Pretreatment and freezing storage effect on antioxidant capacity of sour cherries and correlation with color changes

Received for publication, May 15, 2015
Accepted, September 14, 2015

ANDREEA STAN, MONA ELENA POPA

University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnology, 59 Mărăști Blvd., district 1, 011464, Bucharest, Romania
Corresponding author: andreea_stan88@yahoo.com

Abstract

During smoothie production is mandatory to have raw materials for the whole year, so it is necessary to store it after different pretreatments. Freezing preservation is the most used method for the half-finished products in smoothie industry.

Color, flavor, texture and physico-chemical properties are very important quality attributes of fruits, these affecting the acceptability of fruits, fresh, frozen or processed (concentrates, jam, juice, nectar, syrup, dairy products) and being of major concern in product design.

Sour cherries are commercially important and consumed in a variety of ways, including fresh, frozen and canned, or as juice, brined or dried.

The aim of this study is to observe the influence of freezing storage on color, texture, antioxidant activity, vitamin C content and physico-chemical properties of sour cherry and sour cherries puree.

For this purpose, were realized and analyzed 4 different samples starting with just harvested sour cherries, pressed sour cherries, sour cherries immersed in ascorbic acid 1% for 5 minutes and sour cherries blanched at 95°C for 5 minute. The analyses have been made, before and after freezing and also after 6 month of frozen storage. During frozen storage the lightness index, L, yellowness index, b and the redness index, a, were also measured and it was observed colour changes for all of sour cherry samples. The pH and acidity values showed that the sour cherry samples registered insignificant changes compared to control samples.

Key words: color, antioxidant activity, sour cherry, freezing, shelf life

1. Introduction

Sour cherries (Prunus cerasus) are one of the most popular fruits in almost all world regions, due to the taste, sweetness and health-beneficial compounds (ABRANKÓ & al., 2015 [1]). Sour cherry is a globally significant crop with more than 220,000 ha harvested annually in the world (FAOSTAT [2]) and about 1.2 million tons/year (FAO, 2010 [3]). Sour cherries have unique anthocyanin content, and they are rich in phenolic compounds (ŠUMIC & al., 2013 [4], MELICHACOVA & al., 2010 [5]). These compounds have proved to possess important antioxidant activity and are recommended for inclusion in human diet as health promoters (KHOO & al., 2011 [6]; KIM & al., 2005 [7]; PICCOLELLA & al., 2008 [8]). Phenolic compounds present in foods show considerable diversity in their structures, and, in addition to antioxidant activity, they contribute to flavor, color and sensory properties, such as bitterness and astringency (ŠUMIC & al., 2013 [4]).

In the past decade, sour cherry products had an increased utilization in the food market because of their potential health benefit (MING KHOO & al., 2011 [6]; KIRAKOSYAN & al., 2009 [9]).
Pretreatment and freezing storage effect on antioxidant capacity of sour cherries and correlation with color changes

Sour cherries contain phytochemicals that act as antioxidants. Given the importance of free radicals in disease, antioxidant effects from sour cherry intake may confer significant health benefits. Several in vitro studies have examined sour cherry extracts and/or isolated phytochemicals from sour cherry to address this potential benefit. In vivo studies also supported the antioxidant effects of sour cherries. In a rat model of elevated cholesterol and insulin resistance, feeding 1% of the diet as freeze-dried tart cherry powder significantly increases plasma antioxidant capacity (SEYMOUR & al., 2008 [10]; KIRAKOSYAN & al., 2013 [11]). Recent studies have not only confirmed strong antioxidant potential of sour cherry, but have also proved their anti-carcinogenic, anti-inflammatory and anti-neurodegenerative activity (KONOPACKA & al., 2014 [12]; OU & al., 2012 [13]; TRAUSTADÓTTIR & al., 2009 [14]; KIM & al., 2005 [7]; KANG & al., 2003 [15]).

One human study examined the efficacy of sour cherry juice in preventing the symptoms of inflammation-associated muscle damage following acute exercise. In this randomized, placebo-controlled, crossover design, consumption of tart cherry juice reduced both post exercise pain and loss of muscle strength (CONNOLLY & al., 2006 [16]).

