

## Influence of Freezing on the Chemical Composition and Total Phenols of Some Strawberry Varieties

Amel Selimović<sup>1</sup>, Fatima Djedović<sup>2</sup>, Halid Junuzović<sup>3</sup>, Ernad Kucalović<sup>4</sup>, Amra Selimović<sup>5</sup> and Tijana Brčina<sup>6</sup>

<sup>1</sup>Assoc. Prof. Dr., Department of Food Technology, University of Tuzla/Faculty of Technology,  
BOSNIA AND HERZEGOVINA

<sup>2</sup>BA, Department of Plant Production, University of Tuzla/Faculty of Technology, BOSNIA AND HERZEGOVINA

<sup>3</sup>Sen. Asst, Department of Analytical Chemistry, University of Tuzla/Faculty of Technology,  
BOSNIA AND HERZEGOVINA

<sup>4</sup>Asst, Department of Plant Production, University of Tuzla/Faculty of Technology, BOSNIA AND HERZEGOVINA

<sup>5</sup>Assoc. Prof. Dr., Department of Analytical Chemistry, University of Tuzla/Faculty of Technology,  
BOSNIA AND HERZEGOVINA

<sup>6</sup>Assoc. Prof. Dr., Department of Food Technology, University of Tuzla/Faculty of Technology,  
BOSNIA AND HERZEGOVINA

<sup>3</sup>Corresponding Author: halid.j@hotmail.com

### ABSTRACT

The aim of this study was to examine the content of total soluble substance (TSS), total acidity (TA), pH, ascorbic acid content (vitamin C) and total phenols in fresh strawberries, as well as in strawberries stored for 10 days by freezing. Chemical characterization of fresh and frozen strawberries was done on four different varieties: Clery, Maya, Asia, Joly grown in the area of Čelić, Bosnia and Herzegovina. The obtained results showed that frozen strawberries have slightly lower values of TSS, pH, vitamin C, while the content of TA and total phenols was slightly higher in frozen strawberries than in fresh ones, which can be attributed to increasing acidity.

**Keywords-** strawberry, freezing, chemical composition, total phenols.

### I. INTRODUCTION

One of the most popular garden plants throughout Europe and around the world due to its genotypic diversity and easy adaptation to the environment is certainly the cultivated hybrid strawberry (*Fragaria ananassa*) which has been domesticated for less than 300 years [1], [2]. Strawberry is a perennial, herbaceous, low-growing plant whose fleshy fruit is rich in anthocyanins, polyphenols, vitamin C and other nutrients [3].

The unique taste and aroma of strawberries stem from a specific combination of sugars, acids and volatile organic compounds that varies greatly between different varieties and species of *Fragaria* [4]. Thanks to the unique combination of flavors and aromas that are acquired during the process of ripening strawberries make one of the most popular fruit crops [5].

A significant variation in the chemical composition indices depending on the varietal characteristics was found [6]. The results of the research

in Brazil showed significant differences in chemical composition in relation to the variety, so the content of ascorbic acid was twice as high in the variety Campineiro (85 mg/100 g) compared to the variety Dover (40 mg /100 g). Also, the content of total phenolics varied from 159 to 289 (mean 221) mg /100 g. Also, the chemical composition depends on agricultural practice, storage and processing methods: freezing versus dry heat has been associated with maximum retention of strawberry bioactive ingredients in several studies [7].

The health benefits of strawberries are determined by the abundance of biologically-active compounds that support the natural resistance of the organism [8]. Several in vivo and in vitro studies have shown that strawberries can potentially prevent chronic diseases associated with oxidative stress and inflammation and possess directly and indirectly antimicrobial, anti-allergy and anti-hypertensive properties, as well as the capacity to inhibit the activities of some physiological enzymes and receptor properties [9], [10]. Strawberries could be a potential dietary supplement for vitamin C with minerals [11].

One of the disadvantages of this fleshy fruit is its short shelf life caused by rapid spoilage [12]. Therefore, in order to prolong the shelf life, strawberries are subjected to various technological processes, mainly freezing, and also processing into jams or beverages. Currently, freezing as a technological process is most often used to preserve various types of fruits and berries, especially perishable ones such as strawberries [13].

Freezing as a physical process is connected with products inner moisture transforming into ice as the result of the temperature diminishing under the freezing point.

This physical process reduces the biochemical and microbiological processes in the product [14]. The aim of this paper is to examine the influence of freezing

on the chemical composition and total phenols of some strawberry varieties.

