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Chemical, Microbiological and Sensory Evaluation of Probiotics Beverages Prepared with Permeate and Rosella

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A B S T R A C T

Probiotics have several advantages for human health. While looking for alternative food matrices, Chemical, microbiological and sensory evaluation of probiotics beverages prepared with different ratios of permeate and rosella were evaluated at zero time and during storage periods at 7, 14 and21 days in refrigerator at 4±1°C. Results indicated that the increase gradually of acidity and decreased of pH values by increasing rosella percentage. During storage periods the pH values were decreased gradually and increasing gradually in acidity in all treatments throughout storage period of 21 days. The results revealed to gradually decrease in total polyphenols and flavonoids contents at 7, 14 and21 days, respectively. The highly decrease in total polyphenols and flavonoids contents were shown in formula 2 prepared with2.0% rosella. In contrast, anthocyanin was highly decreased in formula 4 (6% rosella). Also, negligible decrease was noticed in tannins, Zn and Fe contents during refrigeration storage. These results due to bifidobacteria used polyphenols, flavonoidsto stable of bifidobacteria count during storage, while bifidobacteria left saponins, tannins, Zn and Fe without use. Slightly decrease the number of bifidobacteria during storage, but the minimum allowable is due to the use of antioxidants that limit the deterioration of a number of beneficial bacteria. The rosella permeate beverages prepared with 4.0 % rosella (formula 3) gained the highest score for color, appearance and flavor at zero time of storage and after 21 days of storage at 4°C ± 1°C.

Introduction

Permeate is an important by-product of the treat of milk by using ultra filtration process in cheese industry. Permeate contains lactose as the major constituent in addition to water soluble vitamins and the salts of milk. Therefore, permeate can be considered as a solution of nutritious significance. Permeate retains about 80% of the initial lactose of the treated milk. The milk permeate is practically free of N-compounds and thus unsuitable for animal feeding (Marhamatizadeh *et al.*, 2012). Therefore, permeate can be considered as a solution of nutritious significance. In this respect, Renner and Abd El-Salam (1991) reported that permeate appears as a crystal clear, greenish fluid. Besides lactose, minerals and vitamins are fractioned between the retentate and permeate. This is quite important since permeate itself has to produce an electrolyte beverage and different drinks respectively or carrying out fermentation using yogurt culture or different starters (Abd El-Salam *et*

al., 1991) or LAB containing Lactobacillus helveticus, LH100 (Abd El-Khair, 2009) to produce fruit beverages and sports drink respectively. Bifidobacteria are one of the most important probiotics in dairy products (Parvez *et al.,* 2006; Russell *et al.,* 2011). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Oliveira *et al.,* 2011). Bifidobacteria have positive effects on human health: (1) synthesis of vitamins (Crittenden *et al.,* 2003; Beitane and Ciprovica, 2011); (2) immunostimulation (Dong *et al.,* 2010); (3) cholesterol reduction (Ziarno *et al.,* 2007; Beitane, 2008); (4) lactose hydrolysis (He *et al.,* 2007); and (5) prevention of infectious diarrhea (Qiao *et al.,* 2002). Nutritional benefits of bifidobacteria are genetically determined. Bifidobacteria are saccharolytic and produce organic acids (Russell *et al.,* 2011). The growth of bifidobacteria can be promoted with addition of prebiotics (Rastall and Maitin, 2002). Some of the important health benefits attributed for *Bifidobacterium* spp. are control of undesirable microorganisms in intestinal tract, reduction in serum cholesterol levels (Hughes & Hoover, 1991).

