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Production of Functional Ice Milk Supplemented with Watermelon Seeds Embryonic Leaves Powder

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ABSTRACT

The objective of this research was to study the production of ice milk supplemented with watermelon seeds embryonic leaves powder (WSELP). Mixes of ice milk containing 2%, 4% and 6% (WSELP) were prepared. Chemical composition of (WSELP) indicated higher total phenolics and radical scavenging activity (RSA). The highest total solids, fat content, protein content, ash content, caloric value, pH, viscosity and overrun were observed in the samples supplemented with (WSELP) and this increasing was proportional to the supplementation ratio. While ice milk samples containing (WSELP) were the lowest acidity (as lactic acid) scores. The ice milk samples prepared with 6% (WSELP) had the highest scores when judged sensory and the degrees of sensory acceptance matched the control. The total plate count and psychrotrophic bacteria count of all produced ice milk formulations were within the safe levels, and the moulds & yeasts were not detected.

Keywords: Antioxidant activity, *Citrullus Lanatus*, Ice milk mixes, embryonic leaves.

INTRODUCTION

The fruits and vegetables produced worldwide finish up as wastes. Most wastes are generated by industrial processing, the so-called by-products. These by-products still contain many bioactive compounds, such as macronutrients (proteins and carbohydrates) and phytochemicals. Recently, the recovery of those bioactive compounds from industrial by-products had received significant attention mainly due to their possible health benefits (Coman et al., 2020).

By-products obtained from fruit processing wastes contain rich sources of dietary fibers and bioactive compounds (Balasundram et al., 2006). Researchers have been concerned on healthy foods that have functional properties and high in nutraceuticals (Bhat and Bhat, 2012). The consumption of functional food (FF) is increasing rapidly worldwide because of increased consumer's awareness about the importance of health and diet (Salem, et al., 2005). FF is food considered to provide benefits behind basic nutrition importance and can play a role in reducing or minimizing the risk of certain diseases and other health conditions. New food products are being developed to include beneficial components such as probiotics and functional components isolated from plants (Shori and Baba, 2014).

Ice cream is a product commonly accepted and enjoyed by people of all ages due to its cooling effect. The nutritive value of ice cream is high, as it is a milk-based dessert (Karaman et al., 2014). Ice cream market amounted to 5.75 billion liters (USDA, 2012). It comprised 86.7% of the total volume of all frozen desserts (Kilara and Chandan, 2013). However, several chronic diseases found to be correlated with fat rich foods such as obesity, cardiovascular diseases and cancer (Fenelon and Guinee, 2000). Therefore, there is a growing demand for low fat products which retain

the desirable characteristics of full-fat products (Romeih et al., 2002)

Ice milk is a frozen dairy product, which gaining in popularity. Approximately 50% of the ice milk production is sold as a soft-frozen product. Ice milk is a frozen product that contains total milk solids not less than 11 %, and not less than 2% and not more than 7% fat (Bikheet et al., 2018).

The watermelon (*Citrullus Lanatus*) seeds are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for instance used to prepare used for prepare sauces, milled into flour and used for snacks (Oyeleke et al. 2012). In spite of the various potential applications, the watermelon seeds are often discarded while the fruit is eaten. There is also limited literature on the effect of variety on the nutritional, antioxidant properties and phytochemical of the watermelon seeds (Tabiri et al., 2016).

The objective of this study was to evaluate the feasibility of using watermelon seeds embryonic leaves powder as enriching bioactive and important functional components in ice milk processing.

MATERIALS AND METHODS

Materials

Fresh morning cow's milk (4.2 % fat) was purchased from a private farm in Zagazig City, Egypt, and standardized to 3% fat by partial cream separation. The average of total solids content of the standardized cow's milk was 11.9%. The contents of fat, protein, ash and acidity (as lactic acid %) were 3.00, 3.53, 0.75, 0.18%, respectively.

