GROWING STRAWBERRY FROM THE EQUATOR TO THE ARCTIC

Specialized metabolites profile of strawberry fruit during flash-freezing

J. Šic Žlabur, B. Duralija, Z. Emanović, N. Mikulec, S. Voća
University of Zagreb Faculty of Agriculture, Zagreb, Croatia

Contact: bduralija@agr.hr

Strawberry fruit species given the extremely high water content (90-92%) are prone to the rapid spoilage and therefore it is difficult to preserve it fruits for the longer time period. The effective method to preserve strawberry fruits with significant preservation of nutritional and sensory quality is freezing. One of the method increasingly in use, alternative to conventional freezing, is flash-freezing which implies significantly lower freezing temperatures (cryogenic) allowing freezing for just few minutes. The aim of this study was to evaluate the effect of freezing method (conventional, flash-freezing) on the content of predominant specialized metabolites of strawberry fruit. Also, to determine the effect of storage period (10, 20 and 30 days) on the content of specialized metabolites. According to the obtained results, significantly higher values of vitamin C (5 times), total phenolic compounds (2 times), non-flavonoids (1.5 times), flavonoids (50 %), total anthocyanins (3 times) and antioxidant capacity (2.7 %) were determined in strawberry fruits frozen by flash-freezing compared to the conventional method. Also, as expected regardless of the freezing method, content of all analysed specialized metabolites significantly decreased during storage period. It should be emphasized that during storage, however, nutritional quality of flash-frozen samples was better preserved compared to the samples frozen by conventional method. Based on the stated, it can be concluded that flash-freezing significantly preserved content of all analysed specialized metabolites in strawberry fruits, thus can be recommended as effective method for strawberry processing and storage.

INTRODUCTION

Strawberry fruit is significant source of antioxidants, anthocyanins, phenols, flavonoids, vitamins and minerals. Phenolic compounds participate in the biochemical changes that take place during fruit ripening, participate in the formation of colour, taste and aroma. Due to their structure, they show a strong antioxidant effect that is associated with various physiological effects in the human body, such as anti-inflammatory, anti-allergic and anticancer effects (Rotelli et al., 2003, Mazzoni et al., 2016; Ganhão et al., 2019; Perin et al., 2019). Anthocyanins are the most famous phenolic compounds of strawberry, which is where the attractive red colour of strawberry comes from (Ornelas-Paz, 2012). Strawberry fruit species given the extremely high-water content are prone to the rapid spoilage and therefore it is difficult to preserve its fruits for the longer time period. One of the effective methods to preserve strawberry fruits is freezing. Freezing prolongs the shelf life of food and does not significantly affect their nutritional value. Freezing preservation is based on the fact that chemical, biochemical and microbiological processes are practically stopped by separating water in the form of ice crystals and lowering the temperature. However, freezing also causes certain major or minor irreversible changes, which is of particular importance for those who want to preserve the original structure and texture, and thus the quality of the final product. Most of the mentioned changes are in the function of the freezing rate, i.e. the faster the freezing, the smaller the changes (Lovrić, 2003). Conventional freezing methods imply longer time period (usually 24 h) and temperatures around -18 °C, whereby larger ice crystals grow thus damaging cell structures impacting on the degradation of physico-chemical as well bioactive compounds content. Alternative method to conventional freezing is flash-freezing, which implies significantly lower freezing temperatures (cryogenic) allowing freezing for just few minutes. Storage of classically frozen fruits for a long time can affect the content of bioactive compounds, certain organoleptic properties such as colour and antioxidant properties (Cordenunsi et al., 2005; Nursal Tosun and Yücecan, 2007). Chemical, biochemical, and physical reactions cannot be completely stopped during frozen fruit storage, so the rate of these reactions depends on storage temperature and time (Blando et al., 2004; Sahari et al., 2004).

The aim of this study was to evaluate the effect of freezing method (conventional, flash-freezing) on the content of predominant specialized metabolites of strawberry fruit. Also, to determine the effect of storage period (10, 20 and 30 days) on the content of specialized metabolites.

MATERIALS AND METHODS

Plant material

Fresh strawberry fruits cv. 'Sophia' were purchased from the family farm producer at the optimal stage of fruit maturity (July 2019) and the same day transported to the Department of Agricultural Technology, Storage and Transport at Faculty of Agriculture University of Zagreb for intended analysis. Fresh fruits were analysed in the fresh state and prepared for freezing, petiole and fruits with mechanical and visible signs of spoilage were removed. Fresh fruits were washed and pat-dried to remove excess water from the surface of the fruit.

Freezing method

Conventional freezing was conducted at -18 °C for 24 h in classic freezer, while flash freezing at -34° C for 25 min in shock freezer (Tecnomac Sf 1, Italy). Bioactive compounds content was analysed for fresh samples (S1), immediately after defrosting for flash-frozen (Sfl), classical frozen (Sc) and both for flash and classical frozen after storage period of 10 days (Sfl10 and Sc10), 20 days (Sfl20 and Sc20) and 30 days (Sfl30 and Sc30).

