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Effect of Roselle Extract (*Hibiscus sabdariffa*) on Stability of Carotenoids, Bioactive Compounds and Antioxidant Activity of Yoghurt Fortified with Carrot Juice (*Daucus carota* L.)

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Abstract: Rosella are usually consumed as beverages; the red pigment has high antioxidant activity. Carrot has carotenoids which used as functional ingredients for development of products. The aim of this study is to measure effect of added three levels of roselle extract (0%, 0.2% and 0.4%) blended with three levels of carrot juice (5%, 10% and 15%) to incorporate bioactive compounds to produced functional yoghurt and stduy their effect on the shelf life of yoghurt. The physical, chemical, color and antioxidant activity of control yoghurt and yoghurt fortified with different levels of carrot juice and roselle extract yoghurts were investigated. Addition of carrot and roselle to yoghurt caused lowest level of total solids, ash, fat and protein contents but increasing of total phenolic and decrease the reduction of total carotenoids may due to effect of thermal processing of the obtained results showed that carotenoids enhanced the yoghurt color and increased the shelf life of yoghurt. The sensory evaluation recorded most favorable for 10 and 15% carrot juice treatment. Also, added 0.2 and 0.4% of roselle increased the antioxidant component. Addition rosella extract to carrot juice developing the function yoghurt properties and producing healthy dairy products.

Key words: Carrot juice · Roselle extract · Carotenoids · Yoghurt · Roselle

INTRODUCTION

The free radicals which formation from oxidative metabolism by-products causes highest risk for human heath such fatal diseases [1]. So, the best way to protect people from fatal diseases fortified or supplemented the food by ingredient contain antioxidant components especially the regularly consumed food. In Egypt, the yoghurt is one of most popular product for all age especially children the protectors used a lot of addition to provide the color, texture, taste and nutritional value. Heyman [2] reported that yoghurt enhance the health benefits by improve gastrointestinal functions, reduce the of hypertension by lower the level of cholesterol [3]. Carotenoids are a one of most important compounds which had effect on sensory properties of dairy products and also affect by indirectly on their antioxidant properties [4]. Carrot is vegetables classified as vitaminized food content tocopherol, ascorbic acid and carotene which important for human nutrition [5]. Carrots content dietary fiber, minerals and vitamins as well as carotenoids and flavonoids which preventing human from diseases [6]. Carrot has antioxidants component which enhance the humane health, the immune system, protect against stroke, high blood pressure, Osteoporosis, cataracts, arthritis, heart disease, bronchial asthma and urinary tract infections [7]. Using yogurt fortified with carrot-juice has increased the yoghurt shelf life because the carrot juice has antibacterial and antifungal properties [8] and increased consumer acceptable [6]. Roselle (*Hibiscus sabdariffa* L.) is herbal plant from *Malvaceae* family [9]. Roselle has health and medicinal effect [10]. Roselle contains anthocyanins in red calyces of brilliant red pigments [11] with high antioxidant activity [12]. Also, roselle had (on dry weight basis), 555.3 - 23.4 mg/100g calcium, 213.8-11.5 mg/100g, magnesium, 39.8-9.0 mg/100g, phosphorus, 2.3-5.47 mg/100g, sodium, 5.85-0.69 mg/100g zinc, 29.5-1.36 mg/100g iron and 493.5-118.2 mg cyanidin-3-glucoside/100g monomeric anthocyanin, 40.0-86.5 mg/100g vitamin C, 54.6-10.8 mg

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gallic acid/ 100 g as a total phenolic compounds and 1.80-1.37 mmol/L antioxidant activity [13]. Also, roselle contains glycoside hivicin chloride, red pigments (gossipten and hibiscin) and phytosterolin and organic acids (malic, citric, tartaric, ascorbic and hibiscic acids) and used as antispasmodic, hypotensive and antimicrobial agent and for relaxation of the uterine muscle [14]. Rosella provide health benefits to consumers so it used as functional food because the content of phytochemicals [15]. The aim of this work to study the effect fortification of yogurt by three different levels (5%, 10% and 15%) of carrot juice and added roselle extract with three different levels (0% , 0.2% and 0.4 %) for every level of carrot juice to improve the quality and shelf life of the fortified yogurt and their effect on stability of carotenoids and antioxidant component. Also, the sensory properties of the final products were investigated to establish the optimal quantity of carrot juice addition based on consumer prefers. Research results will development the novel of functional dairy products.

MATERIALS AND METHODS

Materials: Freeze dried culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from Chr. Hansens laboratories Denmark. Fresh cow's milk, carrot roots (*Daucus carota L.*) and roselle (*Hibiscus sabdariffa*) were purchased from the local market of Cairo, Egypt.

Methods

Preparation of Carrot and Roselle Juice: For preparation, the carrot juice, wash the carrot fruit well, clean, peel, cut into slices and steam up for five minutes, beat the product in the mixer until you get the juice that is filtered to get the smooth and free of impurities juice. To prepare Roselle extract: soak 100 g of roselle leaves in one liter of distilled water for 12 hours in the refrigerator and then drain the product until get the extract.