## II. MATERIALS AND METHODS

### Materials

#### Plant material

For the purposes of this work, four types of strawberries were used: Clery, Maya, Asia and Joly grown in Čelić in June 2019. Further chemical analyses was performed on fresh and frozen strawberries after 23 days of storage. The freezing process was performed in a freezing chamber at a temperature of  $-20\text{ }^{\circ}\text{C}$ . Strawberries were stored at the mentioned temperature until the moment of analysis.

#### Chemicals

All chemicals used for the experimental part of the work were of analytical grade: Fluka sodium hydroxide, 0,1 mol/L, 2,6, dichloroindophenol, sodium salt hydrate, 90% +, New Jersey, USA, ascorbic acid, min. 99%, lac:ner. Neratovice, Chezh Republic, oxalic acid, min. 99%, Centrohém, Stara Pazova, Folin-Ciocalteu reagens, VWR, Chemicals, France, Ethanol, Merck KGaA, Darmstadt, Germany

#### Instruments

pH meter (Mettler Toledo, Easy Plus Titration), refractometer, (Atago, automatic refractometer), spectrophotometer (UV Unicam Hellios B), analytical laboratory balance (Kern ABT 220 – 4M)

### Methods

#### Preparation of strawberry puree

Samples of fresh and frozen strawberries were homogenized using tarionics and pestles, and the resulting slurry was used for further chemical analysis.

#### Determination of soluble dry matter (TSS)

Refractometrically determined dry soluble substance by direct reading from the device Atago. Jouquand and Chandler also determined TSS refractometrically with digital Atago PR-101 refractometer with a 0% to 45% Brix range [15].

Measurements were performed three times for each sample, and the final result is the mean of all three measurements.

#### Determination of pH value

Determination of pH value was performed according to the by immersing the combined glass electrode in a sample of strawberry puree. The pH meter was calibrated with commercial buffer solutions at pH 7.0 and 4.0. [16].

Aaker et al. The pH of the strawberry samples was determined using a pH meter [17].

#### Determination of total acidity (TA)

After the sample was heated for 30 minutes on reflux and filtered, titration was performed with standard NaOH solution, concentration 0.1 mol/L up to pH 8.1 (Rulebook, Official Gazette of SFRY No. 28/83).

#### Determination of ascorbic acid (vitamin C)

After 10 mL of  $\text{H}_2\text{C}_2\text{O}_4$  solution, 2% and 5 grams of strawberry puree were added to a 100 mL volumetric flask, the flask was made up to 2%  $\text{H}_2\text{C}_2\text{O}_4$  solution. An aliquot of the sample was titrated with 2.6 dichlorophenol-indophenol solution until pink.

#### Determination of total phenols by Folin Ciocalteu method

Determination of total phenols by the Folin Ciocalteu method is performed in the ethanol extract of a strawberry puree sample using the spectrophotometric method at 765 nm.

The total phenolic contents were expressed as gallic acid equivalents, according to a linear calibration curve  $y = 0,0056x + 0,015$ . Kadivec et al. using the Folin Ciocalte method, they determined the content of phenolic compounds in strawberries [18].

## III. RESULTS AND DISCUSSION

Table 1: Chemical composition and total phenols of some fresh strawberry varieties

Strawberry' varieties	TSS (%)	TA (%)	Sweetness index (TSS/TA)	pH	Vitamin C (mg/100 g)	Total phenols (mg/100 g)
Clery	$11,28 \pm 1,20$	$0,82 \pm 0,01$	13,76	$3,53 \pm 0,01$	$82,08 \pm 2,16$	$61,63 \pm 1,11$
Maya	$11,29 \pm 0,97$	$0,86 \pm 0,01$	13,13	$3,46 \pm 0,02$	$77,04 \pm 1,25$	$60,92 \pm 6,31$
Asia	$10,64 \pm 1,04$	$0,59 \pm 0,01$	18,03	$3,63 \pm 0,01$	$66,96 \pm 2,16$	$67,84 \pm 2,07$
Joly	$11,75 \pm 0,51$	$0,78 \pm 0,01$	15,07	$3,51 \pm 0,01$	$82,11 \pm 3,79$	$79,83 \pm 6,04$

95% confidence limit for mean ( $n = 3$ )

