

**THE EFFECT OF STORAGE CONDITIONS  
ON SELECTED QUALITY MARKERS OF FROZEN  
VEGETABLES**

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**Key words:** frozen vegetables, broccoli, sensory evaluation, vitamin C, folate.

**Abstract**

At the end of frozen food distribution chain, there is home storage including storing at refrigerator. However, not much is known how it affects frozen vegetables quality. The aim of the study was to evaluate the effect of storage conditions (2°C and 8°C from 1 to 7 days) on frozen broccoli and green beans sensory properties and vitamin C and folate content. Sensory evaluation was conducted with the use of the 5-point category scale method, while vitamins were determined with the use of HPLC technique. The results showed constant tendency in sensory quality and vitamins content decrease, the rate of which was higher at 8°C than at 2°C. The study concluded, that folate content analysis can be good chemical indicator for storage period of frozen vegetables next to vitamin C and sensory quality. However, each product requires individual assessment of its shelf-life at refrigerating temperature.

**WPLYW WARUNKÓW PRZECHOWYWANIA NA WYBRANE WYRÓŹNIKI JAKOŚCI  
MROŻONYCH WARZYW**

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**Słowa kluczowe:** mrożonki warzywne, brokuły, ocena sensoryczna, witamina C, foliany.

### Abstrakt

Ostatnim etapem dystrybucji żywności mrożonej jest jej przechowywanie w domu, np. w lodówce. Jednak niewiele wiadomo, jaki ma ono wpływ na jakość mrożonych warzyw. Celem pracy była ocena wpływu warunków przechowywania (2°C i 8°C, od 1 do 7 dni) wybranych mrożonek warzywnych, brokułów i zielonej fasolki szparagowej, na ich właściwości sensoryczne, zawartości witaminy C i folianów. Ocenę sensoryczną przeprowadzono z wykorzystaniem 5-punktowej skali kategorii, a zawartość witamin oznaczono z wykorzystaniem techniki HPLC.

Wykazano stałą tendencję obniżania się jakości sensorycznej i zawartości witamin w mrożonkach. Była ona wyższa w temperaturze 8°C niż w 2°C. Wykazano, że analiza zawartości folianów może być dobrym wskaźnikiem chemicznym okresu przechowywania mrożonych warzyw obok zawartości witaminy C i ich jakości sensorycznej. Jednak każdy produkt wymaga indywidualnej oceny okresu przydatności do spożycia podczas przechowywania w temperaturze chłodniczej.

## Introduction

Changes in the consumer preferences and lifestyle related to the lack of free time, make frozen vegetables more and more popular as convenient for quick and easy meal preparation (JOHANSSON et al. 2008). Nowadays, freezing is considered one of the best method for retaining not only sensory properties of the raw material, but also nutritive compound including vitamins. The quality of frozen product is highly dependent on the raw vegetable characteristics, environmental conditions, parameters of freezing processes, storage temperature and time. The lower the temperature is provided, the longer the shelf-life is (GONCALVES et al. 2011). According to official recommendations, the storage temperature must be stable and maintained below -18°C for a storage period between 6 and 24 months (EU Directive 89/108). Information on freezing storage conditions is written on the product packaging. However, according to some manufacturers, consumers are often interested in frozen vegetable shelf-life when it is stored in the refrigerator. Many deep-frozen products, available nowadays in retail, are marked with additional information about the storage time at temperatures above 0°C next to time of storage at -18°C.

However, at higher temperatures, the quality decrease might be observed very quickly. Numerous parameters of frozen food can be affected under such storage conditions. These are not only analytical parameters with nutritive and chemo-protective properties (e.g. vitamins, sugars, amino acid, fatty acid content) but also sensory attributes, which determine consumers acceptance (BERGER et al. 2007). Generally, to establish food products' shelf-life, microbiological criteria are considered. However, in case of frozen vegetables, sensory evaluation is used most of the times together with the use of some objective measurements, such as content of nutritional compounds, e.g. vitamin C (CORBO et al. 2006, GONCALVES et al. 2011). Although literature data on

vitamin C content in frozen vegetables under various storage conditions are available, many are old and the analysis were conducted with the use of antiquated photometric methods instead of the modern methods using high performance liquid chromatography, HPLC. Besides, knowing that chemical parameters can change in the same manner as important sensory attributes, there is a need to determine more of them, including another vitamins, as these can be considered as an important back-up to sensory methods (BERGER et al. 2007).

The aim of the study was to evaluate the effect of various storage conditions at the temperature above 0°C on frozen vegetables selected quality parameters, such as sensory properties, vitamins C and folate content.