Preparation of Fortified Yoghurt:

- Yoghurt was manufactured using *lactobacillus delbrueckii supsp bulgaricus* and *Streptococcus salivarious subsp.thermophilus* (1:1) commercial starter culture. Fresh cow's milk (4.14% protein, 3.66 %fat, 0.75% ash, 86.42% moisture content14.58% total solids and pH 6.42) which added different levels of carrot juice and roselle exteact to prepare fortified yoghurt as following:Carrot juice at level of 5% (w/w) and roselle extract at levels of 0, 2 and 4% (w/w) which were defined as treatments YC1H1, YC1H2 and YC1H3, respectively.
- Carrot juice at level of 10% (w/w) and roselle extract at levels of 0, 2 and 4% (w/w) which were defined as treatments YC2H1, YC2H2 and YC2H3, respectively.
- Carrot juice at level of 15% (w/w) and roselle extract at levels of 0, 2 and 4% (w/w) which were defined as treatments YC3H1, YC3H2 and YC3H3, respectively. All treatment was heated to 90°C for 10 min and cooled to 42°C, add starter and incubation at 43°C about 4 hours or until coagulation occur (4.7 pH). The treatment without roselle extract and carrot juice were considered as the control.

Proximate Analysis: Moisture, total solids and total soluble solids "TSS" percent of carrot juice or roselle extract were determined according to AOAC [16]. Total sugars and titratable acidity (as % citric acid) were determined according to Ranganna [17], total phenolic compounds and total carotenoids and were according to Reddy and Sisrunk [18]. pH value was measured by a digital pH meter (Wissenschaftlich- technische-Werkstätten weilheim, Germany). Yogurt acidity (expressed as lactic acid), total solids, ash, fat and protein percentage was determined according to AOAC [19].

Analysis of Total Phenolic Content: Twenty grams of yoghurt were extracted using petroleum ether, tetrahydrofuran and methanol in succession using soxhlet apparatus according to the methods of Roopalatha and Nair [20] with some modifications. Each extract obtained following extraction step was filtered using filter paper Whitman No 1, dried using rotary evaporator and the yield of each extract thus obtained was recorded. Different extracts were reconstituted in 10 mL dimethylsulfoxide (DMSO) and stored under nitrogen at -30°C till further use the total phenolic content was determined according to the Folin-Ciocalteu procedure Zilic *et al*. [21].

Briefly, the extract (500 µl) was transferred into a test tube and oxidized with the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with Gallic acid and expressed as µg of Gallic acid equivalent (GAE) per ml of sample.

Analysis of Total Flavonoid Content and Total Carotenoids: The total flavonoid content and total carotenoids were determined according to Zilic *et al.* [21]. Briefly, 250 µl of 5% $NaNO_2$ was mixed with 500 µl of extract. After 6 min, 2.5 ml of a 10% $AlCl_3$ solution was added. After 7 min, 1.25 ml of 1 M NaOH was added and the mixture was centrifuged at 5000 *g* for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as µg of catechin equivalent (CE) per ml of sample. Total carotenoids content was determined according to Moore *et al.* [22]

Free Radical Scavenging Activity by DPPH: Free radical scavenging activity was determined according to the method of Zainoldin and Baba [23]. A sample of yoghurt (4 mL) was homogenized with 1 mL of water. One mL of methanol (blank) or homogenized yoghurt was added to 2 mL of 0.02 g/L of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. The mixture was then shaken vigorously and kept standing in the dark room for 30 min at room temperature. The reduction of DPPH was measured at 517 nm against a blank at 30 min. Antioxidant activity was expressed as free radical scavenging activity using the following equation:

Free radical scavenging activity as expressed as following:

$$\text{Inhibition (\%)} = 100 \times [(A_{control}-A_{sample})/A_{control}]$$

Evaluation the Color: The color of the roselle extract, carrot juice and yoghurt which fortified was measured using a Hunter Lab, Easy MatchQC according to Hunter method [24]. The Hunter L*, a* and b* values correspond to lightness, redness (+a) and yellowness (+b), respectively.

Sensory Evaluation: Sensory evaluated according to Iwe [25]. Where the evaluation in terms of odor, color, taste and body & texture in addition to the overall acceptability of fresh and storage fortified yoghurt for 21days at 4°C.

Statistical Analysis: All samples were investigated on 1, 7, 14 and 21 days. Data obtained from analysis of the samples was evaluated statistically using analysis of variance (ANOVA) and the differences among means were compared using the Duncan's multiple range tests (using SPSS statistical software program version 13 (SPSS Inc., Chicago, IL). The statistical significance was determined at level of $P < 0.05$.