Generally, sour cherries are consumed after processing into various products, such as jams, frozen fruits and fruit juices (the most consumed/preferred). However, sour cherry fruit cannot be processed into 100% fruit juice because of its characteristic sour taste (TOYDEMIR & al., 2013 [17]). Sour cherry juice is not palatable in its natural form and the addition of water and sugar is required to obtain a drinkable juice, which is classified as “nectar” (TOYDEMIR & al., 2013 [17]). Nectars are fruit drinks containing 25–99% fruit juice (TOYDEMIR & al., 2013 [17]). The production of sour cherry nectar involves several steps such as heating and filtration. These steps can potentially determine the fate of phenolic antioxidants during processing (TOYDEMIR & al., 2013 [17]).

Freezing is one of the most common methods of preservation of fruits for long-term storage. Frozen fruits are used as ingredients in many food formulations such as jams, jellies, sauces, purées, toppings, syrups, juice concentrates, as well as bakery and dairy products. The freezing process and frozen storage may change the anthocyanin content of fruits, thereby affecting the antioxidant capacity and possible health benefits of the fruit (SABLANI, 2015 [18]).

With rather limited availability of fresh fruits on the market, any novel ideas leading to attractive products which contain sour cherries that could lead to higher consumption of these fruits may be deemed advisable (KONOPACKA & al., 2014 [12]), in this case very good examples being smoothie products without adding preservatives or stabilizers. During smoothie production is mandatory to have raw materials for the whole year, so it is necessary to storage it after different pretreatments. Freezing preservation is the most used method for the half-finished products in smoothie industry.

Therefore, the aim of this study is to study the influence of pretreatment and freezing storage on color, texture, antioxidant activity, vitamin C and physico-chemical properties of sour cherry and sour cherries puree used for smoothie production.

2. Materials and methods
2.1. Samples
Sour cherries were harvested at the commercial maturity stage, having characteristic color, aroma and flavor. All fruits were selected based on the same ripening stage (>90% red surface color), uniform size, absence of any physical damage and fungal infection. Considering that sour cherries are highly perishable fruits and available only for a short time during the season, following standard industrial practice the material of all cultivars was frozen to ensure
availability for off season processing according to KONOPACKA & al., 2014 [12]. Fruits were washed immediately after harvest and packed for freezing within 2 h, or analyzed. Sour cherries were packed in 200 g plastic bags and stored for 6 months at -18°C, until processing. Were tested 4 different samples starting with just harvested sour cherry, pressed sour cherries, sour cherries immersed in ascorbic acid 1% for 5 minutes and sour cherries blanched at 95°C for 5 minutes. These samples were analysed before and after freezing. Thawing of frozen sour cherries were made at 4°C for 24 h before they were analysed.

2.2. Physical-chemical analysis

2.2.1. pH determination

pH was determined with a pH meter WTW INOLAB 720 series type with automatic temperature compensator, whose pH domain is between 0,00-14.00, with a precision of ± 0.01.

2.2.2. Titratable acidity (TA) - STAS 6182/1-79 [28] and SR 6182-1:2008 [29]

Titratable acidity was determined by titrating 10 g of homogenized sample with 0.1 N NaOH to an end point of pH 7.3 using Schott automatic titrator type Titronic basic. TA was analyzed in duplicates and expressed as malic acid/100 g product (factor 0.67).


The level of sugars was measured as Brix by a Krüss Refractometer and correlated with the amount of soluble solids (expressed as sucrose concentration) using the conversion table or read directly on the scale Refractometer.

2.2.4. Dry matter content (D.M. %)

The dry matter content was determined after drying approximately 5 g of pulp at 140°C till a stable weight, with PRECISA XM 60 thermobalance.

2.2.5. Water activity (aw) value

Approximately 2.5 g of chopped or pressed sour cherries were placed in the sample holder of NOVASINA system. Water activity (aw) was measured at 25°C.

