Optimization of Antioxidant Content in Apple (Malus pumila) Squash Formulations during Storage

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Abstract: Apple is known for its vast health benefits, especially antioxidants content. Apple squash is a product of apple pulp, sugar and water and is used as a drink. The objective of this study was to evaluate the impact of formulation and storage time on the antioxidant contents (total phenol and ascorbic acid), physico-chemical parameters (total soluble solids, pH, acidity and browning), and sensory attributes of apple squash. Fresh apple pieces were cooked in 20% (w/w) water and then pulped. Six formulations containing fruit pulp, water, sugar, citric acid, maleic acid, and gaur gum, i.e., T1 (16.67%, 33.33%, 50%, 4.0 g.l⁻¹, 0.0, 0.0), T2 (25%, 25%, 50%, 4.0 g.l⁻¹, 0.0, 0.0), T3 (16.67%, 33.33%, 50%, 4.0 g.l⁻¹, 0.0, 5.0 g.l⁻¹), T4 (25%, 25%, 50%, 4.0 g.l⁻¹, 0.0, 5.0 g.l⁻¹), T5 (16.67%, 33.33%, 50%, 1 g.l⁻¹, 3 g.l⁻¹, 5 g.l⁻¹) and T6 (25%, 25%, 50%, 1.0 g.l⁻¹, 3.0 g.l⁻¹, 5 g.l⁻¹), respectively, were prepared. Ascorbic acid content ranged from 6.06 to 2.58 mg/100 g and was significantly (p<0.001) affected by both formulation and storage. Maximum antioxidant (ascorbic acid and total phenol) content were recorded in treatment T2, during the four month storage. The storage means of total phenol content 550.12, 540.43, 542.85, 539.46 and 534.13 mg.l⁻¹ for 0, 1st, 2nd, 3rd, and 4th month revealed slight decrease in phenolic content but the changes were statistically not significant. Results of pH, acidity and browning revealed significant (p<0.001) differences among formulations and storage periods. Maximum scores for sensory evaluation were recorded for treatment T6 followed by T2. The study revealed that the samples with more pulp and peel (T6, T2 and T4) showed higher antioxidant content and organoleptic acceptability of apple squash during storage.

Key words: Apple squash, Ascorbic acid, Peel, Storage, Quality parameters, Total phenol.

INTRODUCTION

Apple (Malus pumila) is one of the richest source of phenolic compounds and its intake has shown to reduce the risk of generative diseases, such as, cancer, diabetics and cardiovascular complications (Candrawinata et al., 2014). Ascorbic acid content contribute up to 0.4% of the antioxidant activity of apple implying that there are other main contributors, such as, phenolics (Drogoudi et al., 2008). It has been revealed that concentration of phenolic acid flavonoids are found in large quantity with major phenolic compounds, such as, quercetin, catechin, anthocyanins and are influenced by a number of factors, like, cultivars, maturity, environment and a part of the fruit (Yuri et al., 2014). Raudone et al. (2017) studied total phenolic content and antioxidant activity by different methods in six Lithuanias grown apple cultivars. The results depicted that a significant amount of flavonols is present in peel of apple fruit. Similarly, Wang et al. (2015) reported that red fleshed apples had higher amount of total phenolics, flavonols and antioxidant activity, as compared to white fleshed variety. Processing adversely affects the phytochemical content of apple. Apple juice prepared from Jonagold apples by pulping and straight pressing retained only 10% of the antioxidant activity of fresh apples, while juice obtained by enzyming pulp had only 3% of the antioxidant activity. Most of the antioxidant compounds remained in the pomace (Van der Sluis et al., 2002). Similarly, Guyot et al. (2003) found that 42% of the total phenols were availbale in the juice, leaving the rest in the apple pomace. They found that the highest extraction yields in the juice were hydroxycinnamic acids (65%) and dihydrochalcones (80%). Procyanidins had the lowest extraction (32%) in the juice. Apple phenolics, particularly procyanidins, bind with cell wall material, leading to decreased level of polyphenols in apple juice (Ko et al., 2005). Phenolic profiles studies have been conducted on different parts of the apple fruit in cultivars grown in Italy (D’Abrosca et al., 2007) and New Zealand (McGhie et al., 2005). However, little attention has been given to the proper utilization of the phenolic compounds in peel. As discussed above, apple juices retain a very small amount of the original phenols, processed apple peels, largely retain its...
phenolic and flavonoids activity. It can, therefore, be used as a value-added ingredient with potent antioxidant activity (Lata and Tomala, 2007). The objectives of the current work were to standardize a healthy nutritious apple drink by incorporating peel and evaluation of antioxidant and quality parameters during storage.