**Table 2: Chemical composition and total phenols of some frozen strawberry varieties**

Strawberry' varieties	TSS %	TA (%)	Sweetness index (TSS/TA)	pH	Vitamin C (mg/100 g)	Total phenols (mg/100 g)
Clery	11,03±0,00	1,04±0,01	10,30	3,35±0,01	79,2±1,52	71,42 ±1,80
Maya	11,98±0,00	0,95±0,00	12,61	3,33±0,00	71,86±2,31	104,82 ±4,44
Asia	9,5±0,01	0,79±0,02	12,02	3,45±0,00	57,2±1,86	89,07 ±9,49
Joly	11,37±0,00	0,88±0,04	12,92	3,31±0,00	74,06±2,03	106,30 ±15,79

95% confidence limit for mean ( $n = 3$ )

The TSS is mainly influenced by the total sugars and organic acids, which, in turn, are influenced by the cultivar and stage of ripeness [19].

Based on the obtained results, it can be concluded that the fresh strawberry with the highest TSS content is the variety Joly with 11.75%, then Maya with 11.29%, Clery with 11.28% and finally the lowest content of soluble dry matter had the variety Asia with 10.64%. The effect of freezing resulted in a decrease in TSS content in all strawberry cultivars, and an increase in TA in all samples.

Fruit total acidity (TA) depended directly on the concentration of aliphatic and aromatic organic acids in the fruits [20]. It is a major determinant of the taste and quality of most fruits, in combination with sugars and flavor volatiles [21]. The TA in fresh strawberries ranged from 0.78 to 0.86%, while in frozen strawberries these values were higher and ranged from 0.79 to 1.04%.

The pH value of fresh strawberry puree ranged in the weakly acidic range (3.46–3.63), while the slightly lower pH value was for frozen strawberries. Wrostand, 2006 found that pH has a very high correlation with discoloration of strawberries [22].

According to the FDA, the pH value of fresh strawberries ranges from 3.00-3.90, while the pH value of frozen strawberries is lower and ranges from 3.00-3.40 [23]. Nastase et al., 2012, found that the pH values of fresh, frozen and canned strawberries ranged from 3.65 to 3.81 [24].

European epidemiological studies have revealed that the recommended daily intake of vitamin C is 60-100 mg (National Research Council, 198

9) [25]. Fresh strawberries had a higher value of vitamin C content expressed at mg/100 g. The highest value was recorded in the cultivar Clery (82.08± 2.16), while the lowest value was recorded in the cultivar Asia (66.96 ± 2.16). All frozen strawberry samples had lower vitamin C values compared to fresh ones. From the results it can be concluded that strawberries are a good source of vitamin C. Similar results were obtained

(Koyuncu et al., 2010) where the content of vitamin C was reduced in frozen strawberry varieties compared to fresh. [26]. Also, similar results were obtained by Farah et al. where they found that low temperatures have a direct impact on the stability of vitamin C [27]. Degradation of vitamin C in fresh strawberries is a complex process involving oxidation and intermolecular rearrangement reactions and is considered one of the main factors for quality changes and color changes during storage [28].

Zavala et al. investigated the antioxidant capacity and quality of strawberry fruit after harvest at different temperatures: 0 ° C, 5 ° C and 10 ° C. The results of the research showed that the overall quality of the fruit was longer, and the content of aromatic compounds and antioxidant capacity at 0 ° C was lower than at 5 ° C or 10 ° C [29].

The Folin – Ciocalte method is a method used to determine total phenols in various plant materials and is based on molybdotungstophosphoric heteropolyanion reducing reagent which indirectly detects phenolics but lacks specificity [30]. The content of total phenols in fresh strawberries was the lowest in the Maya variety (60.92), while the highest content was in the Joly variety (79.83). The effect of freezing had an effect on increasing the content of phenolic compounds in all varieties of strawberries, so the highest in the variety Joly (106.30), and the lowest in the variety Clery (71.429).

#### IV. CONCLUSION

Based on the obtained results, it can be concluded that the value of TSS (%) is the highest in the cultivar Joly, while the lowest was in the cultivar Asia. Also, the freezing process in three cultivars decreased the TSS content (%), while in the Maya cultivar it increased. In all cultivars, the TA value (%) increased by freezing, while the pH value decreased. The highest content of vitamin C was recorded in the variety Joly,

while the lowest was in the variety Asia. Freezing did not have a high content of vitamin C, so this method of storing strawberries proved to be good in terms of vitamin C content. Freezing increased the content of phenolic compounds in strawberries.