Roselle (*Hibiscus sabdariffa L.*) is a tropical plant which belongs to the family Malvaceae and is in Egypt as Karkadah. It is probably a native of West Africa and is now widely cultivated throughout the tropics and subtropics e. g. Sudan, China, Thailand, Egypt, Mexico, and the West India (El-Saidy *et al.,* 1992). In addition, Roselle juice, which is conventionally made from water extraction of fresh or dried Roselle calyxes, has been reported as being a popular soft drink with daily consumption in many countries including Egypt, Sudan, Mexico, Nigeria and Thailand (Aurelio *et al.,* 2007). The chemical components contained in the flowers of *Hibiscus sabdariffa* include anthocyanins, flavonoids and polyphenols (Tzu-Li Lin *et al.,*2007). The petals are potentially a good source of antioxidant agents as anthocyanins and ascorbic acid (Prenesti *et al.,* 2007). Roselle calyx contains a rich source of dietary fiber, vitamins, minerals and bioactive compounds such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties.(Aurelio *et al.,* 2007). Recently, the biological activities of anthocyanins, such as antioxidant activity and anticarcinogenic activity have been investigated (Tsai *et al.,* 2002).The rosella calyces are rich in anthocyanin, ascorbic acid and hibiscus acid. It is water soluble with brilliant and attractive red color and with sour and agreeable acidic taste which aid digestion (Asolkar *et al.,* 1992).

Studies dealing with the production of fermented rosella extracts used *B. bifidum* bacteria. In this study, the changes of microbial population and some major components in rosella extracts during fermentation with bifidobacteria were investigated. The aim of this is to determine the suitability of rosella extracts as a raw material for the production of UF- permeates based probiotic beverages.

Materials and Methods

Materials

Milk permeates was obtained from Sorad-Garbyia Industrial region, Egypt. It was a by-product from the cow's milk. It was prepared at 50^{o}C using spiral-wound module membrane. The permeate was immediately heated in a water bath at 85^{o}C for 15 min., and then cooled to 4±1^{o}C.

Roselle Calyces (*Hibiscus sabdariffa* L.) were used as source of the natural pigments investigated in the present study. The dried

calyces of Roselle were purchased from a local market in Cairo, Egypt.

The dried Roselle calyces were ground for 3 second using a blender (Braun KMM 30 mill), (Germany). The dried calyces were immediately packed in polyethylene bags and kept at low temperature (4°C) till used.

Bifidobacteria strain (*Bifidibacterium befidum*, Bb.12) was obtained from chr. Hansen, Denmark.

Preparation of Different Formulas

It was prepared by blending cold permeate with2%, 4% or 6% powder rosella calyxes (w/v) and dipping overnight, then the mixing was filtered. After that, sucrose 4% was added to filtrates then pasteurized to 72^{o}Cat 15 min. After pasteurization, the mixtures were cooled at 38^{o}C, and then bifidobacteria (*Bifidibacterium befidum*) were added 1% (10^{8} cfu /g) to mixtures and incubated at 38^{o}C/3 h. The final mixtures were filled into the sterilized bottles and stored at 4^{o}C for21 days. This experiment was carried out in triplicate and all analysis was carried out in triplicate.

Formula (1) permeate+ bifidobacteria,
Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella,
Formula (4) permeate+ bifidobacteria +6% rosella.

Analytical Methods

pH value was measured using a pH meter as described by Ling (1963). Soluble nitrogen (S.N) and titratable acidity was determined by Ling (1963). Total solids were determined using oven drier according to AOAC (2000). Ash, protein and fat contents were determined as reported in AOAC (2000). Lactose was determined in UF-permeate by HPLC (Hewlett Packrd 1040A) detection as given by (Jeon *et al.*, 1984).

Folin-Ciocalteu reagent was used to determine total polyphenols in formulas (Singleton and Rossi, 1965).The content of flavonoids was determined by a Pharmacopeia method (1989). Saponins were determined by the method of Hiai *et al*. (1976). Total anthocyanins content of Roselle extract was determined calorimetrically according to the procedure described by Du and Francis (1973). Whereas, total tannins (as tannic acid) as described by AOAC (2000).

Minerals content were determined after ash using Atomic Absorption Spectrophotometer, Perkin-Elmer 3300 (USA) according to Raganna (1979)

Microbiological analysis

Bifidobacterium bifidum (Bb12) counts were determined according to Dave and Shah (1996) using modified MRS agar supplemented with 0.05% L-cysteine and 0.3% lithium chloride. The plates were anaerobically incubated at 37^{o}C for 48 hrs. Molds &Yeasts were determined according to Standard Methods for Examination of Dairy products (APHA, 1994). Coliforms were determined according to Harrigan and McCance (1996).Spore forms were determined according to Marshall (1993).