MATERIALS AND METHODS

Chemicals and reagents: Folin-Ciocalteu reagent (FCR), ascorbic acid, 2, 6-dichlorophenol-indophenol, sodium hydroxide, phenolphthalein, ethanol, citric acid, maleic acid and gaur gum were purchased from Merck (Germany), (+) catechin from sigma (USA), from BDH and sodium carbonate from RDH. Deionized water was used throughout in assay of all parameters.

Sample collection: Fruits (apples green) were purchased from different shops of local markets (1 kg from each shop), Peshawar, Pakistan. The samples from different markets were pooled to form a composite sample, washed with tap water and spread on stainless steel table for some time for removal of adhering water.

Sample preparation: After coring, the whole fruit (flesh + peel) were cut in to four pieces without peeling. Water (20% of whole weight) was added to the fruit followed by cooking. The slurry or pulp was prepared by passing through pulping machine. Six different formulations T1 (16.67% : 33.33% : 50% : 4 g.l⁻¹ : 0 : 0), T2 (25% : 25% : 50% : 4 g.l⁻¹ : 0 : 0), T3 (16.67% : 33.33% : 50% : 4 g.l⁻¹ : 0 : 5 g.l⁻¹), T4 (25% : 25% : 50% : 4 g.l⁻¹ : 0 : 5 g.l⁻¹), T5 (16.67% : 33.33% : 50% : 1 g.l⁻¹ : 3 g/l : 5 g.l⁻¹) and T6 (25% : 25% : 50% : 1 g.l⁻¹ : 3 g/l : 5 g.l⁻¹) of pulp, water, sugar, citric acid, maleic acid and gaur gum, respectively, were prepared. The samples were placed in pre sterilized air tight bottles, labeled and stored at ambient temperature (four months) for periodic analysis of antioxidants, physicochemical parameters and sensory evaluation.

Biochemical Parameters:

Total phenols: Total phenols were extracted by boiling the samples (5 g) in deionized water (50 ml) for half an hour. The extract was filtered and volume was made to 50 ml by adding deionized water. The extracts were prepared according to previously reported (Ashish et al., 2009) with a small modification of taking sample squash instead of taking dried powder to evaluate the antioxidant content of the squash only and thus avoid the drying effect. Total phenolic content of the samples was measured using Folin-Ciocalteu method (Lee et al., 2003). Deionized water (3.9 ml) and known dilution (0.10 ml) of the extract were added to a screw capped test tube of 10 ml. Folin-Ciocalteu phenol reagent (1.0 ml) was added to the solution and allowed to react for 5 min. Then 5 ml of 20% sodium carbonate was aliquoted into the test tube. The absorbance was measured at 720 nm after 20 min with a spectrophotometer (U-1800, Hitachi). The measurements were made from standard curve of (+) catechin.

Ascorbic acid: Vitamin C was measured using the direct colorimetric method, which is based on the discoloration of 2, 6-dichlorophenol-indophenol solution by ascorbic acid in the apple squash samples as well as standard solution of ascorbic acid (AOAC, 1984 method # 43.064). (AOAC, 1984).

Quality parameters:

Total soluble solids (TSS): TSS is the amount of sugar and soluble minerals. It was estimated by means of a hand refractometer (AOAC, 1984).

pH: pH was measured (AOAC, 1984), using an electrotech pH meter (510).

