Nutritional composition, acceptability, and shelf stability of carrot pomace-incorporated cookies with special reference to total and β-carotene retention

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Abstract: Dehydrated carrot pomace was assessed for the feasibility of incorporating into baked product by partial substitution of refined flour at 4, 8, and 12% level. As carrot pomace is a good source of antioxidant components and dietary fiber, especially soluble fiber, it was added to the cookies. Carrot pomace and products were analyzed for chemical composition, and products were further evaluated for sensory quality, carotene retention and bioaccessibility, and storage stability. The results showed that pomace contained protein, (6.50%); dietary fiber (44.75%); total carotene, (5,456 μg); and β-carotene (607 μg/100 g). Products with 4% pomace incorporation had highest retention in terms of total carotene (75%) and β-carotene (69%). The highest level of replacement (12%) had a significant adverse effect on the product. Overall acceptability indicated that panel members liked the products up to 8% of added carrot pomace compared to control. Thus, it can be stated that value-added cookies could be advantageous as they are nutrient dense containing dietary fiber and carotenoids.

Subjects: Food Analysis; Fruit & Vegetables; Sensory Science

Keywords: sensory attributes; bioavailable carotenoids; storage; free fatty acids; peroxide value

ABOUT THE AUTHORS

The first author was a senior research fellow working at the University and this is a part of her PhD research work. The research team of senior author has been engaged in product development, sensory studies, and compositional analysis of foods with special reference to digestibility and availability of nutrients for many years with many published papers to her credit. The research focus in present study is to develop nutritious products with high nutrient availability keeping in mind the high level of malnutrition in developing countries. This particular problem focused on utilizing carrot pomace, which is a by-product of carrot juice industry for a shelf-stable carotene-rich product.

PUBLIC INTEREST STATEMENT

The research reported in this paper deals with utilization of a bio-waste material from food industry. Detailed investigation deals with development of a product using carrot pomace, sensory studies to understand its acceptability, and shelf stability studies to know whether the prepared product can be stored with no deterioration. In addition, the retention and bioavailability of total and β-carotene from cookies were also studied to eventually understand whether this product would contribute to carotenoids at the physiological level. From the health point of view, carotenoids are very powerful, antioxidant needed not only for regular functions in the body but also for their disease preventing role. The pigment carotenoid is more stable to heat than any other pigment found in fruits and vegetables; hence, it was incorporated in a baked product keeping in mind the high temperatures used for baking.
1. Introduction

Processing of fruits and vegetables results in high amounts of waste materials such as peels, seeds, pulp residues, etc. Plant wastes are prone to microbial spoilage hence, drying is necessary before further exploitation. The cost of drying, storage, and transport poses additional economical limitations to waste utilization (Oreopoulou & Tzia, 2007). However, valuable nutrients contained in agroindustrial wastes are lost if not utilized suitably. Identification of ways to incorporate peels and pomaces, one of the by-products of fruit and vegetable industry, as a health food ingredient in human diet could provide many health benefits. The interest in food, rich in dietary fiber and antioxidants, increased in recent decades and the importance of these food constituents has led to the development of a large market for fiber- and antioxidant-rich products and ingredients. Intake of dietary fiber and phytochemicals such as polyphenols, carotenoids, tocopherols, and ascorbic acid have been related to the maintenance of health and protection from diseases such as cancer, cardiovascular diseases, and many other degenerative disorders (Saura-Calixto, 2011). Therefore, the potential for the industrial exploitation of pomace as a health food ingredient for the bakery industry and selected functional foods is promising. Various attempts have been made at utilizing carrot pomace in food such as bread, cake, dressing, and for production of functional drinks. Biscuits, breads, and cookies are the most popularly consumed bakery items in the world. Cookies are one of the main items consumed among the bakery products. Cookies hold an essential position in snack foods due to variety in taste, crispiness, ready-to-eat nature, availability in different tastes, and longer shelf life. Carrots are a rich source of β-carotene, a precursor of vitamin A and a proven antioxidant. While carrot pomace can be utilized for value addition to baked product, the retention and bioaccessibility of β-carotene from such products is worth investigating due to high temperatures used in baking and the susceptibility of β-carotene to oxidative deterioration at high temperature. Keeping this in mind, present study was planned to assess the nutritional and sensory properties of cookies incorporated with carrot pomace and to determine its shelf stability particularly with respect to retention of carotenoids and also further bioaccessibility of selected samples for total and β-carotenes.