## **Materials and Methods**

### **Material**

Test material, consisting of frozen green beans and broccoli, was purchased from company CHŁODNIA OLSZTYN in Olsztyn city which produces frozen fruit and vegetables. The company prepared 12 bags (450 g each) of both frozen vegetables which were stored in a manufactory cold room at -18°C until delivery to the Department's laboratory for analysis. The sensory evaluation together with determination of vitamin C and folate content were carried out at the vegetables samples after thawing and after 1, 3 and 7 days of storing in refrigerator at 2°C and 8°C.

### **Sensory evaluation**

The sensory properties of vegetables samples were evaluated with the use of the 5-point category scale method by a panel of selected assessors. During the test, the following attributes were evaluated: shape, color, odor and firmness. All the selected properties were characterized, described in words and the appropriate number of points (5 – very good, 4 – good, 3 – satisfactory, 2 – poor, 1 – bad) were assigned to them using previously prepared standard cards (BARYŁKO-PIKIELNA and MATUSZEWSKA 2009). The definitions and evaluation techniques were agreed upon by the assessors during training before evaluation (*Sensory analysis...* ISO 8586-1:1993). All samples of each vegetable were presented to panelists in the single sessions during which they were provided with a response sheet with written instructions for the test. Sample were prepared and coded with three random numbers and the order of their

presentation on the plates was completely randomized among assessors (*Sensory analysis...* ISO 6658:2005). The sensory evaluation took place in the test room (*Sensory analysis...* ISO 8589:2007).

### Folate analysis

**Standards.** 5-Methyltetrahydrofolate (5-CH<sub>3</sub>-FH<sub>4</sub>), tetrahydrofolate (FH<sub>4</sub>), 5-formyltetrahydrofolate (5-HCO-H<sub>4</sub>folate), 10-formyltetrahydrofolate (10-HCO-H<sub>2</sub>folate) were purchased from Sigma-Aldrich (USA) and prepared as described by KONINGS (1999), and concentrations were calculated using molar absorption coefficients given by BLAKLEY (1969).

**Enzymes.**  $\alpha$ -amylase (A-6211) was purchased from Sigma Chemical Co. and prepared as described by GUJSKA et al. (2014), by dissolving in 0.05 M potassium phosphate buffer, pH 6.1, at the concentration of 20 mg ml<sup>-1</sup> 50 ml of rat plasma folate conjugase was prepared according to JASTREBOVA et al. (2003) by dialyzing for 24 h at 4°C in 1 l of 0.05 M phosphate buffer, pH 6.1, containing 2% sodium ascorbate and 0.1% 2-mercaptoethanol.

**Extraction.** Samples of vegetables were blended and the extraction was carried according to CZARNOWSKA and GUJSKA (2012), in a 0.1 M phosphate buffer (pH 6.1) containing 2% sodium ascorbate and 0.1% 2-mercaptoethanol. After homogenizing, samples were placed for 15 min in a boiling water bath, then cooled in ice. The homogenate was subjected to enzyme treatment: 0.25 ml rat plasma folate conjugase and 1 ml  $\alpha$ -amylase (4 h at 37°C). Deconjugation was stopped by keeping tubes for 5 min in boiling water bath, then cooling in ice. Samples were centrifuged twice for 20 min at 12000 rpm, at 4°C. Supernatants were collected, filled to 50 mL with the extraction buffer and filtered, then stored at -80°C until HPLC analysis. All samples were prepared in triplicate.

**Purification and HPLC analysis.** Extracts were purified according to the JASTREBOVA et al. (2003) by solid-phase extraction with the use of Bakerbond SPE columns (500 mg 3 ml<sup>-1</sup>, J.T. Baker 7091-03). HPLC folate analysis were conducted with the method described by CZARNOWSKA and GUJSKA (2012) with Shimadzu Series LC-10A instrument equipped with Phenomenex Synergi C18 column. The chromatographic condition for gradient elution were as described: flow rate: 1 ml min<sup>-1</sup>, volume injection 100  $\mu$ l, column temperature 25°C, fluorescence detector – 290 nm excitation/ 356 nm emission. The mobile phase was acetonitrile and 30 mM phosphoric acid buffer, pH = 2.3. Peak identification was based on the retention time of standards and samples peaks. Quantification was based on calibration curves of identified folate forms.

## Vitamin C analysis

**Materials.** L-ascorbic acid and dithiotreitol were purchased from Sigma Aldrich, while metaphosphoric acid ( $\text{HPO}_3$ ) from P.P.H. "STANLAB" Sp.J.