Crimson sweet watermelon seeds, Sugar (sucrose) and vanilla powder were purchased from local market (Zagazig City, El-Sharkia Governorate, Egypt). Stabilizer

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which was used for ice milk preparation was Lacta 515B (E440, E1422, E471) and was obtained from Mifad (Misr Food additives company Egypt).

Chemicals and reagents for determination of total phenolic content (TPC) and radical scavenging activity (RSA%) were obtained from Sigma-Aldrich Co. (St. Louis, Mo., USA). Other chemicals were obtained from El-Gomhouria company, Egypt.

Methods

Preparation of watermelon seeds powder (WSELP)

Crimson sweet watermelon seeds were dried in hot air oven at 45°C for a period of 48 h. The dried watermelon seeds were shelled manually, and watermelon seeds embryonic leaves ground into fine powder using electrical blender. The fine powder was stored in a plastic container in the deep freezer until used.

Manufacture of ice milk mixes and ice milk

Mixes and ice milk were prepared in the dairy products manufacturing unit, Department of Food Science, Faculty of Agriculture, Zagazig University as described by Marshal and Arbuckle (1996). Table 1 shows the formulation of different prepared mixes. Five kg of the control mix were standardized to contain 3% fat, 15% sugar, 0.7% stabilizer, and 0.0001% vanillin. The other three mixes (T1, T2 and T3) were made by supplementation the mixes with (WSELP) at level of 2, 4 and 6 %, respectively. The prepared ice milk mixes were heated at 72±1°C for 30 min., mixed by using high speed mixer and then kept for 6 h, for aging at 4±1°C. Vanilla powder was added during cooling and aging. The different mixes were aged for 2 h, freezed and whipped in ice cream maker (Taylormate TM Model 152, Taylor Company, Blackhawk Blvd, USA). The resultant ice milk of all treatment was filled in 100 mL plastic cups, covered, hardened at -18°C for 24 h. and stored until analyzed.

Table 1. Formulation of different ice milk mixes

Ingredient (g)	Treatment			
	C	T1	T2	T3
Milk 4% fat	5000	5000	5000	5000
Sugar (WSELP)	750	750	750	750
Stabilizer/Emulsifier	-----	100	200	300
Vanillin	25.00	25.00	25.00	25.00
	0.50	0.50	0.50	0.50

(WSELP): Watermelon seeds embryonic leaves powder

C= Control ice milk ,T1= Ice milk supplemented with 2.00% (WSELP),

T2= Ice milk supplemented with 4.00% (WSELP),

T3= Ice milk supplemented with 6.00 % (WSELP).

Chemical analysis of water melon seeds embryonic leaves powder and ice milk mixes

All ice milk samples were analyzed for total solids contents, total protein, fat, ash, and crude fiber, as described by AOAC (2007). The carbohydrate content was calculated as follows:

$$\text{Carbohydrate} = \text{TS} - (\text{fat} + \text{protein} + \text{ash})$$

Determination of nutritive value

The nutritional values were evaluated as per the formula used by Nile and Khobragade (2009).

$$\text{Nutritive value} = (9 \times \text{percentage of fat}) + (4 \times \text{percentage of protein}) + (4 \times \text{percentage of carbohydrate}).$$

Total phenolic content (TPC)

The concentration of TPC in watermelon seeds powder embryonic leaves (WSELP) was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999) and expressed as mg GAE/100g.

Determination of radical scavenging activity (RSA %)

RSA of watermelon seeds powder embryonic leaves (WSELP) was measured by bleaching of the purple-coloured solution of DPPH, according to Brand-Williams *et al.* (1995).

Percentage of antioxidant activity of DPPH was calculated as follows:

$$\text{RSA (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where

A₀ is the absorbance of the control reaction, and A₁ is the absorbance of the extract. Samples were analyzed in triplicate.

The titratable acidity was measured as described by AOAC (2007). pH values were determined using a digital pH meter (HANNA, Electric Instruments Limited).

The Physical Analysis of ice milk

The specific gravity of each mix was measured by weighing a fixed volume (Goff and Hartel, 2013).