Acidity: Acidity in apple squash samples was measured by taking known quantity of the sample and known volume of distilled water and titrating an aliquot with 0.1 N NaOH using phenolphthalein as indicator (AOAC, 1984).

Browning: Browning values were assayed spectrophotometrically (Srivastava and Sanjeev, 2003).

Sensory evaluation: The apple squash samples were marked with standard random numbers. The samples were then evaluated for appearance, odor and taste using a 10 point hedonic scale by a panel of 10 trained judges (Larmond, 1970). The judges were free to analyze any sample more than once at room temperature under normal laboratory light conditions.

Statistical analysis: The data was statistically analyzed for each of the calculated parameter by analysis of variance (ANOVA - using CRD two-factorial design) and Duncan Multiple Range test (DMR) was used to separate the means by Mstat-C software (SAS, 1996).

RESULTS AND DISCUSSION

Data on effect of treatment and storage on antioxidant content (ascorbic acid) of apple squash are compared in Fig. 1. The average concentration of ascorbic acid was 5.18 mg/100g and was significantly decreased (p<0.001, Table 1) to 2.38 mg/100 g, during four months ambient storage. During 1st month the ascorbic acid content decreased from 6.06 mg/100 g (T2) to 4.56 mg/100g (T5) depicting significant effect (p<0.001, Table 1) of treatment. Total phenolic content is the most important criteria for evaluating antioxidant content and antioxidant activity of apple squash. Effect of treatments on phenolic content is shown in Fig. 2 and is highly significant (p<0.001, Table 1). There were two groups (T1, T3 and T5 with 16.67% pulp and T2, T4 and T6 with 25% pulp), in which the means were not significantly different from one another. The most important and encouraging conclusion from the study is that unlike other
antioxidant (ascorbic content), effect of storage on phenolic content of all samples was not significant (Table 1). During the 1\textsuperscript{st} month maximum total phenol (696.91 mg.l\textsuperscript{-1}) were assayed in T2, followed by T6 (682.37 mg.l\textsuperscript{-1}), T4 (673.65 mg.l\textsuperscript{-1}), T3 (420.77 mg.l\textsuperscript{-1}), T5 (417.87 mg.l\textsuperscript{-1}) and T1 (409.15 mg.l\textsuperscript{-1}), while in the 4\textsuperscript{th} month of storage the values ranged from 406.24 mg.l\textsuperscript{-1} (T3) to 679.47 mg.l\textsuperscript{-1} (T2).

Table 1: Analysis of variance showing mean sum of squares and F-value (parentheses) for antioxidant content of apple squash.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Ascorbic Acid</th>
<th>Total phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>2</td>
<td>0.0574</td>
<td>839</td>
</tr>
<tr>
<td>Storage</td>
<td>4</td>
<td>21.5043***</td>
<td>610</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(690.65)</td>
<td>(0.49)</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>1.2213 ***</td>
<td>316810***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(39.22)</td>
<td>(256.19)</td>
</tr>
<tr>
<td>Storage*</td>
<td>20</td>
<td>0.2028***</td>
<td>140</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>(6.51)</td>
<td>(0.11)</td>
</tr>
<tr>
<td>Error</td>
<td>58</td>
<td>0.0311</td>
<td>1237</td>
</tr>
</tbody>
</table>