RESULTS AND DISCUSSION

Proximate Composition: The physicochemical composition of the carrot juice and roselle extract is given in Table 1. The results showed that carrot juice has high amounts of total solids, total carotenoids and pH value than roselle extract. Roselle extract recorded high amounts of moisture, total soluble solids, total sugars, total phenolic content and titratable acidity than carrot juice. Table 2 show chemical composition of control yoghurt and fortified with different levels of carrot juice and roselle extract. Table 2 shows that there are statistically decreases in total solids, ash, fat and protein contents of yoghurt fortified by different levels of carrot juice 5, 10, 15% level these may be due to the diluted of milk content by the carrot juice and roselle extract. The lowest level of total solids, ash, fat and protein were observed in YC3H1, YC3H2 and YC3H3 comparing to control which may be due to the variations in chemical composition of vegetables which used in fortification [26] these results are in agreement with Salwa *et al*. [7]. The increasing of roselle extract was recorded no difference in total solids, ash, fat and protein contents for all carrot juice treatments that may due to the added small amount of roselle extract 0.2 and 0.4%. The pH values of yoghurt fortified by different levels of carrot juice and roselle extract were decreased statistically ($P<0.05$) with increasing level of carrot juice and roselle extract also, the pH value increased ($P<0.05$) at storage period extended to 21days at 4°C (Table 3). The acidity of yoghurt was decreased ($P <0.05$) by increasing level of carrot juice and roselle extract also, decreased ($P <0.05$) at the end of storage period (21days at 4°C) (Table 3). Increment of acidity and lower pH of yhogurt samples which fortified by different levels of carrot juice and roselle extract may be due to the excessive sugar fermentation [7] and accumulation of lactic acid in the yoghurt [27].

Bioactive Compounds

Total Phenolic Contents: Total phenolic of yoghurt fortified by different levels of carrot juice and roselle extract are shown in Table (4). From the tabulated data, it could be noticed that the total phenolic contents were 28.10±0.07 mg GAE/g and 32.63±0.36 mg GAE/g of carrot juice and roselle extract, respectively. The highest content of total phenols were 0.461±0.07, 0.465±0.06 and 0.469±0.04 mg GAE/g in yoghurt fortified with 15% carrot juice YC3R1, YC3R2 and YC3R3, respectively. The results in Table 4 showed that the total phenolic content increased by increasing the carrot juice level, this may be due to the total phenolic in carrot juice was 28.10±0.07 mg

Table 1: Physico-chemical composition of fresh carrot juice and roselle extract (fresh weight)

Chemical composition	Carrot juice	Roselle extract
Total solids (% w/w)	7.91±0.32	7.53±0.24
Moisture (% w/w)	92.09± 0.27	92.47±29
Total soluble solids (% w/w)	7.1± 0.9	7.28± 0.12
Total sugars, as dextrose (% w/w)	5.85± 0.24	6.12± 0.31
Total carotenoids (mg / kg)	107.65 ± 0.16	22.97 ± 0.34
Total phenols (mg GAE/g)	28.10±0.07	32.63±0.36
Titratable acidity, as citric acid (% w/w)	0.53± 0.05	1.34± 0.024
pH value	6.02± 0.2	5.4± 0.1

Mean ±SD

Table 2: Chemical composition of control yoghurt and fortified with different concentration of carrot juice and roselle extract yoghurts during 21days at 4°C (mean ±standard deviation)

Treatments	Total solids %	Ash %	Fat %	Protein %
Control	14.45± 0.63^{a}	1.02±0.05 ^{a}	3.12 ±0.11^{a}	4.56±0.18^{a}
YC1R1	14.13±0.11^{b}	1.01± 0.09^{ab}	2.97± 0.17^{b}	4.38± 0.12^{b}
YC1R2	14.09 ± 0.24^{b}	1.02±0.09^{a}	2.97±0.09^{b}	4.39±0.11^{b}
YC1R3	14.06 ± 0.16^{b}	1.02±0.00^{a}	2.98±0.09^{b}	4.39±0.14^{b}
YC2R1	13.82 ±0.32^{c}	0.99±0.02^{ab}	2.81±0.04^{c}	4.21± 0.1^{c}
YC2R2	13.79± 0.17^{c}	1.01± 0.01^{ab}	2.81±0.12^{c}	4.23±0.13^{c}
YC2R3	13.77±0.11^{c}	1.01±0.04^{ab}	2.81±0.08^{c}	4.24±0.17^{c}
YC3R1	13.55±0.04^{d}	0.98±0.05^{b}	2.63±0.1^{d}	3.99± 0.1^{d}
YC3R2	13.53±0.25^{d}	0.98±0.07^{b}	2.64±0.05^{d}	4.00±0.11^{d}
YC3R3	13.50±0.17^{d}	0.98±0.04^{b}	2.64±0.08^{d}	4.01±0.09^{d}

*Means within the same row without a common letter (a–q) are significantly different (P<0.05) for each type.