The overrun of the ice milk as the increase in volume of the ice milk over the volume of the mix due to the incorporation of air, this is known as percentages overrun (Arbuckle, 1986). The following formulation could be used.

$$\% \text{ Overrun} = [(\text{Volume of ice milk} - \text{Volume of ice mix}) / \text{Volume of ice mix}] \times 100.$$

Using Rotational Viscometer Type (Lab. Line Model 5437), the viscosity of ice milk was measured according to the method of Aryana (2003), Results expressed as CPS.

Microbiological examination

Ten grams of ice milk were added to 90 ml of sterile buffered peptone water (Merck, Darmstadt, Germany) and homogenized. The samples were serially diluted with maximum recovery diluent. The total plate count (TPC) was determined using the plate count agar method (Houghtby *et al.*, 1992). Samples for the determination of psychrotrophic bacteria count spread on plate count agar + 1% skim milk powder (97% TS, product of Dairy America™, USA). The plates were incubated at 6.5°C for 10 days (ISO, 2001). Yeasts & moulds counts were enumerated according to Marshall (1992).

Sensory evaluation

Sensory evaluation of ice milk treatments were carried out according to (Homayouni *et al.*, 2008), performed by a group of 10 panelists who were experienced academicians from the Department of Food Science Faculty of Agriculture ,Zagazig University. The ice milk samples (100 g) were evaluated using a sensory rating scale of 1-10 for (flavour & taste), and 1-5 for (body & texture) and 1-5 for (colour & appearance).

Statistical analysis

The obtained results were evaluated statistically using analysis of variance as reported by McClave and Benson (1991).

RESULTS AND DISCUSSION

Chemical composition and antioxidant properties of water melon seeds embryonic leaves powder

The proximate chemical composition of (WSELP) is illustrated in Table 2. Fat /dry matter, protein / dry matter, carbohydrate, crude fibers and ash contents of water melon seeds embryonic leaves powder were (26.99, 49.62, 9. 81, 3.49 and 3.91 g/100g respectively, while, nutritional value of water melon seeds embryonic leaves powder was 450.38

K Cal /100 g. These results are in harmony with the results stated by Lakshmi and Purnima, (2011).

Total phenolic content TPC of ethanolic extract of (WSELP) were 3560 mg/100g. While the radical scavenging activity RSA (%) of ethanolic extract was 84.80 %. These results are in agreement with those previously reported by Tabiri *et al.*, (2016) who studied antioxidant activity of (WSELP) extract. Therefore, water melon seeds could be a good source of many of bioactive compounds which have high antioxidant potential.

Table 2. Nutrition value and proximate chemical analysis of water melon seeds embryonic leaves powder.

Parameter	Means ± S.D
Nutritional value	450.38 ± 1.04 K Cal /100 gm
Moisture	6.86± 0.22%
Total solids	93.14± 1.14%
Fat/dry matter	26.99± 1.10%
Protein/dry matter	49.62±1.43 %
Carbohydrate	9.81± 0.20%
Crude fibers	3.49± 0.13%
Ash	3.91± 0.07%
Total Phenol (mg GAE/100g)	3560±85.72
Radical scavenging activity (RSA %)	84.80±0.70

Nutritional value and chemical composition of ice milk supplemented with water melon seeds embryonic leaves powder

Table 3 shows the significant increasing in caloric value with increasing ratio of supplementation with (WSELP) and the highest value was noticed in ice milk supplemented with 6.0% (WSELP) and the lowest value was given for the control. This may be explained on the basis that (WSELP) had high content of protein, fat and carbohydrate. Similar results were stated by Tabiri *et al.*,(2016).

Also, Table 3 shows the gross chemical composition of ice milk as affected by the supplementation with (WSELP) in the internal milk. The results reveal that the total solids content and fat content significantly increased with increasing ratio of (WSELP), and the highest value was found in ice milk supplemented with 6 % (WSELP), whereas the lowest one was given for the control treatment. This may be due to the raising of total solid in (WSELP). Hassan *et al.* (2008) reported that de-hulled watermelon seeds powder contain about 50% fat and 35% protein. The seeds have been reported to be rich in lipids (Wani *et al.*, 2008).