2. Materials and methods

2.1. Preparation of carrot pomace

Carrot (Daucus carota) was obtained from the local market, cleaned, peeled, grated, homogenized, and filtered for separating juice. The pulp left after juice extraction was dried in an oven at 50 ± 1°C and powdered using a lab grinder and stored in air-tight jars under refrigeration at 4°C till use. This powder was taken for further analysis and product development. Chemicals used for the study: β-carotene was purchased from Sigma (Sigma-Aldrich, USA) Chemical Co, and all others were obtained from E-Merck, Mumbai or Qualigens Fine Chemicals, Mumbai, India. Double-glass-distilled water was used for all analysis. All analyses were run in triplicates and averaged.

2.2. Preparation of product, sensory, and storage studies

Refined wheat flour (42 g), sugar (30 g), and baking powder (1 g) were sieved into a pan. Butter (25 g) and milk powder (2 g) were added to the sieved flour mixture and mixed together. Water was added and kneaded well to make stiff dough. The dough was kept aside for 10 min, kneaded again, and divided into small portions. The dough was rolled to about 4 mm thick and cut using a cutter into small circles. The cookies were baked in a preheated oven at 160°C for 10 min. Refined wheat flour was replaced with dehydrated carrot pomace at 4, 8, and 12% levels. Carrot cookies were coded and subjected to sensory analysis by semi-trained panel members (n = 30) with the help of a grading score card. Control and carrot cookies were stored for 60 days in two packaging materials i.e. polyethylene teraphthalate (PET) and metalized foil at both room and low temperature. Cookies were withdrawn at 0, 20, 40, and 60 days and were analyzed for storage studies.

2.3. Chemical composition and free fatty acid value and peroxide value

The carrot pomace and prepared products were analyzed for moisture, protein, ether extractives, and ash by standard AOAC methods (2005). Dietary fiber consisting of insoluble (IDF) and soluble fractions (SDF) was estimated by the enzymatic gravimetric (Asp, Johansson, Hallmer, & Siljestrom, 1983)
method which is equivalent to physiologically indigestible fiber residue. Free fatty acids (FFA) and peroxide value (PV) were estimated in fat extracted from the products as per AOCS (2000). FFA and PV analysis was continued at 20 days intervals for up to 60 days storage period.

2.4. Total and β-carotene content
Total carotene was extracted in acetone, transferred to petroleum ether phase, and read colorimetrically at 452 nm using petroleum ether for baseline correction. β-Carotene was separated by column chromatography using neutral aluminum oxide as the adsorbent in a 10 cm length adsorbent column. β-Carotene, which moves down the column prior to all the pigments, is collected till the desired pigments have moved off the column and the eluent is colorless. Eluent is made up to a known volume and the intensity of the color is measured in a spectrophotometer at 452 nm using 3% acetone in petroleum ether as blank (Ranganna, 1986).

2.5. In vitro bioaccessible β-carotene assay
The bioaccessibility of β-carotene in vitro was determined by the method of Garrett, Failla, and Sarama (1999). Briefly, the method involved subjecting the sample to simulated gastric digestion at pH 2.0 in the presence of pepsin at 37°C (16 g in 100 ml 0.1 M HCl), followed by simulated intestinal digestion in the presence of pancreatin–bile extract mixture (4 g porcine pancreatin) and 25 g by bile extract (porcine) in 1,000 ml of 0.1 M NaHCO3 pH 7.5 at 37°C for 2 h. At the end of simulated intestinal digestion, the micellar fraction, containing the bioaccessible β-carotene, was separated by ultracentrifugation at 70,000×g for 120 min using an ultracentrifuge. Total as well as bioaccessible β-carotene were determined by extraction of the pro-vitamin from the samples with acetone followed by petroleum ether (60–80°C), and fractionated on neutral alumina using 3% acetone in petroleum ether. The color intensity of β-carotene eluent was measured at 450 nm in a spectrophotometer, and compared with that of β-carotene standard.