*: p<0.05, **: p<0.01, ***: p<0.001

The quality characteristics (browning, acidity, pH and TSS) of apple squash are depicted in Table 2 and Figs. 3-5, respectively. Browning value/color of the product was influenced by treatments as well as storage (Fig. 3) and the effects were highly significant (p<0.001). The maximum browning values of 0.005, 0.008 and 0.007 \( \Delta A \)\textsubscript{420} were recorded for T2, T4 and T6 (treatments with 25% pulp), respectively, while minimum values of 0.003 \( \Delta A \)\textsubscript{420} were assayed for T1, T3 and T5 (treatments with 16.67% pulp). Acidity (Fig. 4) and pH (Fig. 5) were significantly (p<0.001) affected by treatment and storage (Table 2). At the end of storage period, acidity increased while the pH values showed a decrease for all the apple samples. The data of total soluble solids (TSS) reflects non significant changes of storage and treatment on the parameter (Table 2) with values ranging from 47.5 (T3) to 47.8 (T1 and T6).
Table 2: Analysis of variance showing mean sum of squares (and F-values in parentheses) physicochemical parameters.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Browning</th>
<th>Acidity</th>
<th>pH</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>2</td>
<td>1.333E-07</td>
<td>0.00050</td>
<td>0.0140</td>
<td>0.09244</td>
</tr>
<tr>
<td>storage</td>
<td>4</td>
<td>4.850E-06*** (7.33)</td>
<td>0.00946*** (30.60)</td>
<td>0.01724*** (96.87)</td>
<td>1.08483*** (17.47)</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>8.461E-05*** (127.80)</td>
<td>0.02127*** (68.77)</td>
<td>0.13735*** (771.58)</td>
<td>1.56544*** (25.21)</td>
</tr>
<tr>
<td>Storage* Treatment</td>
<td>20</td>
<td>1.963E-06*** (2.97)</td>
<td>0.00099*** (3.20)</td>
<td>0.00136*** (7.66)</td>
<td>0.06850*** (1.10)</td>
</tr>
<tr>
<td>Error</td>
<td>58</td>
<td>6.621E-07</td>
<td>0.00031</td>
<td>0.00018</td>
<td>0.06210</td>
</tr>
</tbody>
</table>

Total 89

*: p<0.05, **: p<0.01, ***: p<0.001

The data of sensory evaluation (appearance, odor and taste) is summarized in Table 3 and Figs. 6-8. The data revealed that the effect of treatments was highly significant (p<0.001, Table 3) for appearance, odor and taste while the effect of storage was non-significant. The average scores ranged from 6.73 (T1) – 7.43 (T6) for appearance, 6.69 (T1) - 7.21 (T6) for odor and 6.04 (T4) - 7.22 (T6) for taste. Accumulating the scores of the sensory evaluation resulted in overall acceptability with the highest score of 7.29 (T6) > 7.04 (T2) > 7.02 (T5) > 6.96 (T1) > 6.74 (T4) > 6.67 (T3). Regarding sensory evaluation, product prepared with 25% pulp (T6, T4 & T2) was liked more by the panelists.

Table 3: Analysis of Variance showing mean sum of squares and F-value (in parentheses) for sensory evaluation.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Appearance</th>
<th>Odor</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>2</td>
<td>0.35925</td>
<td>0.23837</td>
<td>0.05560</td>
</tr>
<tr>
<td>storage</td>
<td>4</td>
<td>1.22723</td>
<td>2.16629</td>
<td>0.23497</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.87047***</td>
<td>0.61196***</td>
<td>5.01540***</td>
</tr>
<tr>
<td>Storage* Treatment</td>
<td>20</td>
<td>0.58990**</td>
<td>0.20724*</td>
<td>0.11888</td>
</tr>
<tr>
<td>Error</td>
<td>58</td>
<td>0.09400</td>
<td>0.09736</td>
<td>0.09425</td>
</tr>
</tbody>
</table>

Total 89

*: p<0.05, **: p<0.01, ***: p<0.001

Fig. 6: Effect of processing and storage on sensory evaluation (appearance score 0-10) of apple squash.