**Sample preparation and HPLC analysis.** Vitamin C content in each vegetable sample was analyzed in triplicate according to GÖKMEN et al. (2000). After blending, 1 g of the sample was dissolved in water and  $\text{HPO}_3$  (4:1) and shaken in a water bath, then filtered. To determine total vitamin C content, dehydroascorbic acid reduction to ascorbic acid was carried by adding to the filtrate (1 ml) dithiotreitol solution (2 ml) at the concentration of  $2 \text{ mg ml}^{-1}$ . After keeping in darkness for 2 h, samples were filtered and analyzed on Agilent 1200 Liquid Chromatograph equipped with Lichrospher RP 18 column ( $5.0 \mu\text{m}$ ,  $250 \times 4.6 \text{ mm}$ ). The flow rate was  $0.5 \text{ ml min}^{-1}$ , volume injection –  $50 \mu\text{l}$ , column temperature –  $30^\circ\text{C}$ , UV spectroscopic detector ( $\lambda = 245 \text{ nm}$ ) was used. Peak identification was based on the retention time of standards and samples peaks. Quantification was based on ascorbic acid calibration curve.

## Statistical analysis

Data were analysed using the Analysis of Variance (Statistica version 10). Duncan multiple range test, with the significance level at  $p < 0.05$ , was applied to the results to test the effect of different storage conditions on folate and vitamin C content in tested vegetables.

## Results and Discussion

### Sensory evaluation

Sensory properties of the product are crucial for consumers. Meanwhile, one of the main change leading to reduction of frozen vegetables quality, is decrease in sensory properties due to degradation of natural colors, flavor changes and browning. The result of sensory evaluation of frozen vegetables is strongly effected by storage conditions, temperature and time (PUKSZTA 2013).

The sensory quality of broccoli immediately after thawing was assessed quite low at 3.7 (Figure 1). Similarly, as in the studies conducted by GONCALVES et al. (2011), the highest impact on that score had product color, which at the same time had the most influence on consumer choice. Color alternations in green vegetables during storage, including frozen storage, are attributed to the fade of the vivid green color of chlorophyll to an olive brown, characteristic

of pheophytin (MARTINS and SILVA 2002). In turn, the main features of lowering the total score of broccoli stored at 2 and 8°C for 1–7 days, were aroma and firmness. Also in MARTINS and SILVA (2004) model studies, the flavor retention has shown to be always the limiting factor of shelf-life at +5, -6 and -12°C. In our test samples after one day storing at 8°C, an unpleasant odor was noticed, firmness was also unsatisfactory. However, the product was assessed as poor no sooner than after 7 days of storage at 8°C with 50% decrease of total score (Figure 1).

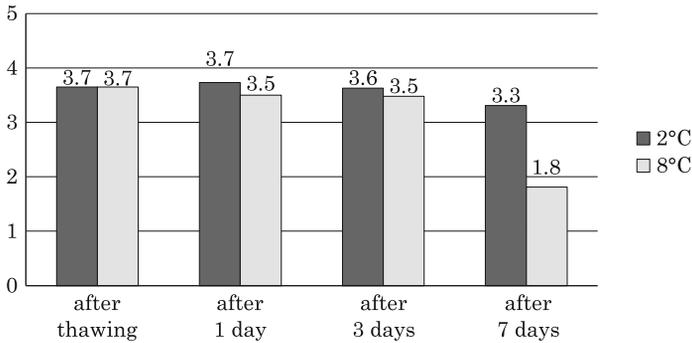


Fig. 1. Total score of broccoli sensory evaluation in the 5-point scale

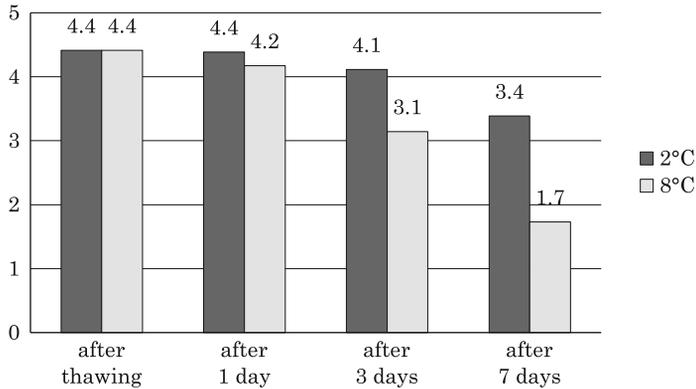


Fig. 2. Total score of green beans sensory evaluation in the 5-point scale

Sensory quality of green beans after thawing was assessed as good (4.4). The main feature of lowering the overall sensory score of this product throughout the storage period were color and firmness. The sensory quality decrease of almost 30%, was noticed no sooner than after 3 days of storing at 8°C and after 7 days at 2°C (Figure 2). Similarly, in the model studies conducted by MARTINS and SILVA (2004), only slow retention of flavor, texture and color was observed during 24 h storage at 5°C. After 7 days at 8°C, our test samples with quality decrease of 60%, received a “poor” note (Figure 2).