2.3. Color

Color assessment of the samples was conducted at room temperature using a HunterLab colorimeter, Miniscan XE Plus. This instrument was calibrated using the black and white tiles provided. Instrumental color was measured using Illuminant D65 and 10° observer angle. Samples were filled into a low reflectance sample container and placed over the colorimeter chamber. For each sample, measurements were made in ten different points and results were averaged. Therefore the total color change ($\Delta E$) was calculated with the following equation:

$$\Delta E = \left[ (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2}.$$  

2.4. Antioxidant activity

The effect of antioxidant activity on DPPH (2,2-diphenyl-1-picryl-hydrazyl) was estimated according to the procedure described by VILLAÑO et al., (2007) [19], with some modification. To obtain DPPH solution (60 μM), 2.36 mg DPPH were diluted in 100 ml ethanol. Samples were diluted appropriately in ethanol. Sample preparation was done by maceration in ethanol (75%) for 2-3 days in the dark at room temperature. All measurements were performed in triplicate. For each measurement, 0.05 ml sample in ethanol was added to 1.95 ml DPPH ethanolic solution (60 μM). These solutions were vortexed thoroughly, and incubated in dark at room temperature for 30 min (GÜLÇİN, 2010 [20]). After 30 min, sample absorbance was measured at 515 nm (t=30min) against DPPH ethanolic solution alone (t=0 min). For calibration curve were used six different concentrations of Quercetin (QE) (100-3.125 μM). Absorbance measurements were recorded on a UV/Vis spectrophotometer Unicam Helios Gamma. Results were expressed as quercitin equivalents using the following equations:
AAR (QE) = (%ΔA515 –3.4954) / 0.0811

where:
AAR (QE) - antiradical activity expressed in quercitin equivalents
%ΔA515 = [(A515 (t=0)-A515 (t=30))/A515 (t=0)] x 100

2.5. Ascorbic acid spectrophotometric determination

The content of ascorbic acid from sour cherry samples was determined by using a UV/VIS spectrophotometer Unicam Helios Gamma at 500 nm. 10 g of fruit pulp was extracted with 100 ml of 2% oxalic acid in a homogenizer for 1 min. The extract was filtered through a filter paper. After filtration, 2 ml from extract solution, 1 ml oxalic acid 2%, 5 ml tampon solution, 2 ml indophenol (2, 6-Dichloro phenol Indophenol) and 20 ml xylene, were placed in a centrifuge tube and centrifuged 20 min at 4°C and 9000 rpm. After absorbance measurements, ascorbic acid content was expressed in milligrams at 100 g product, and is calculated with following equation:

Vitamin C (mg/100g) = [(V0 -V1) xV3 x C / (V4 xV2)] x 100

where:
V0 - indophenol solution volume added for reduction,
V1 - indophenol solution excess volume read on the standard curve,
V3 - sample volume for analysis,
V4 – acid extract volume used for analysis,
C - Ascorbic acid corresponding quantity for 1 ml indophenol solution.

2.6. Statistical analysis

All experiments were performed in triplicates and results presented in the tables and figures were shown with standard deviations. All data were statistically assessed using SPSS software. The test of statistical significance was based on the total error criteria with a confidence level of 95.0% (p< 0.05).

3. Results and discussions

3.1. Pretreatment and freezing storage effect on physico-chemical properties of sour cherries

The results showed that the pH value of the all samples (P1, P2, P3, P4) registered insignificantly decreases in regard to just harvested samples (P1). The highest difference was recorded at just harvest sour cherry samples (fresh: 3.78 (±0.01) and frozen: 3.72 (±0.005)) and for P3 (sour cherries immersed in ascorbic acid 1% for 5 minutes) (fresh: 3.57 (±0.01) and frozen: 3.49 (±0.01)). In this case, pH value is correlated with acidity and apparently these are not responsible for color changes during on freezing storage. Acidity, expressed as malic acid content, which is the main acid of sour cherries (DAMAR & EKSI, 2012 [21]), ranged between 0.8 (±0.05) and 0.91 (±0.03) for P1 before and after freezing, and for P2, between 1.08 (±0.02) (fresh) and 1.22 (±0.003) (after freezing). Brix values for all sour cherry samples, recorded insignificant changes during on freezing storage. For dry matter content, all sour cherry samples recorded significant decreases from 73.95% to 67.36% for P1 samples before and after freezing and from 62.63% to 17.51% for P3 (before and after freezing) (Tabel 1).

