Supplemented Ice Milk with Natural Bioactive Components from Roselle Calyces and Cinnamon Extracts

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ABSTRACT

The objective of this research was to study the feasibility of using roselle calyces and cinnamon extracts as enriching bioactive components in ice milk making. Formulations of ice milk containing 2%, 4% roselle extract and 10%, 15% cinnamon extract was carried out. The chemical analysis of roselle extract indicated higher total phenolic, total flavonoids, tannic acid, total antioxidant and anthocyanin contents, compared with cinnamon extract. The highest overrun was observed in the control sample and ice milk with roselle calyces extract of 2%. The control samples had the lowest flavonoid, anthocyanin and tannic acid contents, while ice milk samples with roselle extracts 2 and 4% additive ratio were of the greatest concentration of flavonoid, anthocyanin and tannic acid. Phenolic content of most ice milk samples showed slight increases during the storage at -18°C for 30 days. The total antioxidant content (TAC) increased in the ice milk by increasing the ratio of roselle calyces extracts and cinnamon extract. The differences in sensory scores of ice milk samples were no statistically significant (P>0.05) in the colour, flavour, taste, mouth feel and texture. The total bacterial count of the ice milk formulations was within the safe levels.

Keywords: Ice milk, bioactive components, Roselle Calyces, Cinnamon.

INTRODUCTION

Ice cream is a very popular frozen dessert among consumers of all ages in many countries (Makarem, et al., 2014, Vadiveloo, et al., 2014). It is commonly enjoyed by people of all ages due to its cooling effect and the nutritive value of ice cream is high, as it is a milk-based dessert.

Ice milk, sherbets, mellorine- and parevine-type products, other frozen dairy products, and water ices have been responsible for over 75% of the rise in per capita sales of all frozen desserts. Ice milk is gaining in popularity. Approximately 50% of the ice milk production is sold as a soft-frozen product, and ice milk accounts for 75% of the soft-frozen product gallonage (Douglas and Richard, 2013). Ice milk is a frozen product that contains not less than 2% and not more than 7% fat, and not less than 11% total milk solids (Arbuckle, 1986).

Ice cream is one of the most consumed dairy products in the world (Gorski, 1997) but the ice cream available commercially is generally poor in natural antioxidants like vitamin C, colors and polyphenols. Improvement of the nutritional attributes of ice cream using ingredients with health benefits, focusing on natural antioxidants, natural colorants, vitamins, low fat and freedom from synthetic additives in light of consumer expectations (Sun-Waterhouse et al., 2013).

Bioactive compounds are phytochemicals found in foods that are capable of modulating metabolic processes and resulting in the promotion of better health. They exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression (Correia, et al., 2012).

Bioactive compounds (phytonutrients) are health-promoting compounds that can lower the risk of heart disease, cancer and other diseases; they vary widely in chemical structure and function and are grouped accordingly. Phenolic compounds, including their sub category, flavonoids, are present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, and tea. Many phenolic compounds have antioxidant properties, and some studies have demonstrated favorable effects on thrombosis and tumorgenesis and promotion (Kris-Etherton et al., 2002).

Later, many studies are available where in flowers or their extracts have been shown to exhibit rich bioactive and antimicrobial properties (Shyu et al., 2009; Jo et al., 2012).

Roselle (Hibiscus) pertain to the family of Malvaceae, it is high quality source of natural bioactive content and color, providing even higher levels compare with traditional sources such as raspberries and blueberries (Daniel et al., 2012). Hibiscus flower extract (HFE) have been reported to contain higher antioxidant property when extracted with water (Prenesti et al., 2007).

Cinnamon (Cinnamomum sp.) is one of the very frequently used spices in many countries since ancient time. It is often added to food for better taste and aroma of food. It is even used as part of Ayurvedic treatments in India. Cinnamon bark is a common culinary spice used in Malaysian curry formulations. Its availability along the year makes it further popular. Lately, cinnamon bark has been reported to be possessing potent antioxidants (Singh et al., 2007; Chan et al., 2012a); comparable to that of synthetic antioxidants (Mathew and Abraham, 2006).