Table 3: pH and total acidity (%) control yoghurt and fortified with different levels of carrot juice and Roselle extract yoghurts during storage for 21days at 4°C (mean ±standard deviation)

	Fresh		7 days		14 days		21 days	
Treatments	pH	Total acidity	pH	Total acidity	pH	Total acidity	pH	Total acidity
Control	4.45±0.006^{a}	0.744±0.1^{i}	4.42±0.005a	1.147±0.03^{i}	4.33±0.005^{a}	1.258±0.02^{g}	4.22±0.01^{a}	1.41±0.02^{h}
YC1R1	4.38± 0.005^{b}	0.751±0.07^{h}	4.36±0.002^{b}	1.231±0.09^{h}	4.28±0.002^{b}	1.273±0.04^{f}	4.15±0.005^{b}	1.416±0.06^{g}
YC1R2	4.33±.0.001^{d}	0.771±0.011^{g}	4.31±0.005^{c}	1.337±0.11^{fg}	4.23±0.005^{c}	1.394±0.02^{e}	4.11±0.001^{d}	1.440±0.02^{f}
YC1R3	4.32±0.002^{e}	0.776±0.013^{f}	4.28±0.005^{d}	1.346±0.07^{e}	4.20±0.001^{e}	1.403±0.07^{d}	4.09±0.001^{e}	1.480±0.06^{d}
YC2R1	4.36±0.005^{c}	0.782±0.01^{e}	4.31±0.005^{c}	1.334±0.01^{g}	4.22±0.005^{d}	1.395±0.05^{e}	4.14±0.005^{c}	1.478±0.14^{e}
YC2R2	4.28±0.002^{g}	0.793±0.02^{d}	4.25±0.001^{e}	1.338±0.01^{f}	4.16±0.005^{g}	1.465±0.04^{a}	4.06±0.01^{f}	1.490±0.022^{b}
YC2R3	4.25±0.01^{h}	0.803±0.01^{c}	4.22±0.001^{f}	1.400±0.14^{c}	4.14±0.005^{h}	1.461±0.03^{b}	4.05±0.001^{g}	1.493±0.03^{b}
YC3R1	4.29±0.01^{f}	0.785±0.002^{e}	4.25±0.005^{e}	1.392±0.04^{d}	4.17±0.002^{f}	1.427±0.01^{c}	4.09±0.005^{e}	1.487±0.05^{c}
YC3R2	4.22±0.01^{i}	0.807±0.02^{b}	4.20±0.002^{g}	1.43±0.17^{b}	4.11±0.01i	1.464±0.02^{a}	4.01±0.01^{i}	1.497±0.01^{a}
YC3R3	4.20±0.005^{j}	0.816±0.03^{a}	4.17±0.001^{h}	1.455±0.11^{a}	4.09±0.005j	1.466±0.001^{a}	4.02±0.005^{h}	1.510±0.09^{a}

*Means within the same row without a common letter (a–q) are significantly different (P<0.05) for each type

Table 4: Total phenolic compounds and total flavonoids, total carotenoids of control yoghurt fortified with different levels of carrot juice and Roselle extract yoghurts (mean ±standard deviation)

Treatments	Total phenol (mg GAE/g)	Total flavonoids (mg CE/g)	Total carotenoids (mg / kg)
Control	0.040±0.01^{d}	0.001±0.000^{d}	6.23±0.36^{g}
YC1R1	0.180±0.07^{c}	0.005±0.002^{d}	6.77±0.55^{f}
YC1R2	0.184±0.07^{c}	0.011±0.003^{c}	6.81±0.28^{ef}
YC1R3	0.189±0.05^{c}	0.015±0.004^{bc}	6.86±0.64^{e}
YC2R1	0.320±0.08^{b}	0.009±0.004^{c}	7.30±0.65^{d}
YC2R2	0.325±0.02^{b}	0.014±0.008^{bc}	7.35±0.38^{cd}
YC2R3	0.329±0.07^{b}	0.020±0.006^{a}	7.39±0.94^{c}
YC3R1	0.461±0.07^{a}	0.013±0.006^{bc}	7.84±0.68^{b}
YC3R2	0.465±0.06^{a}	0.018±0.007^{b}	7.89±1.02^{ab}
YC3R3	0.469±0.04^{a}	0.023±0.003^{a}	7.93±0.74^{a}

Table 5: Percentage of free radical scavenging activity of control yoghurts and fortified with different concentration of carrot juice and Roselle juice yoghurts during 21days at 4°C (mean ±standard deviation)

	Storage days			
Treatments	1	7	14	21
Control	32.19±0.57^{d}	31.94±0.12^{e}	30.81±0.18^{e}	29.64±0.27^{e}
YC1R1	32.96±0.23^{cd}	32.12±0.41^{e}	31.68±0.15^{de}	30.87±0.24^{de}
YC1R2	38.87±0.18^{b}	38.17±0.24^{b}	37.70±0.34^{b}	37.11±0.07^{b}
YC1R3	33.26±0.09^{c}	32.66±0.09^{d}	32.08±0.10^{d}	31.57±0.17^{d}
YC2R1	33.89±0.15^{c}	33.21±0.18^{d}	32.85±0.28^{d}	32.14±0.24^{d}
YC2R2	39.11±0.22^{b}	38.40±0.17^{b}	37.96±0.20^{b}	37.28±0.19^{b}
YC2R3	34.14±0.26^{c}	33.64±0.21^{d}	33.09±0.15^{d}	32.69±0.29^{d}
YC3R1	35.67±0.11^{c}	35.05±0.09^{c}	34.58±0.21c	34.11±0.23^{c}
YC3R2	39.42±0.32^{b}	38.61±0.13^{b}	37.88±0.32^{b}	37.09±0.11^{b}
YC3R3	43.25± 0.13^{a}	42.71±0.23^{a}	41.02±0.17^{a}	41.38± 0.61^{a}