## Folate

Folate, vitamin B, are reduced derivatives of folic acid, which naturally occur in foods, and are necessary for proper functioning of the body. The rich source of folate in the human diet are vegetables, especially green leafy ones (spinach, broccoli) but also legumes, cereals, dairy products and liver (RAMPERSAUD et al. 2003). Unfortunately, these vitamins are very unstable compounds and quickly lose their biological activity. They undergo degradation at high temperature, sunlight, during storage time and food product preparation (JOHANSSON et al. 2008, XUE et al. 2011). The results available in literature indicate high folate lability in some food matrices at refrigeration temperatures. Researchers have called for further studies to explain the relationship between storage temperature and food matrix with which folate are bound in various foodstuffs. According to HAWKES and VILLOTA (1989), in vegetables due to the use of different technological treatments (soaking, cooking, blanching, steaming, freezing, thawing), folate reduction can vary from 22% in asparagus up to 84% in cauliflower. PUUPPONEN-PIMIÄ et al. (2003) and STEA et al. (2006), noticed that folate loss due to pea blanching (98°C/ 2 min) may range from 12 up to 35%. In another studies, CZARNOWSKA and GUJSKA (2012), observed folate reduction with the time of frozen storage (up to 12 months) at -18°C. For example, in frozen cauliflower, folate loss exceeded 95% just after three months of frozen storage while in green and yellow beans, significant loss (75% and 95%, respectively) was observed no earlier than after nine months.

In our study in all broccoli and green beans samples two folate forms were identified, tetrahydrofolate  $\text{FH}_4$  and 5-methyltetrahydrofolate,  $5\text{-CH}_3\text{-FH}_4$ . The conducted studies showed that frozen broccoli and green beans were good folate sources and its content after thawing was at the level of 129.3 and 91.1  $\mu\text{g } 100\text{g}^{-1}$ , respectively. In case of frozen broccoli stored at 2°C and 8°C up to 7 days, the significant folate loss of 20% was observed after 3 days at 2°C. The highest decrease of 30%, was noticed after 7 days at 8°C, while after the same time at 2°C, the loss didn't exceed 20%. In green beans significant folate loss of 10% was observed after 1 day at 2°C and almost 20% at 8°C. Folate decrease during storage for 3 and 7 days remained at the level of 20% and was not significantly different to the loss noticed after 1 day at 8°C (Table 1).

## Vitamin C

Vitamin C is a water soluble vitamin that is an important antioxidant in the human body, which can prevent cancer (MARTINS and SILVA 2004). Due to its high sensitivity, it is widely considered as an appropriate marker for monitoring changes during processes including storage in the whole frozen chain.

Table 1  
The effect of selected storage conditions on folate and vitamin C content in frozen broccoli and green beans\*