MATERIALS AND METHODS

Raw materials:
Roselle calyces extract (RE)
Freshly harvested roselle calyces (Hibiscus sabdariffa) was obtained from Horticulture Research Laboratory, Mallawi Agricultural Research Station (Minia, Egypt). They dried on the day of purchase, using a mixed-mode forced convection solar dryer at 50±3°C., roselle calyces were ground for using a grinder.

The proportion of roselle calyces to water extraction included 1:10 ratio of dried calyces to water. Meanwhile, the temperature of 50°C for 30 min rosellewas extraction, extract was filtered with a cheese cloth. (Chumsri et al., 2008).

Cinnamon extracts (CE)
Dried cinnamon barks were ground for using a grinder, added to hot water (100 °C) at ratio of 1:20. The mixture was stirred using a magnetic stirrer for 30 min and filtered through a cheese cloth (Chan et al., 2014).
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Milk sample

Cow milk was obtained from the Mallawi Agricultural Research station, Minia, Egypt.

Procedure for manufacturing ice milk

Ice milk mix with cow’s milk, flavoring materials (vanilla at the rate of 2%), 0.07% stabilizer (gelatin), 19.8% sugar, 0.79% corn syrup solids was prepared. The mix was pasteurized at 72°C for 15 s (Stork Friesand) and then aged overnight at 4°C. The required amounts of Roselle calyces extract and cinnamon extract were added throwing frozen in an ice milk machine (model) at 2%, 4% for Roselle extract and 10%, 15% for cinnamon extract. The resultant ice milk was packaged in cups (100 cc) and kept in a freezing cabinet at −18°C for 24 hours at least before evaluation, as suggested by Sommer (1951).

Cont: control without addition ratio, R2: Ice milk with Roselle calyces extract addition ratio 2%, R4: Ice milk with Roselle calyces extract addition ratio 4%, C10: Ice milk with Cinnamon extract addition ratio 10%, C15: Ice milk with Cinnamon extract addition 15%.

Analytical methods

Total soluble solids

was determined according to AOAC (2002).

Total solid content

Total solid content of the both ice milk were determined gravimetrically by drying a sample to constant weight in an oven at 105°C. The difference in weight before and after drying for 4-5 hours at 105°C gives the results of total solid content (Method 33.2.44; 990.20, AOAC, 2002).

pH values

of samples were determined according to the methods of AOAC (2002).

Titratable acidity

was determined according to Adekunle et al., (2010).

Ash content:

Ash were determining according to AOAC (2002). Weight the sample and ash was prepared in muffle furnace.

Total bacterial count

Count of bacteria were determined using NA (nutrient agar) and the count of fungi, yeast were determined using PDA (potato dextrose agar) (APHA, 1994).

Determination of anthocyanins

Anthocyanin as cyanidin-3-glycoside mg/100g pigment was measured following the method described by Ranganna (1977).

Determination of total phenols

Estimation of total phenols was carried out according to Musa et al., (2011) using Folin-Ciocalteu reagent, the results were expressed as mg of gallic acid equivalents/100g of sample.

Determination of total flavonoids

Total flavonoid content was determined by the colorimetric method as described by Abu Bakar et al., (2009), the results were expressed as mg of quercetin equivalents/100g of sample.

Determination of total tannin

Method of Schanderl (1970) was employed for determination of water soluble tannins by the colorimetric method. Water soluble tannins were estimated and calculated with the help of standard curve of tannic acid (0.1mg/mL) and expressed as mg.100g⁻¹.

Determination of total antioxidant capacity

The determination of total antioxidant activity was done as per the phospho-molybdenum method with some modifications (Kanika et al., 2015). 0.3 ml extract was combined with a mixture of 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were then capped and incubated at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the solution was then measured at 695 nm against blank. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the mg of equivalents of ascorbic acid.