Protein content also significantly increased with increasing ratio of supplementation with (WSELP), the ice milk supplemented with 6% (WSELP) had a higher value than the other treatments. This may be due to that (WSELP) contains high fat and protein more than cow’s milk. Tabiri *et al.* (2016) reported that watermelon seeds powder contained high protein content.

The ash content also increased as the proportion of (WSELP) increased. This could be due to the fact that (WSELP) der has high ash content and minerals (Tabiri *et al.*, 2016). The high ash values in ice milk supplemented with (WSELP) agree with the results obtained by (Akalın *et al.*, 2018), who produced probiotic ice cream with different dietary fibers.

Also, Table 3. shows the titratable acidity of control ice milk and ice milk supplemented with (WSELP). The acidity significantly decreased and pH increased with increasing ratio of (WSELP), the control ice milk had a higher value than the treatments. These may be due to antimicrobial activity of (WSELP) as reported by (Adelani *et al.*, 2015). These results are in agreement with Abd El-Aziz *et al.* (2015), who produced ice cream supplemented with cress seed and flaxseed mucilage.

Physical analysis of ice milk supplemented with (WSELP)

Results presented in Table 4 revealed that supplementation of ice milk with (WSELP) caused a significant increase in the viscosity compared with control treatment, and this increasing was proportional to the supplementation ratio .These results might be due to a high content of total solids, fiber and fat of (WSELP). Results are in agreement with those reported by El-Dardiry and Gab-Allah (2016) who found that supplementation of ice cream with quinoa powder increased the viscosity of ice cream mixes.

Table 3. Chemical composition of ice milk supplemented with (WSELP) after storage at –18°C for 24 h.

Property	Treatment			
	C1	T1	T2	T3
Calories K Cal /100 gm	135.99±2.10 ^b	140.1±5.18 ^a	144.78±5.08 ^a	145.68±5.22 ^a
Total solids (%)	28.34±2.08 ^c	30.45±2.00 ^b	32.50±1.98 ^{ab}	33.80±1.84 ^a
Fat (%)	5.10±0.64 ^a	5.16±0.58 ^a	5.22±0.60 ^a	5.28±0.66 ^a
Total protein(%)	3.62±1.02 ^c	4.60±0.88 ^b	5.68±0.94 ^a	5.90±0.82 ^a
Ash (%)	0.725±0.08 ^d	1.81±0.06 ^c	2.83±0.10 ^b	3.98±0.05 ^a
Carbohydrate	18.90±1.18 ^a	18.88±1.12 ^a	18.77±1.08 ^a	18.64±1.02 ^a
Acidity (as lactic acid %)	0.42±0.02 ^a	0.38±0.01 ^b	0.33±0.06 ^c	0.30±0.02 ^d
pH	6.5±0.04 ^d	6.60±0.01 ^c	6.62±0.02 ^b	6.68±0.05 ^a

(WSELP): Watermelon seeds embryonic leaves powder
 C= Control Ice milk,T1= Ice milk supplemented with 2.00% (WSELP),
 T2= Ice milk supplemented with 4.00% (WSELP),
 T3= Ice milk supplemented with 6.00 % (WSELP).

The proportional supplementation with (WSELP) in ice milk mixes processing was associated positively whereas, it caused a significant increase in the specific gravity of the mix compare with the control. Similar results were reported in 75% ultrafiltration retentates ice cream by Mohamed (1997), while (Khalil and Blassey, 2016) noticed that the specific gravity was decreased with added roasted date seeds powder which may attributed to air incorporation into ice milk mix in the pre-freezing process decreased the specific gravity.