GAE/g (Table 1), 26.6±1.70 µg/g [28] and 772±119 mg/l [29]. Furthermore, added roselle extract caused more increasing in total phenolic content. These results are agreement with Mgaya *et al*. [13] who reported that addition of roselle extract for tropical fruit juices increased of quantity of total phenolic content. Also, the present study showed that roselle extract increased the total phenols of yoghurt fortified by different lvels of carrot juice and roselle extract which reduce during fermentation in yoghurt [30].

Total Flavonoids Contents: The importance of flavonoid compounds because their ability as function power antioxidants, which known as secondary metabolites in plants [31] and highest antioxidant activity due to their chemical structure [32]. Table 4 shows the total flavonoids content of yoghurt fortified with different levels of carrot juice and roselle extract. The total flavonoids were 0.001 mg CE/g in control yoghurt sample and increased significantly ($P<0.05$) by increasing level of carrot juice and roselle extract added to yoghurt to be 0.023 mg CE/g in YC3R3. Also, added roselle extract to carrot juice caused more increasing significantly ($P<0.05$) of total flavonoids with increasing roselle extract in all carrot juice levels. This increasing of total flavonoids due to the carrot total flavonoids 0.047 mg/g DW [33]. Fermentation process reduce the total flavonoids content [30], the present study reported that addition roselle extract to yoghurt fortified with carrot caused increased the flavonoids content. Flavonoids had highest antioxidant activity due to their chemical structure. Among dietary flavonoids, isoflavones, especially genistein, shown one of the highest antioxidant activities [32].

Total Carotenoids: The total carotenoids (TC) of yoghurt fortified with different levels of carrot juice and roselle extract shows in (Table 4). The total carotenoids were increased by increasing levels of carrot juice. The treatment YC3R3 was recorded the highest value of TC 93±0.74 mg / kg, whereas, the control yoghurt sample was recorded the lowest TC value which was 6.23±0.36 mg / kg. Additionally, roselle extract caused increasing in total carotenoids in all carrot juice level. These reduce in TC may due to effect of thermal processing effect of carotenoids [34]. So, Addition 0.2% and 0.4% roselle extract caused prevent the carotenoids from reducing during fermentation period that may due to vitamins C and E in roselle extract [15].

Free Radical Scavenging Activity by DPPH Assay: The free radical scavenging activity of yoghurt fortified with different level of carrot juice and roselle extract, in fresh yoghurt and during storage for 21 days at 4°C are shown in Table (5). YCR samples had recoded significantly ($p<0.05$) increased in free radical scavenging activity by increasing levels of carrot juice and roselle extract. comparing to control yoghurts in fresh and stored yoghurt. The highest present of free radical scavenging activity was 29.64±0.27% in control yoghurt and lowest present of free radical scavenging activity was 43.25± 0.13 % in YC3R3 after storage at 4°C for 21 days. This may due to the antioxidant capacity of carrot juice and roselle extract for their phenolic compounds [28, 29]. Melo *et al*. [35] analyzed 15 vegetables for their antioxidant activity and recorded that carrot has lowest capacity for scavenging DPPH-radical.

Color Characteristics: Color is very important indicate the quality of product by consumers [36, 37]. Also, the properties of color were often related to the properties of the antioxidant or index of heat treatments [38]. The color characteristics of roselle extract, carrot juice and yoghurt fortified with different levels of carrot juice and roselle extract in fresh yoghurt shows in (Table 6).

Table 6: Color characteristics of roselle juice, carrot juice, control yoghurts and fortified with different concentration of carrot juice and Roselle juice yoghurts (mean ±standard deviation)

Treatments	L*	a*	b*	Δ E
Carrot juice	33.36±0.001^{e}	13.58±0.020^{b}	41.29±0.01^{a}	54.7
Roselle extract	28.25±0.021^{f}	70.78±0.014^{a}	13.86±0.021^{bc}	77.45
Yogurt (control)	84.12±0.004^{a}	0.96±0.002^{g}	5.24±0.012^{i}	84.28
YC1R1	81.14± 0.120^{b}	1.87±0.140^{f}	8.09± 1.200^{h}	81.56
YC1R2	77.21±0.210^{cd}	6.74±0.390^{d}	8.91± 0.670^{gh}	78.01
YC1R3	76.04±0.130^{d}	10.54±0.23^{c}	9.34±0.240^{fgh}	77.33
YC2R1	80.36±0.190^{b}	2.35±1.90e^{f}	10.42±0.600^{efg}	81.06
YC2R2	76.25±0.360^{cd}	7.26±0.040^{d}	11.29±0.020^{def}	77.42
YC2R3	75.41±0.230^{d}	11.37±1.100^{c}	11.91±0.410^{cde}	77.09
YC3R1	79.25±0.170b^{c}	03.91±0.570^{e}	12.93±0.330^{bcd}	80.39
YC3R2	75.33±0.110^{d}	8.60 ± 0.690^{d}	13.57±1.200^{bc}	77.02
YC3R3	75.06±0.09^{0d}	12.68± 0.310^{bc}	14.02±0.510^{b}	77.40