The Overrun

Overrun in the ice milk as the increase in volume of the ice milk over the volume of the mix due to the incorporation of air. When expressed as a percentage of the volume of the mix, this is known as percentage overrun. There are two basic ways to calculate the percentage of overrun: by volume and by weight. Each way has three variations. Calculation by Volume (Arbuckle, 1986) simple formulation. When calculating the percentage overrun for plain ice milk or when an approximation is all that is required for a flavored ice milk, the following formulation could be used:

\[
\% Overrun = \frac{Volume \ of \ ice \ milk - Volume \ of \ mix}{Volume \ of \ mix} \times 100
\]

Sensory Analysis

Sensory properties of the ice milk samples were tested by 15 trained panelists using a sensorial rating scale Color and flavor, taste, mouthfeel, texture and experience of ice milk samples were evaluated at the end of the storage by sensory analysis. (Amerine et al., 1965)

Statistical analysis

Experimental results were means ± SE of three parallel measurements. Analysis of variance was performed by ANOVA procedures. GraphPad Software, San Diego, CA, USA (Motulsky, 1999).

RESULTS AND DISCUSSION

The composition of cow’s milk was fat 5%, TS 13.3%, Ash 0.7%, moisture 86.6%, pH 6.3 and acidity 0.19% (Table 1).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Milk</th>
<th>Roselle calyces extract (RE)</th>
<th>Cinnamon extract (CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.3</td>
<td>2.90</td>
<td>6.5</td>
</tr>
<tr>
<td>Moisture%</td>
<td>86.6</td>
<td>99.5</td>
<td>93</td>
</tr>
<tr>
<td>T.S%</td>
<td>13.3</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>Acidity%</td>
<td>0.19</td>
<td>0.7</td>
<td>0.014</td>
</tr>
<tr>
<td>Ash%</td>
<td>0.7</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Fat%</td>
<td>5</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

The Total soluble solid, pH value and titratable acidity of Roselle calyces extract (RE) and cinnamon extract (CE) are presented in Table (1).
The results showed that the addition of roselle calyces extract resulted in the highest content of total soluble solid and titratable acidity (7 and 0.7%), however the lowest value of pH (2.90) was detected, Cinnamon extract had the lowest content of total soluble solid and titratable acidity (0.5 and 0.014%), and the highest value of pH (6.5).

These results were in agreement with the findings of Chumsri, et al. (2008), Abou-Arab et al., (2011) and Da-Costa-Rocha et al., (2014), who reported that roselle calyces extracts were of high content of organic acids.

Table 2. Bioactive components and total antioxidant as mg/100ml of roselle calyces extract (RE) and cinnamon extract (CE).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Samples</th>
<th>Total phenolic</th>
<th>Total flavonoid</th>
<th>Tannic</th>
<th>Total antioxidant</th>
<th>Anthocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytocemical</td>
<td>Roselle calyces</td>
<td>294.34</td>
<td>675.12</td>
<td>150.99</td>
<td>125.46</td>
<td>35.12</td>
</tr>
<tr>
<td>Components</td>
<td>extract (RE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cinnamon extract</td>
<td>110.96</td>
<td>442.67</td>
<td>34.97</td>
<td>33.74</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Roselle calyces extract and cinnamon extract are rich source of bioactive compounds such as organic acids, anthocyanin, flavonoid and polyphenols, some of them with antioxidant properties. (Chumsri, et al., 2008, and Lim T.K. 2014).

The resultent ice milk contains 71.74% moisture, 28.26% T.S and 0.617 % ash. On the other hand rosalla ice milk (2%) contain 71.61 % moisture, 28.39 % T.S and 0.613 % ash, rosalla ice milk (4%) contain 72.69 % moisture, 27.31% T.S and 0.556 % ash, cinnamon ice milk (10%) contain 74.44 % moisture, 25.56% T.S and 0.554 % ash and cinnamon ice milk (15%) contain 75.41 % moisture, 24.59 % T.S and 0.529 % ash, (Table 3).