Effect of supplemented with (WSELP) on volume expansion (overrun)

Air cells are incorporated into ice milk mix in the pre-freezing process and lead to an increase in ice milk volume. Overrun is an important parameter in ice milk evaluation. This due to a rising in the volume of ice milk during processing (Cruz *et al.*, 2009). As shown in Table 4, the highest overrun were noticed in the samples supplemented with (WSELP) and this increasing was proportional to the supplementation ratio, while the lowest overrun was determined in control sample. This may be due to increasing the viscosity of (WSELP) supplemented ice milk. An increase in the viscosity of the serum phase

enhances the stabilization of air cells and allows them to be reduced to smaller sizes Den Engelsen (2002). Smaller dispersed air cells produce a creamier mouth feel during consumption Eisner *et al.* (2005), and higher overrun values (Sofjan and Harte, 2003). In turn, higher overrun values result in slower melting, since air cells act as an insulator medium, and overrun increased in reduced-fat ice creams employing inulin was reported by Akalin *et al.* (2008). Dervisoglu and Yazici (2006) found that the use of citrus fiber led to the significant improvement of the melting quality of ice cream but failed to improve viscosity, overrun, and texture.

Table 4. Physical analysis of ice milk supplemented with (WSELP) after storage at -18°C for 24 h.

Properties	Treatment			
	C	T1	T2	T3
Viscosity (CPS)	3400±98.16 ^d	3940±100.11 ^c	4560±94.00 ^b	5100±110.14 ^a
Specific gravity (g/cm ³)	1.1482±0.03 ^d	1.1564±0.04 ^c	1.1650±0.02 ^b	1.1830±0.06 ^a
Overrun (%)	57.5±1.34 ^d	59.0±1.0452 ^c	60.5±1.40 ^b	62.0±1.24 ^a

(WSELP): Watermelon seeds embryonic leaves powder
 C= Control ice milk, T1= Ice milk supplemented with 2.00% (WSELP),
 T2= Ice milk supplemented with 4.00% (WSELP),
 T3= Ice milk supplemented with 6.00 % (WSELP).

Sensory evaluation of ice milk supplemented with (WSELP)

(Flavour & taste), (body & texture), and (colour & appearance) properties of ice milk samples were evaluated as sensory characteristics, as shown in Table 5. In terms of (flavour& taste), a significant difference was found among the control and the experimental ice milk treatments enriched with (WSELP). The ice milk samples prepared with (WSELP) had higher flavour & taste scores. This could be because the good (flavour& taste) of watermelon seeds embryonic leaves. In addition, the panelists perceived an astringent (flavour & taste) in the high ratio of watermelon seeds powder embryonic leaves samples, Generally, the supplementation of ice milk with (WSELP) significantly (P > 0.05) increased the texture characteristic of the samples.

Table 5. Sensory evaluation of ice milk supplemented with (WSELP) after storage at -18°C for 24 h.

Property	Treatment			
	C	T1	T2	T3
Flavour & taste (10)	9.50±0.66 ^a	8.80±0.44 ^a	9.00±0.62 ^a	9.30±0.84 ^a
Body & texture (5)	5.00±0.06 ^a	4.30±0.12 ^d	4.50±0.08 ^c	4.90±0.014 ^b
Colour & appearance (5)	4.50±0.28 ^{bc}	5.0±0.22 ^a	4.90±0.34 ^{ab}	4.80±0.20 ^b
Total acceptability (20)	19.00±0.14 ^a	18.10±0.20 ^c	18.40±0.16 ^b	19.00±0.18 ^a

(WSELP): Watermelon seeds embryonic leaves powder
 C= Control ice milk ,T1= Ice milk supplemented with 2.00% (WSELP),
 T2= Ice milk supplemented with 4.00% (WSELP),
 T3= Ice milk supplemented with 6.00 % (WSELP).

All treatments of ice milk showed similar scores with respect to colour & appearance. In contrast, the highest scores were given to samples supplemented with 6.0% (WSELP), and that on a disagreement with the findings of Abd El-Aziz *et al.* (2015), who produced ice cream supplemented with cress seed and flaxseed mucilage.

Microbiological evaluation of ice milk supplemented with (WSELP)

Microbiological quality of ice milk and ice milk supplemented with (WSELP) are recorded in Table 6. The total plate count of the ice milk and ice milk formulations was within the safe levels. The total plate count for an ice milk product must not be over 150.000 colonies per gram according to Egyptian organization for standardization and Quality (2005).