Table 7: Sensory evaluation control yoghurts and fortified with different levels of carrot juice and Roselle extract yoghurts during 21days at 4°C (mean ±standard deviation)

Storage days	Treatments	Odor	Color	Taste	Body & texture	Over all acceptance
Fresh	Control	8.70^{b}	8.72^{c}	8.66^{e}	8.71^{b}	8.75^{c}
	YC1R1	8.91^{a}	8.84^{b}	8.88c^{d}	8.87^{a}	8.91^{a}
	YC1R2	8.89^{a}	8.90^{a}	8.92^{abc}	8.89^{a}	8.87^{b}
	YC1R3	8.92^{a}	8.94^{a}	8.86^{d}	8.70^{b}	8.91^{a}
	YC2R1	8.88^{a}	8.91^{a}	8.93^{ab}	8.89^{a}	8.87^{b}
	YC2R2	8.89^{a}	8.91^{a}	8.94^{a}	8.91^{a}	8.90^{ab}
	YC2R3	8.91^{a}	8.91^{a}	8.93^{ab}	8.91^{a}	8.89^{ab}
	YC3R1	8.90^{a}	8.89^{ab}	8.89^{bcd}	8.88^{a}	8.78^{c}
	YC3R2	8.93^{a}	8.90^{a}	8.89^{bcd}	8.90^{a}	8.90^{ab}
	YC3R3	8.93^{a}	8.91^{a}	8.89^{bcd}	8.91^{a}	8.92^{a}
7 days	Control	8.39^{f}	8.31^{g}	8.20^{i}	8.25^{f}	8.17^{g}
	YC1R1	8.47^{e}	8.37^{f}	8.27^{h}	8.29^{ef}	8.21^{f}
	YC1R2	8.54^{d}	8.42^{ef}	8.29^{h}	8.30^{de}	8.24^{e}
	YC1R3	8.53^{e}	8.47^{e}	8.36^{f}	8.32^{de}	8.25^{e}
	YC2R1	8.59^{cd}	8.38^{f}	8.27^{h}	8.29^{ef}	8.21^{f}
	YC2R2	8.61^{c}	8.39^{f}	8.30^{g}	8.34^{cde}	8.22^{f}
	YC2R3	8.62^{c}	8.39^{f}	8.34^{fg}	8.36^{cd}	8.26^{e}
	YC3R1	8.59^{cd}	8.52^{d}	8.30^{g}	8.35^{cd}	8.22^{f}
	YC3R2	8.60^{c}	8.56^{d}	8.33^{fg}	8.38^{c}	8.25^{e}
	YC3R3	8.66^{b}	8.56^{d}	8.36^{f}	8.39^{c}	8.31^{d}
14 days	Control	7.71^{gh}	7.64^{i}	7.49^{m}	7.22^{gh}	7.40^{6}
	YC1R1	7.75^{g}	7.66^{i}	7.54^{l}	7.24^{gh}	7.50^{i}
	YC1R2	7.78^{g}	7.66^{i}	7.55^{k}	7.25^{g}	7.51^{h}
	YC1R3	7.79^{g}	7.68^{hi}	7.55^{k}	7.25^{g}	7.51^{h}
	YC2R1	7.77^{g}	7.66^{i}	7.56^{jk}	7.22^{gh}	7.52^{h}
	YC2R2	7.81^{g}	7.69^{hi}	7.57^{jk}	7.25^{gh}	7.54^{gh}
	YC2R3	7.82^{g}	7.7^{hi}	7.57^{jk}	7.26^{g}	7.55^{gh}
	YC3R1	7.81^{g}	7.67^{hi}	7.55^{k}	7.^{23}gh	7.54^{gh}
	YC3R2	7.82^{g}	7.69^{hi}	7.58^{jk}	7.26^{g}	7.56^{g}
	YC3R3	7.82^{g}	7.72^{h}	7.61^{j}	7.27^{g}	7.56^{g}
21 days	Control	-*	-	-	-	-
	YC1R1	-	-	-	-	-
	YC1R2	7.46^{k}	7.18^{m}	7.11^{q}	7.15^{i}	7.09^{l}
	YC1R3	7.49^{j}	7.26^{kl}	7.18^{p}	7.21^{h}	7.14^{k}
	YC2R1	-	-	-	-	-
	YC2R2	7.53^{j}	7.28^{l}	7.21^{op}	7.22^{g}h	7.12^{k}
	YC2R3	7.54^{j}	7.30^{jkl}	7.22^{nop}	7.22^{gh}	7.15^{jk}
	YC3R1	7.55^{j}	7.29^{kl}	7.18^{p}	7.2h	7.10
	YC3R2	7.62^{i}	7.34^{jk}	7.23^{no}	7.24^{gh}	7.15^{jk}
	YC3R3	7.67^{hi}	7.35^{j}	7.26^{n}	7.27^{gh}	7.18^{j}