## REFERENCES

- [1] Michael A. H., Anne L., Dominique D. A. P., Mitchell J F., Randi A F., Charlotte B A., Seonghee L., Sujeet V., Vance M. W., Nahla B., Jason Z., Glenn S. C., Kevin B., Patrick P. E., Steven J. K., (2021). Unraveling the Complex Hybrid Ancestry and Domestication History of Cultivated Strawberry. *Molecular Biology and Evolution*, 38 (6), 2285–2305.
- [2] Kirk D. Larson (1994). *Handbook of Environmental Physiology of Fruit Crops*. (1<sup>st</sup> ed.). Boca Raton: Bruce Schaffer & Peter C. Andersen.
- [3] Baoguo X., Jianan C., Essodézam S. T., Weiqiang Y., S.M. R., Jun Y., Benxi W., Cunshan Z., Haile M., (2021). Effect of multi-mode dual-frequency ultrasound pretreatment on the vacuum freeze-drying process and quality attributes of the strawberry slices. *Ultrasonics Sonochemistry*. 78.
- [4] José F. S. S., Eduardo C. R., Victoriano V., Miguel A B. Iraida A., (2014). Deciphering gamma-decalactone biosynthesis in strawberry fruit using a combination of genetic mapping, RNA-Seq and eQTL analyses. *BMC Genomics volume*. 15.
- [5] Carmen M. P., José G. V., Sonia O., Victoriano M., María U., Jeremy P., Ana C., Catharina M., Iraida A., Lothar W., Alisdair R. F., James J. G., Miguel A. B., Victoriano V., David P., (2021). The NAC transcription factor FaRIF controls fruit ripening in strawberry. *The Plant Cell*. 33 (5), 1574-1593.
- [6] M.U. Akimov, E.V. Zhanova, V.N. Makarov, I.B. Perova, L.V. Shevyakova, O.A. Vrzhesinskaya, N.A. Beketova, O.V. Kosheleva, M.N. Bogachuk, E.V. Rylina, I V Luk'yanchuk, A. M. Mironov., (2019). Nutrient value of fruit in promising strawberry varieties. *National Library of Medicine*. Available at: <https://pubmed.ncbi.nlm.nih.gov/31233690/>
- [7] Aripa B., Angel N., Nancy B., Timothy J.L., (2014). Strawberry As a Functional Food: An Evidence-Based Review. *Critical Reviews in Food Science and Nutrition*. 54 (6), 790-806.
- [8] Jolanta K., Hanna K., Agata M., Tomasz B., Kinga S. Andrzej L., (2018). Dried strawberries as a high nutritional value fruit snack. *Food Science and Biotechnology*. 27. 799-807.
- [9] Maurizio B., Francesca G., Danila C., Johura A., Xiumin C., Di Z., Emilio G., Tamara F. H., (2021). The roles of strawberry and honey phytochemicals on human health: A possible clue on the molecular mechanisms involved in the prevention of oxidative stress and inflammation. *Phytomedicine*. 86. Available at: <https://www.sciencedirect.com/science/article/abs/pii/S0944711320300039>.
- [10] Francesca G., Josè M. A.S., Luca M., Stefania R., Stefano B., Jacopo D., Franco C., Bruno M., Josè L. Q., Maria S. F., Sara T., Maurizio B. (2012). The potential impact of strawberry on human health. *Natural Product Research: Formerly Natural Product Letters*. 27 (4-5),
- [11] Ashrafi H., Parveen B., M. Salma Z., Md. Hafizur R., Monira A., Sheikh N. I. (2016). Nutrient composition of strawberry genotypes cultivated in a horticulture farm. *Food Chemistry*. 199. 648-652.
- [12] Asad R., Rana M A., Abdou M. O. A., Mohanad B., Muhammad A., Malik M. H., (2020). Application of chitosan-based apple peel polyphenols edible coating on the preservation of strawberry (*Fragaria ananassa* cv Hongyan) fruit. *Journal of Food Processing and Preservation*. Available at: <https://ifst.onlinelibrary.wiley.com/doi/abs/10.1111/jfpp.15018>.
- [13] Irina S., Ludmila R., Ludmila D., Albina V., (2021). Changes in analytical characteristics of pectic substances when freezing berries. *International Conference on Advances in Agrobusiness and Biotechnology Research (ABR 2021)*. 285. Available at: [https://www.e3s-conferences.org/articles/e3sconf/abs/2021/61/e3sconf\\_abr2021\\_03016/e3sconf\\_abr2021\\_03016.html](https://www.e3s-conferences.org/articles/e3sconf/abs/2021/61/e3sconf_abr2021_03016/e3sconf_abr2021_03016.html).
- [14] Imants A. S., Uldis G., Martins R., Aivars A., (2008). The research of heat transfer process during freezing of berries. *3rd Baltic Conference on Food Science and Technology, FOODBALT-2008*.
- [15] Celine J. and Craig C., (2008). A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality. *J. amer. soc. hort. sci.* 133 (6), 859–867.
- [16] Anica B. M., Predrag P., Boris D., Adela K., Martina I., Ivana M. A., Iva P. B., Branimir P., Jose M. L. Danijela B. K., (2022), Chemometric Valorization of Strawberry (*Fragaria xananassa*Duch.) cv. ‘Albion’ for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. 11. 640.
- [17] Reda A. A., Wafaa A. A., Ramadan S. A., (2021). Enhancement of color stability in strawberry nectar during storage. *Annals of Agricultural Sciences*. 66 (2), 121-130.
- [18] Mirta K., Špela M. B., Tomaz P., Lea D., Janez H., Tomaz P., (2013). Phenolic Content of Strawberry Spreads during Processing and Storage. *Journal of Agricultural and Food Chemistry*. (61), 9220–9229.
- [19] Nicole R. G., Rosanela B., Priscila A., Cristiana P., (2018). Preliminary evaluation of day-neutral strawberry cultivars cultivated in Italy using a qualitative integrated approach. *Hort. Sci. (Prague)*. 45 (1), 29-36.
- [20] Zlatan R., Jasmin T., Ismet T., Aida Š., Emin S. Total content of phenols and anthocyanins in edible fruits from Bosnia. *Bosn J Basic Med Sci*. 7 (2), 119-122.
- [21] Shahar C., Maxim I., Yelena Y., Galil T., Vitaly P., Rotem H. B., Shery L., Uzi S., Rachel D.R., Nadine B.,