Product/ storage conditions	5CH <sub>3</sub> FH <sub>4</sub> µg 100 g <sup>-1</sup>	FH <sub>4</sub> µg 100 g <sup>-1</sup>	Total folate* µg 100 g <sup>-1</sup>	Vitamin C mg 100 g <sup>-1</sup>
Broccoli				
After thawing	121.7 ± 9.9	7.6 ± 0.5	129.3 <sup>a**</sup>	20.7 ± 0.9 <sup>a</sup>
After 1 day at 2°C	122.9 ± 2.9	7.9 ± 0.3	130.8 <sup>a</sup>	20.7 ± 0.3 <sup>a</sup>
After 1 day at 8°C	117.6 ± 7.0	7.2 ± 0.6	124.7 <sup>a</sup>	18.7 ± 1.5 <sup>b</sup>
After 3 days at 2°C	94.8 ± 1.2	10.6 ± 0.3	105.4 <sup>b</sup>	16.6 ± 0.5 <sup>c</sup>
After 3 days at 8°C	95.5 ± 5.7	11.1 ± 0.1	106.6 <sup>b</sup>	14.5 ± 0.7 <sup>d</sup>
After 7 days at 2°C	95.1 ± 3.7	10.9 ± 0.1	105.9 <sup>b</sup>	13.5 ± 0.3 <sup>d</sup>
After 7 days at 8°C	79.9 ± 1.5	8.7 ± 0.6	88.6 <sup>c</sup>	10.7 ± 0.1 <sup>e</sup>
Green beans				
After thawing	65.4 ± 2.7	25.8 ± 1.3	91.1 <sup>a</sup>	12.0 ± 0.6 <sup>a</sup>
After 1 day at 2°C	64.7 ± 6.3	18.1 ± 0.3	82.9 <sup>b</sup>	11.7 ± 0.1 <sup>a</sup>
After 1 day at 8°C	57.6 ± 1.8	17.5 ± 0.5	75.1 <sup>c</sup>	12.2 ± 0.9 <sup>a</sup>
After 3 days at 2°C	55.3 ± 2.3	20.7 ± 1.1	75.9 <sup>bc</sup>	11.5 ± 0.3 <sup>a</sup>
After 3 days at 8°C	53.4 ± 0.9	21.2 ± 0.8	74.6 <sup>c</sup>	11.5 ± 0.8 <sup>a</sup>
After 7 days at 2°C	55.1 ± 2.4	21.5 ± 1.3	76.6 <sup>bc</sup>	9.5 ± 0.5 <sup>b</sup>
After 7 days at 8°C	55.8 ± 4.1	16.7 ± 1.2	72.5 <sup>c</sup>	4.7 ± 0.1 <sup>c</sup>

\* Measured as the sum of 5CH<sub>3</sub>FH<sub>4</sub> and FH<sub>4</sub>, expressed as folic acid

\*\* Means with the same letter are not significantly different ( $p < 0.05$ )

Vitamin C degradation is caused by two factors: L-ascorbic acid oxidation and by destructive effects of specific enzymes (SERPEN et al. 2007). According to PALICH and PUKSZTA (2001), the interaction of both factors is greatly enhanced under temperature fluctuations during storage. The authors observed that even the increase of storage temperature from -18°C to -8°C, resulted in a significant increase in the rate of enzymatic reactions causing vitamin C degradation and led to a reduction in the nutritional value of frozen vegetables and fruits. Also in GONCALVES et al. (2011) study, frozen storage of broccoli under isothermal conditions (from -30°C to -5°C within 57 days) caused 51% loss in vitamin C content.

In our study after freezing operations, frozen storage and thawing, broccoli presented vitamin C content of 20.7 mg 100 g<sup>-1</sup>, which was lower than 34 mg 100 g<sup>-1</sup> and 36.07 mg 100 g<sup>-1</sup> reported by SIKORA et al. (2008) and GONCALVES et al. (2011), respectively. In green beans after thawing, vitamin C content was at the level of 12.0 mg 100 g<sup>-1</sup>, which corresponds well with results obtained for fresh product (10–25 mg 100 g<sup>-1</sup>) by MARTINS and SILVA (2002) and SERPEN et al. (2007). In our broccoli samples significant vitamin C reduction of almost 10% was observed after 1 day at 8°C. After 7 days at refrigerator, the loss was 35 and 49% at 2°C and 8°C, respectively. In green beans the significant reducing was observed after 7 days at 2°C and 8°C at the level of 21 and 61%, respectively. Significant loss in vitamin C content in raw

green beans after 4 days storage at 20°C was also noted by BERGER et al. (2007). The author observed the reduction of around 20% after one day of storage. FAVELL (1998) found that 40% of the original amount of the total ascorbic acid content in fresh green beans was lost after 3 days of storage at 4°C. These differences of his results to BERGER et al. (2007) study, can result from different experimental procedures applied for vitamin C determination. According to BERGER et al. (2007), the reducing trend for vitamin C is the same during deep-frozen green beans storage. When stored for 12 months at -18°C, 75% of the total ascorbic acid content was left.

## Conclusions

Both time and temperature had a significant effect on vitamins content and sensory properties of frozen green beans and broccoli. The total ascorbic acid content is considered to be good chemical indicator for the storage period of frozen vegetables. Our study showed that also another vitamins, folate for instance, seemed to be an important quality parameter of frozen products during their shelf-life at refrigerating temperatures. In case of sensory evaluation it was noticed that total quality of analyzed products is determined by different quality attribute.

For all tested parameters it was observed, that lower temperature (2°C) at refrigerator, extended the frozen vegetable shelf-life comparing to 8°C. However, the dynamics of these changes, were different for each product. Because of this, the knowledge of the exact influence of storage for each frozen vegetable would help to provide the optimal conditions to enhance quality and prolong frozen vegetables shelf-life during home storage, which includes storing at refrigerator.

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