Means within the same row without a common letter (a–q) are significantly different (P<0.05) for each type. *Dash means: excluded for spoilage samples

The addition of roselle extract and carrot juice to the yoghurt production significantly ($p<0.05$) increased the effect on the color values such as a* and b* which increased by increasing carrot and roselle extract.

Sensory Evaluation: The sensory properties of yoghurt fortified with carrot juice and roselle extract were good after manufacture and the sensory scores decreased significantly by increasing storage period (21 day at 4°C). The present study recorded significantly ($p<0.05$) increasing with increased carrot juice level in most sensory properties comparing with the control sample. The increasing in scores of the teast in carrot and roselle yoghurt due to the present of carbohydrate was 5.85± 0.24 and 6.12± 0.31 % w/w (Table 1) in carrot and roselle, respectively these results are agreement with Salwa *et al.* [7]. After 21days of storage the control, YC1 R1 and YC2R1 were rejected by panelist because the test were exchanged however the other treatments show penalties acceptance.

CONCLUSIONS

Fortified yoghurts with different levels of carrot juice and roselle extract showed significant ($p<0.05$) effect on yogurt antioxidant, increased total phenolic, total carotenoids, enhanced the yoghurt color and increased the shelf life of yoghurt. The present study reported that addition 0.2 to 0.4% of roselle extract to carrot juice developed the yoghurt functional properties and increased sensory evaluated of yoghurt for 21 day.

REFERENCES

1. Gjorgievski, N., J. Tomovska, G. Dimitrovska, B. Makarijoski and M. A. Shariati, 2014. Determination of the antioxidant activity in yogurt. Journal of Hygienic Engineering and Design, 8: 88-92.
2. Heyman, M., 2000. Effect of lactic acid bacteria on diarrheal diseases. J. Am. Coll Nutr, 19: 137S-146S.
3. Taylor, G.R. and C.M. Williams, 1998. Effects of probiotics and prebiotics on blood lipids. Br. J. Nutr., 80: 225-230.
4. Nozière, P., B. Graulet, A.Lucas, B. Martin, P. Grolier and M. Doreau, 2006. Carotenoids for ruminants: From forages to dairy products. Animal Feed Science and Technology, 131(3): 418-450.
5. Hashimoto, T. and T. Nagayama, 2004. Chemical composition of ready to eat fresh carrot. J. Food Hyg. Soc. Japan, 39: 324-328.
6. Cliff, M.A., L. Fan, K. Sanford, K. Stanich, C. Doucette and N. Raymond, 2013. Descriptive analysis and early-stage consumer acceptance of yoghurts fermented with carrot juice. J. Dairy Sci., 96(7): 4160-4172.
7. Salwa, A.A., E.A. Galal and A.E. Neimat, 2004. Carrot yoghurt: sensory, chemical, microbiological properties and consumer acceptance. Pakistan Journal of Nutrition, 3(6): 322-330.
8. Daneshi, M., M.R. Ehsani, S.H. Razavi, M. Labbafi and M.S. Rezaee, 2012. Effect of Cold Storage on Viability of Probiotic Bacteria in Carrot Fortified Milk. J. Nutr. Food Sci., 2(9): 1-4.
9. Cisse, M., F. Vaillant, A. Kane, O. Ndiayea and M. Dornier, 2011. Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. J. Sci. Food Agricult., 92: 1214-1221.
10. Du, C.T. and F.J. Francis, 1973.Anthocyanins of roselle (*Hibiscus sabdaniffa*, L). J. Food Sci., pp: 810-812.
11. Mazza, G.E. and E. Miniati, 2001. Anthocyanin in fruits vigetables and Grains, CRC press: Boca Raton, Florida USA., pp: 209-311.
12. El-Sherif, F., S. Khattab, E. Ghoname, N. Salem and K. Radwan, 2011. Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus sabdariffa* L. Life Sci. J., 8: 220-229.
13. Mgaya, K.B., S.F. Remberg, B.E. Chove and T. Wicklund, 2014. Physio-chemical, mineral composition and antioxidant properties of Roselle (*Hibiscus sabdariffa* L.) extract blended with tropical fruit juices. African Journal of Food, Agriculture, Nutrition and Development, 14(3).
14. Khalid, H., W.E. Abdalla, H. Abdelgadir, T. Opatzand and T. Efferth, 2012. Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. Natural Products and Bioprospecting, 2(3): 92-103
15. Cid-Ortega, S. and J.A. Guerrero-Beltrán, 2015. Roselle calyces (*Hibiscus sabdariffa*), an alternative to the food and beverages industries: a review.Journal of Food Science and Technology, 52(11): 6859-6869.
16. A.O.A.C., 1990. Official Methods of Analysis, 15th Edition Association of Official Analytical Chemists Inc., Arlington, Virginia. USA.
17. .Ranganna, S., 1979. Manual of Analysis of Fruit and Vegetable Products. Tata MC Grow-Hill Publishing Company, New Delhi, pp: 9-15.