Water activity (aw) is a physical property that has a direct impact on microbiological safety of food. Knowledge of water activity is very useful to predict the stability of foods, to select formulations and storage conditions for new products (VULLIOUD & al., 2004 [22]).

Microorganisms generally grow best between (aw) values 0.99–0.98, while most microbes cease growth at (aw) < 0.90. The (aw) of fresh sour cherries is in the range 0.915 to
0.922, lower values in comparison with result obtained by BARBOSA-CANOVAS & al. (2007) [23]. From Table 1 it can be seen that \((a_w)\) value was significantly influenced by pretreatments and freezing storage.

Table 1. Pretreatment and freezing storage effect on physico-chemical properties of sour cherries
(P1 – just harvested sour cherries, P2 – pressed sour cherries, P3 – sour cherries immersed in ascorbic acid 1% for 5 minutes, P4 – sour cherries blanched at 95°C for 5 minutes)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Titratable acidity (g citric acid/100g product)</th>
<th>Brix</th>
<th>( (a_w) )</th>
<th>D.M. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>3.78 ±0.01</td>
<td>3.72 ±0.005</td>
<td>0.8</td>
<td>0.91 ±0.03</td>
<td>12.3</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td>0.915</td>
<td>0.92 ±0.01</td>
<td>0.915</td>
</tr>
<tr>
<td></td>
<td>73.95</td>
<td></td>
<td>±0.005</td>
<td>±0.0005</td>
<td>±0.1</td>
</tr>
<tr>
<td></td>
<td>67.36</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td>P2</td>
<td>3.58 ±0.01</td>
<td>3.57 ±0.01</td>
<td>1.15</td>
<td>1.17 ±0.02</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.6</td>
<td>0.917 ±0.01</td>
<td>0.919</td>
</tr>
<tr>
<td></td>
<td>61.84</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>24.4</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td>P3</td>
<td>3.57 ±0.01</td>
<td>3.49 ±0.01</td>
<td>1.08</td>
<td>1.22 ±0.01</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.6</td>
<td>0.917 ±0.05</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>62.63</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>17.51</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td>P4</td>
<td>3.54 ±0.01</td>
<td>3.54 ±0.02</td>
<td>1.00</td>
<td>0.95 ±0.004</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.4</td>
<td>0.916 ±0.02</td>
<td>0.922</td>
</tr>
<tr>
<td></td>
<td>58.34</td>
<td></td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>18.41</td>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
</tbody>
</table>

Data are the means of three replicates followed by their standard deviations.

3.2. Pretreatment and freezing storage effect on color

Besides texture and economic considerations, color is one of the most important factor in the perception of sour cherry fruit quality, affecting consumer acceptance (ABD-ELHADY, 2014 [24]). In general, several factors are believed to affect the color and stability of sour cherry anthocyanin’s include structure and concentration, \( \text{pH} \), temperature, light, presence of co pigments, self-association metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulfur dioxide. During the 6 months of freezing storage period were not observed visually detectable color changes for any of the analyzed sour cherry samples. Changes were observed when the color characteristics were analyzed with colorimeter Hunter Lab according to Universal Software V4.01 MiniScan™ XE Plus program. During freezing storage period, the \( L \) (lightness), \( a \) (redness) and \( b \) (yellowness) values of just harvested sour cherries (P1), pressed sour cherries (P2), sour cherries immersed in ascorbic acid 1% for 5 minutes (P3) and sour cherries blanched at 95°C for 5 minutes (P4) tended to decrease, indicating color changes, as can be observed in figure 1. For all sour cherry samples (P1, P2, P3 and P4), the \( L \) (lightness), \( a \) (redness) and \( b \) (yellowness) values tended to decrease during freezing period, indicating a discoloration of the samples (Table 2). These are in correlation with pretreatments used and freezing storage period. The \( \Delta E \) values, which are an indicator of total color difference (Table 2), showed that freezing storage for 6 months affected color attributes for all sour cherry samples.