2.6. Statistical analysis
The data were analyzed statistically using suitable tests wherever required. Standard deviation and ANOVA were used. The sensory analysis data were subjected to ANOVA and Tukey’s tests to determine the statistically significant differences among the products that were developed. The statistical package used for the above analysis was Excel Stats.

3. Results and discussion

3.1. Chemical composition
The results of the study are summarized in Tables 1–4 and Figures 1–4. The compositional analysis of carrot pomace indicated following values—moisture: 6.54%; protein: 6.50%; soluble fiber: 14.75%; insoluble fiber: 30%; ether extractives: 2.12%, and ash: 5.12% (Table 1). Cookies were prepared from dehydrated pomace at different levels of 4, 8, and 12% and without carrot pomace served as control. Moisture content of the control sample was 2.57% and as the pomace incorporation increased, the moisture content increased i.e. at 12% level, cookies had 4.79%. Protein content was almost similar in control and pomace-incorporated cookies. On applying statistical method, no significant differences were noticed. Fiber content increased as the percentage of carrot pomace increased. TDF content of control samples was 2.48 g/100 g and in 12% pomace-incorporated product, it increased up to 10.18 g/100 g. Highly significant differences were noticed for both soluble and insoluble fiber. Ether extractives for the samples were around 24 g/100 g. Statistically, there was no significant difference observed. Lowest ash content was observed in control sample (0.77 g/100 g), whereas the highest ash content was obtained for 12% carrot-incorporated cookies (1.45 g/100 g). Reports are available on the use of oat bran, wheat bran, and rice bran as a source of dietary fiber content in bread and other bakery products (Laurikainen, Härkönen, Autio, & Poutanen, 1998; Sidhu, Al-Hooti, & Al-Saqer, 1999; Sudha, Baskaran, and Leelavathi (2007) reported the influence of different cereal brans on sensory quality of biscuits. On the other hand, fruit dietary fiber concentrates have better nutritional quality than those found in cereals due to higher proportion of SDF and significant content of dietary fiber-associated bioactive compounds (Chau & Huang, 2003; Grigelmo-Miguel,
The improvement in nutritional properties of cookies and bread with the incorporation of mango dietary fiber obtained from unripe mango fruit (whole fruit) was observed (Vergara-Valencia et al., 2007).

### 3.2. Sensory evaluation

The sensory evaluation is very important criterion in food industry. The cookies prepared with different levels (4, 8, and 12%) of dried carrot pomace were evaluated for their various sensory attributes (Table 1). Analysis was carried out using 30 semi-trained panelists. For the quality of appearance, the range of scores given was 15.53–16.63. Cookies with 4 and 8% levels of carrots had slightly higher range of scores, though statistically there was no difference. Similar trend was seen for color with closer range of 15.13–15.83 of scores with no significant difference. These results indicate that incorporation of carrot pomace did not influence the visual appeal of cookies and the orange color imparted was appreciated by panelists. For the attribute of tastes, the cookies with 4 and 8% carrot were similar to control; however, cookies with 12% carrot had lower scores of 12.53. A similar trend was seen for texture with scores being 12.66, while rest of samples had better scores. This difference was statistically significant. This indicates that a higher level of incorporation of carrot pomace in cookies influenced the taste and texture adversely. This could be due to extremely high content of fiber in carrot pomace, which would tend to make the product rough. For the attribute of aroma, though 12% carrot cookies scored less, the difference was not significant indicating that quality of aroma was not affected. The overall acceptability indicated that panel members liked the products with 4 and 8% carrot more than control, and product with 12% carrot was significantly lesser acceptable than others. Hence, it can be concluded that carrot pomace can be used up to 8% level to incorporate into cookies.