Table 3. Effect of the addition of the roselle and cinnamon extract on the physicochemical properties of ice milk.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Samples</th>
<th>Ash</th>
<th>Moisture</th>
<th>T.S</th>
<th>Over run %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytocemical</td>
<td>Control</td>
<td>0.617</td>
<td>71.74</td>
<td>28.26</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.613</td>
<td>71.61</td>
<td>28.39</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td>0.554</td>
<td>72.69</td>
<td>27.31</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>C10</td>
<td>0.556</td>
<td>74.44</td>
<td>25.56</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>R15</td>
<td>0.529</td>
<td>75.41</td>
<td>24.59</td>
<td>19</td>
</tr>
</tbody>
</table>

Control: R2: Roselle calyces extract 2%  R4: Roselle calyces extract 4%  C10: Cinnamon extract 10%  C15: Cinnamon extract 15%

Overrun is an important parameter for evaluating an ice milk product. This situation relates to a rising in the volume of ice milk during processing (Cruz et al., 2009). Table (3) showed that the highest overrun were observed in the control sample and R2, while the lowest overrun was determined in other samples. In this study, other sample which has high water content is the source of this experiment. (Sakurai et al., 1996)

The effect of addition of roselle calyces extract, cinnamon extract and storage at 18°C for 30 days on pH value and acidity% of vanilla ice are shown in Fig (1 and 2). As expected, this indicates that the addition of roselle calyces extract lowered the pH value of ice milk to 6.49 and 6.29, in addition 2% and 4% roselle calyces extract to ice milk, compared with control sample (6.71) and increased the acidity content to 2.10 and 2.48 %, compared with control sample. On the contrary, cinnamon extract decreased acidity content with increased addition percentage, compared with control sample . This results might be due to the high content of organic acids in roselle calyces extracts.

Fig 1. The effect of additive of roselle calyces extract, cinnamon extract and storage at -18°C for 30 days on pH value and acidity% of ice milk.

Cont: control R2: Roselle calyces extract 2%  R4: Roselle calyces extract 4%  C10: Cinnamon extract 10%  C15: Cinnamon extract 15%

Additionally, increase of pH values were in most ice milk samples during the storage, however acidity content of most ice milk samples decreased. These results were in agreement with those recorded by Forough et al., (2015), who reported that the reduction in pH could be due to an increase in the concentration of organic acids.
Increases were observed at pH values in all ice cream samples during the storage (Sagdic et al., 2012).

Figure (2) recorded the total phenolic contents of ice milk samples. Control samples had the lowest phenolic contents, however ice milk with cinnamon extract had the highest content. Total phenolic contents of ice milks were in the range of 2533.43 – 2818.71 mg/100g. Slight increase of phenolic content of all ice milk samples during the storage at -18°C for 30 days was detected. These results were in agreement with those recorded by Topdas et al., (2017) and Forough et al., (2015).

Figure (4) recorded the anthocyanin content (AC) mg/100g of ice milk samples. Control samples had the lowest anthocyanin contents, however roselle calyxes extracts 2 and 4% additive ratio had the highest content. Anthocyanin contents of ice milks were in the range of 0.09 – 1.57 mg/100g. Considerable decrease of anthocyanin content of all ice milk samples was found during the storage at -18°C for 30 days. These results were in agreement with those recorded by Manuel et al., (2014), who declared that the higher content ratio of roselle calyxes extracts added to the ice cream imparts more anthocyanins.
antioxidant content (TAC) mg/100g as ascorbic acid of ice milk is illustrated in Fig. (6).