Sensory evaluation

Sensory evaluation of the prepared formulas was carried out to fresh and during storage at 21 days by panel tests of 10 judges. The maximum attainable scoring point 10, 10 and 10 points for Color, Taste, Flavour and Overall acceptability (Fasoyiro, *et al.*, 2005).

Statistical Analysis

SPSS for version 17.0 (2011) computer programs was used for statistical analysis.The probability $p< 0.05$ was considered as significant.

Results and Discussion

Chemical composition of dried Roselle calyxes and permeate are presented in Table (1). It could be noticed that dried Roselle calyxes contains 89.20 total solid 12.89 ash, 0.43 fat and 8.22% protein, while UF-permeate contained 5.69 total solid , 0.54 ash, 0.21 protein and 4.42% lactose. On the other hand, calcium, iron and zinc were 604.66, 33.05 and 5.61 mg/100g in dried Roselle calyxes and 40.71, 0.073 and 0.03 mg/100g in permeate, respectively. The permeate contained the highest content of potassium 48.63mg/100g. These results are in agreement with (Adenipeku, 1998) that showed that the rosella calyces contain 11.33 % moisture and 6.90 % protein. The results indicate the nutritional content of calyces compared well literature value. Typical literature values are; carbohydrates (68.75 %), protein (6.71 %) and fat 1.01 %). This may be attributable to the source of calyces (Ameh *et al.*, 2009)

The effect of added rosella calyxes on pH values and titratable acidity of different formula during storage periods at 4 °C are illustrated in Table (2). Total acidity was increased gradually by increasing rosella percentage compared to formula 1 (control). While, the pH value ranged from 6.10 to 5.40 in formula contained gradient rosella percentage. Results indicated that the increase gradually of acidity and decreased of pH values by increasing rosella calyxes percentage. During storage periods the pH values were decreased gradually and increasing gradually in acidity in all treatments throughout storage period of 21 days. The reason for increase in acidity and decrease in pH is due to conversion of lactose to lactic acid during storage period (Nuzhat *et al.*, 2003).

Total polyphenols and flavonoids contents in permeate beverages prepared with adding 2.0, 4.0 and 6.0% dried roselle calyxes during cold storage periods after 21 days at (4 ± 1^{o}C) are presented in Table (3). Data indicated to gradually decrease in total polyphenols and flavonoids contents at 7, 14 and 21 days, respectively.

The highly decrease in total polyphenols and flavonoids contents was found in formula 2 which contained 2% rosella. The microbial (e.g. *Bifidobacterium* sp.) in colon is hydrolyze non-absorbed polyphenols which can further be metabolized to aromatic acids like phenyl acetic, phenyl propionic, phenyl valeric and benzoic acids (Manach *et al.*, 2004, Kroon *et al.*, 2004 and Bosscher *et al.*, 2009), which explain the cause of lowering level of polyphenols and flavonoids content.

Total anthocyanin and tannin contents in permeate beverages prepared with adding 2.0, 4.0 and 6.0% dried rosella calyxes during cold storage periodafter21 days at (4 ± 1^{o}C) are illustrated in Table (4). Data indicated slightly decrease in anthocyanin and negligible decrease in tannins contents during refrigerator storage. In parallel, no change of Zn and Fe contents were noticed within storage Table (5). These results are due to the use of bifidobacteria to polyphenols, flavonoids, to stable of bifidobacteria count during storage, while bifidobacteria left anthocyanin, tannins, Zn and Fe without using.

Table.1 Chemical Properties of *Rosella calyxes* and Permeate (%)

Items	Dry rosella calyxes	Fresh Permeate
Total solid	89.20	5.69
Fat	0.43	--
Protein	8.22	--
Soluble nitrogen	ND	0.43
Ash	12.89	0.61
Lactose	ND	4.42
Potassium mg/100g	22.98	48.63
Iron mg/100g	33.05	0.07
Zinc mg/100g	5.61	0.03
Calcium mg/100g	604.66	40.71

Table.2 Change on Acidity% and pH Values for Formulas during Cold Storage Periods after 21 days at (4±1°C)