Determination of bioactive compounds and antioxidant capacity

For all analysed samples, fresh and freeze, dry matter content (%) was determined by drying to constant mass at 105 °C according to AOAC (1995) for the purpose of final recalculation of the bioactive compounds content on the dry weight. The following bioactive compounds in strawberries samples were analysed: vitamin C content (mg/100 g DW) by titration with 2,6-dichloroindophenol according to AOAC (2002), where vitamin C was isolated from the fruits by homogenizing the 10 g ± 0.01 of strawberry fruit with 100 mL of 2% (v/v) oxalic acid. Prepared solution was filtered through Whatman filter paper, the filtrate was obtained in a volume of 10 mL and used for titration; total phenolic compounds content (including flavonoids and non-flavonoids, mg CAE/100 g DW) spectrophotometrically (Shimadzu, UV 1650 PC) at 750 nm by colorimetric method based on a coloured reaction than phenols develop with Folin-Ciocalteu reagent (Ough and Amerine, 1988), where extraction of phenols was made as follows: 10 g ± 0.01 of fruit was weighed into an Erlenmeyer flask and the first 40 mL of 80% EtOH (v/v) was added. The prepared sample was heated to boiling point and additionally heated for 10 min with reflux. After 10 min, sample was filtered through Whatman filter paper in a volumetric flask volume of 100 mL. The rest of the sample was transferred in the Erlenmeyer flask and an additional 50 mL of 80% EtOH (v/v) was added, and the procedure with reflux was repeated for 10 min. Filtrates were combined and the flask was filled to the mark with 80% EtOH (v/v); and total anthocyanin content (mg/kg DW) spectrophotometrically at 520 nm by the bisulphite bleaching method according to Ough and Amerine (1988), where anthocyanins were isolated as follows: 2 g ± 0.01 of fruit was weighed into cuvette volume of 50 mL, 2 mL of 0.1% HCl (diluted with 96% EtOH) and 40 mL of 2% HCl (v/v) was added. Prepared samples were centrifuged 10 min at 4500 rpm, 10 mL of supernatant was separated into one test tube and 10 mL into another test tube. For the purposes of antioxidant capacity determination, ethanolic extracts obtained from total phenol determination, were used, while antioxidant capacity was determined spectrophotometrically at 734 nm using ABTS radical cation (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) according to method described by Re et al. (1999).

Statistical analysis

Obtained data were analysed using the SAS® version 9.3 (2010). Experiment was arranged in a randomized complete block design with 3 replications. For analysis of obtained data one-way analysis of variance (ANOVA) was conducted. Mean values were compared by t test (LSD), and considered significantly different at P≤0.01. In the tables, in addition to the mean values, the value of the standard deviation is also expressed.

Table 1. Bioactive compounds content and antioxidant capacity of fresh (S1), flash-freeze (Sfl) and classically freeze (Sc) strawberry fruits

Sample	Vitamin C	TPC	TNFC	TFC	TAC	ANT_CAP
S1	726.2b±0.7	2480.5b±2.3	918.8c±0.1	1292.4c±0.1	4342.7b±1.2	1740.1b±0.1
Sfl	1050.2a±0.6	4231.8a±2.2	1700.7a±0.2	2531.0a±2.3	6260.4a±3.6	1748.3a±0.2
Sc	205.3c±1.3	2211.2c±2.0	967.1b±1.1	1513.4b±1.5	3036.2c±2.1	1702.8c±0.1
ANOVA	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001

Table 2. Bioactive compounds content and antioxidant capacity of flash-freeze and classically freeze strawberry fruits during 10, 20 and 30 days of storage

Sample	Vitamin C	TPC	TNFC	TFC	TAC	ANT_CAP
10 days storage						
Sfl10	499.52a	2821.37a	1298.81a	1552.56b	5621.9a	1775.40
Sc10	419.21b	1779.41c	1146.73c	632.68c	3699.7c	1768.32
20 days storage						
Sfl20	321.79c	2796.73b	1282.00b	1514.37c	5580.0b	1736.13
Sc20	199.24e	1736.87d	1090.53d	646.34b	3310.8d	1710.46
30 days storage						
Sfl30	257.24d	2796.39b	1143.54c	1652.85a	3075.3de	1674.93
Sc30	236.18de	1363.76e	1003.43e	359.33d	3013.3e	1666.17
ANOVA	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001	p≤0.0001

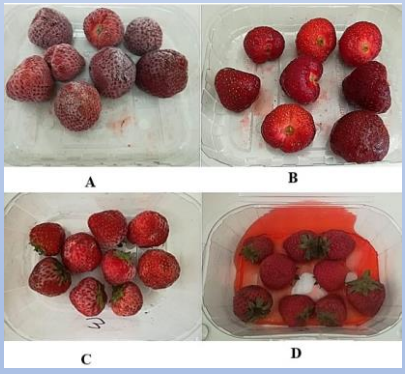

Fig 1. Frozen strawberry fruit cv. 'Sofia':

A) flash-frozen fruits;

B) thawed flash-freeze fruits;

C) classically frozen strawberry fruits;

D) thawed classically frozen strawberry fruits

CONCLUSIONS

Based on the obtained results can be concluded that flash-freezing is an effective method in the terms of preservation nutritional components of strawberry fruit. Namely, in flash-freeze samples significantly higher content of all analysed bioactive compounds: vitamin C, total phenols, total anthocyanins and antioxidant capacity was recorded compared to the samples classically freeze. Also, in the case of strawberries frozen by the flash-freezing method, the defrosting period is significantly shorter (approximately 2 hours) compared to the samples classically freeze, which proves that small ice crystals formed during flash-freezing and during thawing do not contribute to such significant degradation of important nutrients. As expected, during storage period, in total for a 30 days, significant loss of bioactive compounds content was recorded both in flash and classically-freeze samples, but it must be emphasized that this decrease was significantly less pronounced in flash-freeze strawberry samples. Based on all stated, flash-freezing can be recommended as effective method for strawberry processing and storage with significant preservation of overall nutritional quality.