![Fig 6: The effect of additive of roselle calyces extract, cinnamon extract and storage at -18°C for 30 days on total antioxidant content (TAC) mg/100g as ascorbic acid of ice milk. Cont: control R2: Roselle calyces extract 2% R4: Roselle calyces extract 4% C10: Cinnamon extract 10% C15: Cinnamon extract 15%](image)

Results showed that supplementing ice milk with roselle calyces and cinnamon extract increased the TAC in the ice milk by increasing the rate of their addition. Cinnamon extract 15% additive ratio was paramount in TAC (549.62 mg/100g) to come after roselle calyces extract 4% additive ratio (479.02 mg/100g), cinnamon extract 10% (422.14 mg/100g), roselle calyces extract 4% (391.03 mg/100) and control samples was 333.05 mg/100g). However, the content of TAC decreased gradually during storage at -18°C for 30 days for all treatments. These results were in same line with those cleared by Kanika et al., (2015), who reported that the capacity antioxidant of soy ice cream (505.61 ±9.7) is higher than traditional ice cream (351.1033 ± 4.2 mg Ascorbic acid equivalent/100g ).

Sensory scores of ice milk samples are shown in Table (4). There was no statistically significant difference (P>0.05) in the colour, flavour ,taste, Mouth feel Texture, Experience and overall “liking” scores given by the tasting panels for the ice milk formulas with roselle and cinnamon extract added. All ice milk samples had relatively high sensorial scores after the storage period. indicating that supplements did not have negative effect on overall acceptability of ice milk significantly.

### Table 4. Sensory characteristics of ice milk samples

<table>
<thead>
<tr>
<th>sample</th>
<th>Color</th>
<th>Flavour</th>
<th>Taste</th>
<th>Mouth feel</th>
<th>Texture</th>
<th>Experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.73±0.3</td>
<td>8.53±0.3</td>
<td>8±0.3</td>
<td>8.14±0.4</td>
<td>8.62±0.3</td>
<td>8±0.3</td>
</tr>
<tr>
<td>R2</td>
<td>8.13±0.2</td>
<td>8.47±0.2</td>
<td>8.64±0.3</td>
<td>8.29±0.2</td>
<td>8.62±0.2</td>
<td>8.43±0.3</td>
</tr>
<tr>
<td>R4</td>
<td>7.80±0.3</td>
<td>7.80±0.3</td>
<td>7.93±0.4</td>
<td>7.43±0.4</td>
<td>8.08±0.3</td>
<td>8.00±0.2</td>
</tr>
<tr>
<td>C10</td>
<td>8.27±0.4</td>
<td>8.20±0.3</td>
<td>7.50±0.5</td>
<td>7.50±0.4</td>
<td>8.00±0.4</td>
<td>7.86±0.4</td>
</tr>
<tr>
<td>C15</td>
<td>8.40±0.3</td>
<td>8.27±0.4</td>
<td>7.71±0.5</td>
<td>8.00±0.3</td>
<td>8.08±0.3</td>
<td>7.92±0.3</td>
</tr>
</tbody>
</table>

Microbiological qualities of ice milk with roselle and cinnamon extract are presented in Table (5). The total bacterial count of the ice milk formulations was within the safe levels, the total bacterial count for an ice milk product must not be over 150,000 colonies per gram according to Egypton organization for standardization and Quality (2005).

### Table 5. Microbiological qualities of ice milk with roselle and cinnamon extract

<table>
<thead>
<tr>
<th>control R2: Roselle calyces extract 2% R4: Roselle calyces extract 4% C10: Cinnamon extract 10% C15: Cinnamon extract 15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Zero time</td>
</tr>
<tr>
<td>15 day</td>
</tr>
<tr>
<td>30 day</td>
</tr>
<tr>
<td>Fungi</td>
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<tr>
<td>Zero time</td>
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<tr>
<td>Yeast</td>
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<tr>
<td>Zero time</td>
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<tr>
<td>15 day</td>
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<tr>
<td>30 day</td>
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</tbody>
</table>

CONCLUSION

The results of the current study, led to that the roselle extract and cinnamon are commercially traded as low-cost rich of biologically effective compounds (bioactive compounds) during the preparation of new healthy foods or in nutrition applications. In addition, ice milk products containing roselle and cinnamon extract suggested healthy benefits and were accepted by consumers. The addition of the roselle and cinnamon extract gave the ice milk a more increasing the phytochemicals content (total phenolics, total flavonoids, tannic acid, total antioxidant and anthocyanin content) of the ice milk made.

REFERENCES