18. Reddy, N. and W.A. Sisrunk, 1980. Effect of cultivars, size, storage and cooking method on carbohydrates and some nutrients of sweet potatoes. J. Food Sci., 45: 682-684.
19. A.O.A.C., 2005. Official Methods of Analysis, Association of Official and Analytical Chemists Washington DC (12th ed).
20. Roopalatha, U.C. and V.M. Nair, 2013. Phytochemical analysis of successive reextracts of the leaves of moringa oleifera lam. International Journal of Pharmacy and Pharmaceutical Sciences, 5: 629-634.
21. Zilic, S., A. Serpen, G. Akıllıoglu, M. Jankovic and V. Gökmen, 2012. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J Cereal Sci., 56: 652-658.
22. Moore, J., Z. Cheng, J. Hao, G. Guo, J. Liu, C. Lin and L. Yu, 2007. Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. Journal of Agricultural and Food Chemistry, 55: 10173-10182
23. Zainoldin, K.H. and A.S. Baba, 2009. The effect of Hylocereus polyrhizus and Hylocereus undatuson physicochemical, proteolysis and antioxidant activity in yoghurt. World Academy of Science, Engineering and Technology, 60: 361-366.
24. Hunter R.S., 1975. In the Measurement of Appearance. New York: John Wiley & Sons, Inc: 348
25. Iwe, M.O., 2010. Handbook of Sensory methods and analysis, 75-78. Enugu Nigeria Rejoint Communication Science Ltd.
26. Januário, J.G., I.C. Da Silva, A.S. De Oliveira, J.F. De Oliveira, J.N. Dionísio, S.J. Klososki and T. C.Pimentel, 2017. Probiotic yoghurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: Physicochemical and texture evaluation, probiotic viability and acceptance .International Food Research Journal, 24(1).
27. Radiati, L.E., F. Jaya and H. Oktavia, 2016. Effect of carrot-juice on exo-polysaccharides and β-D galactosidase activity in yogurt. Journal of Animal Production, 18(3): 173-179.
28. Oviasogie, O.P., D. Okoro and C.L. Ndiokwere, 2009. Determination of total phenolic amount of some edible fruits and vegetables. Afr. J. Biotechnol., 8: 2819-2820.
29. Karakaya, S., El SN and A.A. Tas, 2001. Antioxidant activity of some food containing phenolic compounds. Int. J. Food Sci. Nutr., 52: 501-508.
30. Darwish, A.Z., H. Bayomy and M. Rozan, 2016. Effect of baked, whipped and fermentation on antioxidant activity in red raspberries. Journal of Food Processing and Technology, 7(9): 621-625.
31. Ghasemzadeh A., H.Z. Jaafar and A. Rahmat, 2010. Identification and concentration of some flavonoid components in Malaysian young ginger (Zingiber officinale Roscoe) varieties by a high performance liquid chromatography method. Molecules, 15: 6231-6243.
32. Heim, K.E., A.R. Tagliaferro and D.J. Bobilya, 2002. Flavonoid antioxidants: Chemistry, metabolism and structure activity relationships, The Journal of Nutritional Biochemistry, 13: 572-584.
33. Ghasemzadeh, A., M. Azarifar, O. Soroodi and H.Z. Jaafar, 2012. Flavonoid compounds and their antioxidant activity in extract of some tropical plants. Journal of Medicinal Plants Research, 6(13): 2639-2643.
34. Hartman, A.M. and L.P. Dryden, 1965. Vitamins in Milk and Milk Products. American Dairy Science Association, Champaign, IL, USA.
35. Melo, E.A., M.I. Maciel, V.L. Lima, F.L. Leal, A.C. Caetano and R. J. Nascimento, 2006. Antioxidant capacity of vegetables commonly consumed. Food Sci. Technol., 26: 639-644.
36. Renuka, B., S.G. Kulkarni, P. Vijayanand and S.G. Prapulla, 2009. Fructooligosaccharide fortification of selected fruit juice beverages: effect on the quality characteristics. LWT- Food Science and Technology, 42(5): 1031-1033.
37. Pathare, P.B., U.L. Opara and F.A. Al-Said, 2013. Colour measurement and analysis in fresh and processed foods: A review. Food Bioprocess Technol., 6: 36-60.
38. Manzocco, L., S. Calligaris, D. Mastrocola, M.C. Nicoli and C.R. Lerici, 2000. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci. Technol., 11: 340-346.