### 3.3. Total and β-carotene content

Cookies were stored in two different packaging materials viz. metalized foil and PET, for a period of 60 days both at room (RT) and low temperatures (LT). Total and β-carotene content of the cookies were measured (Table 1). Total carotenoids of the cookies stored at RT and LT were 5,456 ± 23.78 and 272 ± 2.87, respectively. Cookies stored at RT had higher total carotenoids compared to those stored at LT. Similarly, β-carotene content of the cookies was also higher at RT (607 ± 12.03) compared to LT (ND). These results indicate that packaging material and storage temperature significantly influence the carotenoid content of cookies.
were analyzed at intervals of 0, 20, 40, and 60 day periods and the results are tabulated in Table 2. The initial total carotene content of carrot pomace was 5,456 μg/100 g. The initial total carotene content of cookies in the control sample was 272 μg/100 g, in 4%: 1,278; 8%: 1,550; and 12%: 3,076 μg/100 g (Table 1). Highly significant differences were observed among the samples. The total carotene content during the storage period decreased in both control and treated samples. Thus, it can be said, as the duration of storage increased, there was partial loss of total carotene in the stored product. Highest retention was observed in cookies stored in foil at LT in control (71%) and in samples with 4% (75%) and 8% (72%) carrot pomace-incorporated cookies at the end of 60 days period (Figure 1(A–C)). But in 12% pomace-added cookies, only 40% retention was observed at the end of 60 days storage (Figure 1(D)). It was observed that cookies stored at LT in metalized foil were better compared to PET. Similar results were observed in case of RT-stored cookies which were again better in metalized foil compared to PET storage. Therefore, the percent loss was more in RT.
Table 4. Effect of storage on bioavailable total and β-carotene content of carrot cookies (μg/100 g)

<table>
<thead>
<tr>
<th>Storage</th>
<th>0 day</th>
<th>20 day</th>
<th>40 day</th>
<th>60 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carotene (RT PET)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36 ± 0.57</td>
<td>22 ± 0.57</td>
<td>18 ± 0.43</td>
<td>8 ± 0.14</td>
</tr>
<tr>
<td>4%</td>
<td>46 ± 1.08</td>
<td>23 ± 0.16</td>
<td>15 ± 0.76</td>
<td>11 ± 0.66</td>
</tr>
<tr>
<td>8%</td>
<td>131 ± 1.33</td>
<td>44 ± 0.36</td>
<td>37 ± 0.36</td>
<td>27 ± 0.41</td>
</tr>
<tr>
<td>12%</td>
<td>147 ± 0.23</td>
<td>66 ± 0.07</td>
<td>61 ± 0.55</td>
<td>41 ± 0.01</td>
</tr>
<tr>
<td>β-carotene (RT PET)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>117 ± 0.74</td>
<td>56 ± 1.74</td>
<td>47 ± 1.15</td>
<td>31 ± 0.20</td>
</tr>
<tr>
<td>8%</td>
<td>132 ± 1.18</td>
<td>100 ± 1.32</td>
<td>88 ± 1.30</td>
<td>68 ± 0.60</td>
</tr>
<tr>
<td>12%</td>
<td>206 ± 1.13</td>
<td>128 ± 1.33</td>
<td>120 ± 1.97</td>
<td>97 ± 1.07</td>
</tr>
</tbody>
</table>

Note: RT is the room temperature.

Figure 1. Total carotene retention (%) during the storage period.
Figure 2. β-carotene retention (%) during the storage period.

Figure 3. Effect of storage on FFA content of cookies (g/100 g oil).
compared to LT. Thus, during the 60 days of analysis, the loss was observed in all the samples irrespective of storage in both metalized foil and PET containers. Ranhotra, Gelroth, Langemeier, and Rogers (1995) studied the stability and contribution of β-carotene added to whole wheat bread and crackers and found that the carotene stability was reduced when products were baked, with losses ranging between 4.3 and 14.8% for bread products (proofed doughs vs. fresh breads) and between 17.9 and 22.8% for crackers (sheeted doughs vs. baked crackers). Losses in crackers were higher probably because of their greater relative surface area and more severe baking conditions (lower final product moisture).