Fig. 7: Effect of processing and storage on sensory evaluation (odor score 0-10) of apple squash.
Aromatic rings in phenolic compounds have one or more hydroxyl groups. These are categorized as phenolic acids, flavonoids, stilbenes, coumarins and tannins. Phenolics are important as secondary metabolites in plants and provide vital functions in the growth of plants. Apart from their role in plants, phenolic compounds provide health benefits to humans by reducing the risk of chronic diseases (Rui, 2004). The antioxidant (total phenolic and ascorbic acid) content vary considerably among parts of the fruit as well as cultivar. A comparison of the values obtained in this study with those of other studies suggests higher values of total phenolic which may be considered as an achievement of the methodology adopted. The phenolic content (mg gallic acid equivalent/100 g) of the flesh, whole fruit and skin of three apple cultivars (Epagri, COOP24 Fuji, and Epagri F5 P283) cultivated in Southern Brazil (Lee et al. (2003) ranged from 141-215, 167-233 and 577-640 mg/100 g, respectively. These studies revealed that apple skin possessed elevated quantity of phenolic content in comparison to whole fruit and flesh. A similar tendency was found in the total phenolic compound in various parts of apple in the studied cultivars (7, 8, 9, 12 and 15). According to Lee et al. (2003), the composition of phytochemicals in different varieties of apples has great variability, and that minute alterations in phytochemicals occur during the maturation and ripening of the fruit. Although, storage has little effect, the processing greatly affected phytochemicals in apple fruit. Researchers found great variability in total flavonoid and total phenolic content of various apple varieties. Apple sauce prepared from Rome Beauty variety had the highest, while Cortland had the lowest phenolic content among the four common varieties namely Golden Delicious, Rome Idared, Beauty and Cortland, used applesauce (Wolfe et al., 2003). Results of our study regarding effect of storage on phenolic content agreed with those reported earlier. It has been reported (Lee et al., 2003), that the concentration of total phenolic content on the skin of Golden Delicious apple increased after 60 days of cold storage but it began to decrease after 100 days. However, even after 200 days in storage, the total phenolic contents were the same as those at the time of harvest (Francilene et al., 2009). Similarly, another (Guyot et al., 2003) study found that only 42% of the total phenols were extracted in the juice and more than half remained in the pomace. Apple phenolics, especially procyanidin is known to bind with cell wall material that can result in decreased levels of polyphenols in the juice (Ko et al., 2005). Mean concentrations of main phenolic content and ascorbic acid content in 6 cultivars of apple were: chlorogenic acid, 9.02; phloretin glycosides, 5.59; quercetin glycosides, 13.20; epicatechin, 8.65; procyanidin B3, 9.35; vitamin C, 12.80 (mg/100 g of fresh weight) Srivastava and Sanjeev (2003). Vitamins as essential micronutrients cannot be synthesized and, therefore, must be ingested to prevent metabolic disorder. Apple is a rich source of vitamin A and vitamin C. Outer skin has more concentration of vitamin A, as compared to flesh. Vitamin C concentration, just like potatoes, is higher just beneath the peel of the apples. Thus, the peel of apple needs to be fully utilized. Srivastava and Sanjeev (2003) reported the value of ascorbic acid as 1 mg/100g. Kim and Lee (2004) reported the antioxidant activity as 100, 25.2, 24.0 and 6.5 mg VCEAC/L for ascorbic acid, beta carotene, alpha tocopherol and vitamin A, respectively. The reduction of ascorbic acid during storage in the current study is also supported by other scientists. Breakdown of ascorbic acid during storage of the products may be another possible reason for the development of brown color and decrease of ascorbic acid through formation of dehydroascorbic acid followed by 2, 3-diketogulonic acid and furfural on polymerization resulted in brown pigment (Pietro et al., 2007). The different values of ascorbic acid content can be attributed to varietals differences, stage of maturity and time between harvesting and product development. Similar to our findings, it has been previously reported (Bibi et al., 2009) in a study on storage stability of tomato (Solanum lycopersicum L.) paste, that pH decreased during four months of ambient storage.

CONCLUSION

It can be inferred from this study that samples (T2, T4 and T6) with more pulp content showed maximum quantities of antioxidants. The most important conclusion from this study is that contrary to other nutrients, the changes in apple phenolics are statistically non significant during four months ambient storage. The inclusion of peel in pulp preparation for further processing not only enhanced the antioxidant levels of the squash but also improved the sensory properties and stability with respect to quality parameters of the samples.

REFERENCES