Psychrotrophic bacteria constitute the bacterial species capable of growing at 5°C and below but multiply quite rapid rapidly at 10 to 25°C. psychrotrophic bacteria most commonly associated with refrigerated foods and cause food spoilage by producing various enzymes (Ray, 2004). The enumeration of psychrotrophic bacteria count in refrigerated foods gives an indication of potential spoilage, keeping quality, or safety of food Cousin *et al.* (2001). However, according to Cousin *et al.* (2001), the psychrotrophic bacteria count in food if less than 10⁴ cfu/mL may be useful in order to predict keeping quality. In this research all samples were in safe value.

Table 6. Microbiological qualities of ice milk supplemented with (WSELP) after storage at -18°C for 24 h.

Property	Treatment			
	C	T1	T2	T3
Total plate count	38 x 10 ³	30 x 10 ³	24 x 10 ³	16 x 10 ³
psychrotrophic bacteria count	26 x 10 ²	34 x 10 ²	54 x 10 ²	64 x 10 ³
Moulds	ND	ND	ND	ND
Yeasts	ND	ND	ND	ND

(WSELP): Watermelon seeds embryonic leaves powder
 C= Control ice milk,T1 = Ice milk supplemented with 2.00% (WSELP),
 T2 = Ice milk supplemented with 4.00% (WSELP),
 T3 = Ice milk supplemented with 6.00 % (WSELP).

Moulds & yeasts were not detected in all ice milk treatments. These results are in agreement with Bikheet *et al.* (2018), who produced ice milk supplemented with natural bioactive components

CONCLUSION

Results recommended the using watermelon seeds embryonic leaves powder in supplementation ice milk up to 6%. The resultant ice milk formulations were accepted in sensory evaluation, high in nutrition value and microbiological results in the safe levels.

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إنتاج مثلج لبني وظيفي مدعم بمسحوق الأوراق الجنينية لبذور البطيخ السيد عبدالستار¹، نهال عبداللطيف عبدالغفار² و ماهيتاب فوزي رمضان³ ¹ قسم تكنولوجيا الأغذية والألبان – كلية التكنولوجيا والتنمية- جامعة الزقازيق. ² قسم الاقتصاد المنزلي – كلية التربية النوعية- جامعة الزقازيق. ³ قسم علوم الأغذية – كلية الزراعة- جامعة الزقازيق.

الهدف من هذا البحث هو دراسة إمكانية إنتاج مثلج لبني وظيفي مدعم بمسحوق الأوراق الجنينية لبذور البطيخ (WSELP). حيث تم عمل مخاليط مثلج لبني تحتوي على 2، 4 و 6% (WSELP) بجانب الكنترول. أشار التحليل الكيميائي لـ (WSELP) إلى ارتفاع محتواه من المركبات الفينولية والنشاط الجامح للشقوق الحرة (RSA). سجلت النتائج أعلى قيم للمواد الصلبة الكلية، محتوى الدهون، محتوى البروتين الكلي، محتوى الرماد، قيمة السرعات الحرارية، الرقم الهيدروجيني، اللزوجة في حالة العينات المدعمة بـ (WSELP) وكانت هذه الزيادة متناسبة طردياً مع نسبة مسحوق الأوراق الجنينية لبذور البطيخ. بينما كانت عينات المثلج اللبني المحتوية على (WSELP) أقل في درجات الحموضة المقطرة كحمض اللاكتيك%. سجلت عينات المثلج اللبني المحضرة باستخدام 6% (WSELP) أعلى درجات عند تحكيمها حسباً وكان درجات القبول الحسي مطابقة للكنترول. ولوحظ أن العد الكلي للبكتيريا وكذلك البكتيريا المحبة للبرودة لكل تركيبات المثلج اللبني الناتج في الحدود الآمنة. وكذلك اثبتت النتائج ان المثلجات اللبنية المتحصل عليها خالية من الفطريات والخمائر.