخارجية الجافة للبصل الأصفر، وجلود بذور الفول السوداني الداخلية كمصادر للأنتوسيانين ومكونات وظيفية أخرى مثل مركبات الفينول والفلافونويد لتدعيم مشروبات الزبادي. تم تقدير مضادات الأكسدة، الفينولات الكلية، ومركبات الفلافونويد الكلية في المستخلصات النباتية. تم تقييم ثبات اللون في مشروبات الزبادي المدعمة بالأنتوسيانين المستخلص خلال فترة التخزين عند 4 ± 1 درجة مئوية لمدة ثلاثة أسابيع. تم تقدير إجمالي محتوى الفينول والفلافونويد والأنتوسيانين وتقدير الخصائص المضادة للأكسدة أعلى فعالية DPPH radical-scavenging activity كانت 3.9 ± 7.96 ، 3.2 ± 7.89 ، 3.0 ± 7.82 (بتلات الكركديه الجافة، قشور البصل الخارجية الجافة وجلود بذور الفول السوداني الجافة، على التوالي) عند تركيز 2000 ميكروجرام/مل. ولوحظ أكبر قدر من المحنوى الفينولي الكلي في مستخلص جلود بذور الفول السوداني (1.645 ملجم/مل)، يليه قشور البصل الخارجية الجافة (1.5 ملجم/مل)، وأظهرت صبغة الكركديه أقل محتوى من الفينول الكلي (1.3 ملجم/مل). أظهرت قشور البصل الخارجية الجافة أعلى محتوى من الفلافونويد مقارنة بجلود بذور الفول السوداني وبتلات الكركديه. لوحظ أعلى كمية من الأنتوسيانين الكلي في كركديه (3.877 ملجم/100 جم)، تليها المستخلص المائي من قشور البصل (0.635 ملجم/100 جم). خلال فترة التخزين عند 4 ± 1 درجة مئوية لمدة ثلاثة أسابيع، تم تقييم الخصائص الفيزيائية والكيميائية لمشروبات الزبادي. أدى تدعيم مشروبات الزبادي بالأنتوسيانين إلى تحسين لون ومظهر ومقبولية مشروبات الزبادي بشكل عام. أشارت النتائج الإجمالية إلى أنه من الممكن إنتاج مشروبات زبادي عالية الجودة مدعمة بإضافة 0.2% من مستخلصات جلود بذور الفول السوداني الجافة، وبتلات الكركديه الجافة، والقشور الخارجية الجافة للبصل الأصفر، والتي أدت إلى الحصول على درجة اللون الأحمر البرتقالي، لون الكرز الأحمر، والأصفر البرتقالي، على التوالي، وثبات اللون خلال فترة التخزين لمدة ثلاثة أسابيع عند 4 ± 1 درجة مئوية.