Einat B., Dalia W., Marina P., Shmuel S., Shifra B. D., Ilana R., Asaph A., Tslil A., Maya S., Eduard B., Ravit E., Ron O., Amir S., Benedikt F., H. Ekkehard N., Yimin X., Zhangjun F., Jim G., Efraim L., Yaakov T., Harry S. P., Nurit K., Yosef B. Arthur A. S., (2014). The PH gene determines fruit acidity and contributes to the evolution of sweet melons. *Nature Communications*. 5.

[22] Ronald E.W., Teryl P.P., G.W.V., (2006). Color quality of frozen strawberries: Effect of anthocyanin, pH, total acidity and ascorbic acid variability. *Journal of Food Science*. 35 (4), 448-452.

[23] FDA. (2003). Approximate pH of Foods and Food products. Available at: [https://www.webpal.org/SAFE/aaarecovery/2\\_food\\_storage/Processing/lacf-phs.htm](https://www.webpal.org/SAFE/aaarecovery/2_food_storage/Processing/lacf-phs.htm).

[24] Raluca N., Mira T., Mihaela G., (2012). Comparing some characteristics of fresh, frozen and canned strawberries. *Scientific Bulletin, Series F, Biotechnologies*. 156-159.

[25] Janja K. & Metka S., (2010). Ascorbic acid content in different cultivars of strawberries. *Pomology, Viticulture and Enology. 45th Croatian & 5th International Symposium on Agriculture*. 1114-1118.

[26] Mehmet A. K., Tuba D., (2010). Determination of Vitamin C and Organic Acid Changes in Strawberry by HPLC During Cold Storage. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 38 (3), 95-98.

[27] Husni S. F., Jehad F. A., Atef Al. O., Khalid M. A., Ali M. A., Khalid S., Ashok S., Talal AlQ. (2020). Effect of pH, temperature and metal salts in different storage conditions on the stability of vitamin c content of yellow bell pepper extracted in aqueous media. *Systematic Reviews in Pharmacy*. 11 (9), 661-667.

[28] Lanny S. & Lie H., (2013). Study on the Kinetics of Vitamin C Degradation in Fresh Strawberry Juices. *International Conference and Workshop on Chemical Engineering UNPAR 2013, ICCE UNPAR. Procedia Chemistry*. 9. 62-68.

[29] J. Fernando A. Z., Shiow Y. W., Chien Y. W., Gustavo A. G. A., (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT - Food Science and Technology*. 37 (7), 687-695.

[30] Gene E. L., Kim S. L., Marjorie B. M., Robert A. S., (2012). Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin–Ciocalteu: Assay interference by ascorbic acid. *Journal of Food Composition and Analysis*. 27 (1). 102-107.