Table 2. Instrumental color variables of sour cherry samples before and after freezing

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>( \Delta E )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>P2</td>
<td>31.05</td>
<td>22.14</td>
<td>14.64</td>
<td>12.54</td>
</tr>
<tr>
<td>P3</td>
<td>29.7</td>
<td>22.44</td>
<td>20.48</td>
<td>14.05</td>
</tr>
<tr>
<td>P4</td>
<td>38.46</td>
<td>29.44</td>
<td>33.04</td>
<td>32.57</td>
</tr>
</tbody>
</table>
Consumers prefer visible quality, the color of the product. According to FILIMON & al., 2011 [25], if ΔE between two samples is less than 1.0, it is assumed that the difference would not be sensorial perceptible.

**Figure 1.** Graphical representation of the values of L, a, b, according to Universal Software V4.01 MiniScan™ XE Plus program for sour cherry samples before and after frozen 6 months. (P1 - just harvested sour cherries, P2 - pressed sour cherries, P3 - sour cherries immersed in ascorbic acid 1% for 5 minutes, P4 - sour cherries blanched at 95°C for 5 minutes)

### 3.3. Pretreatment and freezing storage effect on antioxidant activity

DPPH assay is one of the best known, frequently employed and accurate method of assessing free radical scavenging activity. DPPH is a stable free radical because of its spare electron delocalization over the whole molecule. Decolourization causes deep violet color with maximum around 520 nm. When a solution of DPPH is mixed with a substrate acting as a hydrogen ion donor, a stable non-radical form of DPPH is obtained with simultaneous change from violet to pale yellow (MANDAVE & al., 2014 [26]). Antioxidant activity content in just harvest sour cherry samples (P1) decreased from 854.59 μM quercetin equivalents to 416.27 μM quercetin equivalents for frozen sample. Highest value for DPPH content it was seen also at just harvest sour cherries. The most significant decrease (p < 0.05) it was recorded at P2 sample from 813.2 μM quercetin equivalents to 276.4 μM quercetin equivalents. For blanched sour cherry samples (P4), DPPH values recorded insignificant changes, from 831.17 μM quercetin equivalents to 779.38 μM quercetin equivalents after 6 months of freezing storage. In conclusion, antioxidant capacity of blanched sour cherry samples (95°C for 5 min) is more stable (fresh: 831.17 μM quercetin equivalents and frozen: 779.38 μM quercetin equivalents) than P1, P2 and P3 samples. In this case, antioxidant activity of sour cherry samples it was negatively affected by pre-treatments and freezing storage for 6 months.
3.4. Vitamin C (Ascorbic Acid spectrophotometric determination)

Generally, fruits and vegetables show a gradual decrease in vitamin C content as the storage temperature or duration increases (KOYUNCU & DILMAÇÜNAL, 2010 [27]). Vitamin C content in just harvest sour cherry samples (P1) significantly decreased (p < 0.05) from 56.82 (±0.07) mg vitamin C/100 g product up to 47.13 (±0.05) mg vitamin C/100 g product for frozen sample. Lowest values for vitamin C content was recorded from immersed and pressed sour cherry samples (P3) from 52.63 (±0.02) mg vitamin C/100 g product to 46.74 (±0.02) mg vitamin C/100 g product. All changes in vitamin C content of sour cherry during freezing storage is shown in Figure 3.
4. Conclusions

During smoothie production is mandatory to have raw materials for the whole year, so it is necessary to storage it after different pre-treatments and freezing.

Color is one of the most important attributes in the perception of sour cherry fruit quality, affecting consumer acceptance and preference, but only after texture and economic considerations.

During the 6 months of freezing storage period were not observed visually detectable color changes for any of the analysed sour cherry samples. Significant changes (p < 0.05) were observed when the color characteristics were analysed with colorimeter.

The obtained results showed that the pH, titratable acidity, Brix degrees and water activity characteristics of the sour cherry samples were not affected, during the freezing period. Significant decreases (p < 0.05) was registered for dry content (D.M. %), vitamin C and antioxidant activity of all samples (P1, P2, P3 and P4).

Vitamin C and antioxidant capacity of blanched sour cherry puree (95°C for 5 min) has shown more stable values than the rest of analysed samples (P1, P2 and P3). Therefore, thermal pre-treatment is more efficient in order to maintain the quality of the frozen sour cherry puree used for smoothie production.

Acknowledgements:

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

References


28. STAS 6182/1-79, Titratable acidity (TA) determination. Titrimetric method.