Treatments	Acidity %				pH Values			
	0 time	7 days	14days	21 days	0 time	7 days	14 days	21 days
Formula 1	0.16^{d}	0.20^{c}	0.24^{d}	0.27^{d}	6.65^{a}	6.51^{a}	6.30^{a}	5.91^{a}
Formula 2	0.20^{c}	0.22^{bc}	0.27^{c}	0.30^{c}	6.41^{b}	6.10^{b}	6.06^{b}	5.80^{b}
Formula 3	0.25^{b}	0.28^{b}	0.31^{b}	0.39^{b}	5.80^{c}	5.60^{c}	5.15^{c}	4.90^{c}
Formula 4	0.29^{a}	0.32^{a}	0.37^{a}	0.39^{a}	5.84^{d}	5.30^{d}	5.15^{d}	4.88^{cd}

Values with different letters in the same column are significant different at *P*<.0.05

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella

Table.3 Total Polyphenols and Flavonoids Contents (Mg/100ml) in Different Formulas During Cold Storage Periods after 21 Days at (4±1°C)

Treatments	Total Polyphenols				Flavonoids			
	0time	7 days	14 days	21 days	0 time	7 days	14 days	21days
Rosella calyxes	49.20mg/g dry weight				30.40 mg/g dry weight			
Formula 1	2.80^{d}	2.30^{d}	1.80^{d}	1.60^{d}	0.20^{d}	0.18^{d}	0.17^{d}	0.15^{d}
Decrement %		*17.86*	*35.71*	*42.85*		*10.00*	*15.00*	*25.00*
Formula 2	95.08^{c}	70.10^{c}	50.30^{c}	40.28^{c}	60.80^{c}	54.30^{c}	40.16^{c}	33.70^{c}
Decrement %		*25.47*	*47.10*	*57.63*		*10.96*	*33.95*	*44..57*
Formula 3	189.20^{b}	150.30^{b}	120.24^{b}	100.40^{b}	121.20^{b}	109.16^{b}	89.28^{b}	77.24^{b}
Decrement %		*20.56*	*36.45*	*46.39*		*9.93*	*26.33*	*36.27*
Formula 4	278.40^{a}	220.60^{a}	200.16^{a}	175.80^{a}	180.60^{a}	167.20^{a}	140.32^{a}	126.40^{a}
Decrement %		*20.76*	*28.10*	*36.85*		*7.42*	*22.30*	*30.01*

Values with different letters in the same column are significant different at *P*<.0.05.

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella

Table.4 Anthocyanin and Tannins Contents (Mg/100ml) in Different Formulas during Cold Storage Periods after 21 Days at (4±1°C)

Treatments	Anthocyanin				Tannins			
	0 time	7 days	14 days	21 days	0 time	7 days	14 days	21 days
Rosella calyxes	8.20 mg/g dry weight				0.80 mg/g dry weight			
Formula 1	0.0				0.0			
Formula 2	15.14^{c}	14.23^{c}	13.65^{c}	12.59^{c}	1.48^{c}	1.40^{c}	1.32^{c}	1.30^{c}
Formula 3	30.30^{b}	28.33^{b}	26.30^{b}	25.48^{b}	2.60^{b}	2.55^{b}	2.50^{b}	2.44^{b}
Formula 4	46.73^{a}	45.70^{a}	43.45^{a}	42.26^{a}	4.80^{a}	4.73^{a}	4.68^{a}	4.62^{a}

Values with different letters in the same column are significant different at *P*<.0.05.

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella

Table.5 Iron and Zinc in Different Formulas (mg/100ml) during Cold Storage Periods after 21 days at (4±1°C)

Treatments	Fe				Zn			
	0 time	7 days	14 days	21 days	0 time	7 days	14 days	21 days
Rosella calyxes	0.37 mg/ g dry weight				0.0 3mg/ g dry weight			
Formula 1	0.12^{d}	0.12^{d}	0.11^{d}	0.10^{d}	0.06^{d}	0.06^{d}	0.06^{d}	0.06^{d}
Formula 2	0.76^{c}	0.75^{c}	0.74^{c}	0.74^{c}	0.18^{c}	0.18^{c}	0.18^{c}	0.17^{c}
Formula 3	1.42^{b}	1.42^{b}	1.41^{b}	1.40^{b}	0.28^{b}	0.28^{b}	0.28^{b}	0.27^{b}
Formula 4	2.21^{a}	2.20^{a}	2.20^{a}	2.19^{a}	0.40^{a}	0.40^{a}	0.39^{a}	0.39^{a}