The initial β-carotene content of dehydrated carrot pomace was 607 μg/100 g. β-Carotene was absent in control samples. In 4% carrot-incorporated cookies, initial β-carotene content was 126; 8%: 215; and 12%: 333 μg/100 g (Table 1). β-Carotene content decreased during the storage period as in the case of total carotenes (Table 3). Highest retention was observed in cookies stored in foil at LT with 4% (69%), 8% (45%), and in 12% (37%) of added carrot pomace at the end of 60 days storage period (Figure 2(A–C)). The least retention was observed in the case of 12% incorporated cookies. Thus, as the storage period increased, there was significant decrease in the β-carotene content of carrot cookies. The β-carotene content decreased more drastically over a period of 60 days. This loss of β-carotene could be due to non-oxidative changes (cis–trans isomerization, epoxide formation, or heat degradation of tissues) or oxidative changes on exposure to light and oxygen (Aruna, Vimala, & Dhanalakshmi, 1999). Thus, the cookies were best stored at LT in foil compared to PET. In case of RT-stored products also, storage was better in foil compared to PET.
Various factors such as the matrix in which the carotenoids are incorporated, the content of dietary fat, fiber, the particle size, the food processing method, etc. influence the bioavailability of carotenoids from foods (Castenmiller et al., 2000). Selected products which were stored for carotenoid stability studies were taken for further bioaccessibility experiments. On the 0 day in control sample, the bioavailable total carotene was 36 μg/100 g which decreased up to 8 μg/100 g on 60 days of storage. In case of 4% carrot-incorporated cookies, the value was 46 and reduced to 11 μg/100 g, in 8% carrot cookies, the initial value was 131 and on the 60th day, it was 27 μg/100 g and in 12% carrot cookies, the initial value was 147 and decreased to 41 μg/100 g (Table 4). Thus, in control and treated samples, 22–27% retention was observed. In 4, 8, and 12% carrot cookies, there was retention of 26, 51, and 47% of bioavailable β-carotene, respectively. Hence, it can be seen that there was a decrease in bioavailable carotenoids on storage.

3.4. Storage studies
The results of FFA analysis for the products are presented in Figure 3(A–D). Cookies were stored in two different packaging materials i.e. metalized foil and PET jars for a period of 60 days both at low and room temperatures. Cookies were analyzed for lipid oxidation by means of measuring FFA and PV by extracting fat from the cookies on completion of 20, 40, and 60 days. The 0 day values served as control. The initial values or FFA for all products was very low ranging from 0.09 to 0.15% in all cookies. Thereafter, cookies were stored in different containers at room and low temperatures. In general, FFA values did not show major change throughout the storage period. This trend was observed in all the variations prepared. The FFA content of the control sample ranged from 0.09 to 0.14% during storage period. Cookies with 4% level of pomace varied in FFA content from 0.09 to 0.15%, and in cookies with 8 and 12% carrot pomace, the FFA content ranged from 0.10 to 0.15%. Hence, the carrot pomace-incorporated and control samples had similar ranges for the FFA content. A similar trend was seen in case of cookies stored at room temperature in metalized foils.

The cookies stored in PET at low temperature also showed a similar trend. The values were quite comparable with the room temperature-stored cookies. Overall, results indicate that there is an increase in FFA content of all stored products as the duration of storage increased. However, the extent of increase was very low. Among the packaging containers, there was not much difference between the metalized foil and PET in which the cookies were stored. The increase in FFA content could be caused by an increase in the rate of triacylglycerol hydrolysis when moisture content of the product and air inside the container react with absorbed oil of the product. It is reported that during prolonged heating and in the presence of food moisture, hydrolysis of oil occurs and ester linkages are broken to yield FFA resulting in an increase in their concentration (Choe & Min, 2007).

PV is a measure of the amount of peroxides formed in fats and oils through autoxidation and oxidation processes. Indirectly, it measures the initial oxidation of the product. In all the variations and in control, there was a sudden increase in the value at 60 days of storage irrespective of room and low temperatures. This shows that there is slight rancidity occurring at 60 days of storage of cookies. The PV content varied from 0.09 to 0.24 meq/100 g in control samples and in 4, 8, and 12% carrot pomace-incorporated cookies, the PV values ranged from 0.09 to 0.23 meq/100 g samples. But in low-temperature storage, the PV values were less i.e. 0.22 meq/100 g (Figure 4(A–D)) at the end of 60 days storage. This was observed in both metalized foil and PET-stored cookies. Hence, cookies can be stored best under low-temperature storage.

4. Conclusion
The results of the present study indicate that carrot pomace could be considered as an alternative source for formulating products. Products up to 8% were highly acceptable with high carotenoid retention. And also, cookies were better stored in foil and at low temperature. It may be noted that since the incorporation is in dry form, it contributes a significant amount of micronutrients and fiber to the cookies making the product very nutritious.
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