Values with different letters in the same column are significant different at *P*<.0.05

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella

Table.6 Bifidobacterial Counts (log cfu/g) for Formulas during Cold Storage Periods after 21 days at (4±1°C)

Microorganisms	Treatments	Period storage			
		0-time	7 days	14 days	21 days
Bifidobacterial count (log cfu/g)	Formula 1	8.97^{a}	8.86^{a}	8.74^{a}	8.32^{a}
	Formula 2	8.94^{b}	8.31^{b}	8.02^{b}	7.78^{b}
	Formula 3	8.94^{b}	8.11^{c}	7.75^{c}	7.24^{c}
	Formula 4	8.91^{d}	7.41^{d}	7.12^{d}	7.06^{d}

Values with different letters in the same column are significant different at *P*<.0.05.

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella
CFU= Colony for Unit

Table.7 Sensory Evaluation of Different Formulas during Cold Storage at 4 ±1°C For 21 Days

Treatment	Colour (10)	Taste (10)	Flavour (10)	Over acceptability (30)
Zero Time				
Formula 1	4^{c}	3^{a}	4^{b}	11^{d}
Formula 2	6^{d}	6^{d}	7^{c}	19^{b}
Formula 3	10^{b}	9^{c}	10^{a}	29^{a}
Formula 4	10^{b}	8^{c}	7^{c}	25^{a}
After 21 days				
Formula 1	4^{a}	2^{d}	3^{c}	9^{b}
Formula 2	6^{d}	6^{b}	6^{a}	18^{c}
Formula 3	10^{c}	8^{a}	10^{b}	28^{a}
Formula 4	10^{c}	6^{b}	7^{a}	23^{d}

Values with different letters in the same column are significant different at *P*<.0.05.

Formula (1) permeate+ bifidobacteria Formula (2) permeate+ bifidobacteria +2% rosella
Formula (3) permeate+ bifidobacteria +4% rosella Formula (4) permeate+ bifidobacteria +6% rosella

Microbial Count

The effect of added 2.0, 4.0 and 6.0% rosella calyxes on Bifidobacterial counts (cfu/g) for permeate beverages during cold storage periods at 4 °C are illustrated in Table (6). The change of the viable counts of bifidobacteria during storage period is represented in the same table. Data clearly indicated that the viability of bifidobacteria remained high for 14 days then started to decline. Concerning the therapeutic benefits the product should contain at least 10^{6}viable cell of bifidobacteria per gram to realize its therapeutic properties (Lee *et al.,* 1996; and Salem and El-Shirbiny, 2003). As can be seen in the same table probiotic beverage fortified with rosella showed this count remained more suitable during storage till 21 days. The stability of bifidobacterium bifidum counts during storage may be due to the use of antioxidants by bifidobacteria to defiance the degradation of useful bacterial counts.

As result of high hygienic conditions during process and storage, Mould and Yeast counts, Coliform bacterial count and spore forms count were not detected in all treatment when fresh during storage period for permeate beverage.

Sensory Evaluation

Sensory evaluation of permeate beverages prepared with 2.0, 4.0 and 6.0% dried rosella calyxes during cold storage period are shown in Table (7). The rosella permeate beverages prepared with 4.0 % rosella (formula 3) gained the highest score for color, appearance and flavor at zero time of storage and after 21 days of storage at 4°C ± 1°C. Therefore, these samples came in the first order on comparison with other prepared beverages up to 21 days of storage. This treatment was the most acceptable formula followed with permeate beverages prepared with 6.0% dried rosella (formula 4) and permeate beverages prepared with 2.0% dried rosella (formula 2) while permeate beverages prepared without dried rosella (formula 1) were unacceptable blends since they had been given the lowest scores.

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