CHAPTER 9

Impact of long-term storage on the quality of frozen pickled sweet peppers

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Abstract

Vegetables are produced seasonally; therefore, freezing is one of the main methods of preserving them while maintaining their quality. Both fresh vegetables and semi-finished or ready-to-eat vegetable products are commonly frozen. Such products are popular among consumers, as they provide rapid preparation and consumption. Vegetables, particularly sweet peppers, are often pickled in marinades with various spices to enhance sensory properties, preserve nutrients, extend shelf life, and expand the range of available products. A promising approach for storing pickled sweet peppers is freezing. In this study, sweet peppers were pickled in a marinade containing the following ingredients: water, sunflower oil, sugar, natural honey, salt, citric acid, and spices (dried dill and parsley, bay leaves, fresh garlic, and allspice). After washing with water, cleaning, and cutting, sweet pepper slices were pickled in the marinade for 12 h at room temperature and subsequently frozen at −20°C. Experimental results showed that during 270 days of frozen storage of pickled sweet peppers, losses of ascorbic acid ranged from 12.0 to 19.8%, carotenoids from 5.8 to 15.1%, dry matter from 0.7 to 4.9%, total sugars from 0.6 to 6.1%. At the same time, the contents of water-soluble pectin, anthocyanins, catechins, and total flavonoids increased during storage. Freezing also had a positive effect on the microbiological safety of the product, as the number of microorganisms in the frozen product during storage was lower than that in fresh sweet peppers. The sensory quality of the frozen product, including appearance, color, aroma, taste, and consistency, was highly rated by expert evaluators. To bring the product quickly to a ready-to-eat state, microwave thawing is recommended. Pickled sweet peppers thawed using this technique exhibited high sensory quality. However, prolonged storage of the product at room temperature after thawing is not recommended, as it leads to

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a decrease in ascorbic acid content and an increase in polyphenol oxidase activity and microbial growth. Although the product remained safe for consumption after 24 h of storage at 20°C, the number of microorganisms did not exceed the permissible limits for quick-frozen vegetables (the maximum bacterial and mold counts in the thawed pickled sweet peppers were 48,400 CFU/mm^2 and 19.0 CFU/mm^2, respectively).

Keywords

Sweet pepper, marinade, freezing, thawing, pickled vegetable, frozen vegetable, thawed vegetable, microwave-thawed vegetable, microflora, oxidative enzymes.

9.1 Introduction

Freezing is a widely used method for preserving vegetables [1]. This preservation method extends the shelf life of vegetables and helps maintain their quality. The use of freezing storage (below –20°C) increases the seasonal availability and distribution range of vegetables beyond the region where they are grown [2]. Physicochemical parameters, sensory attributes, and the texture of frozen vegetables are strongly affected by the freezing process. The most commonly used methods for freezing vegetables include air-blast, fluidized bed, immersion, and cryogenic freezing [3]. Conventional freezing method is characterized by relatively low process efficiently [4]. During freezing and frozen storage, vegetable cells are damaged, which leads to the degradation of released antioxidant compounds through chemical and enzymatic oxidation reactions [5]. Among the methods of pre-processing vegetables before freezing, blanching is commonly used, as it preserves the natural taste and color by inactivating enzymes. However, it also leads to the reduction in the content of vitamin C and other heat-labile compounds due to leaching into the blanching water [6]. To shorten the freezing process and prolong the shelf life of vegetables, osmotic dehydration is used as a pretreatment. During this process, vegetables are soaked in an aqueous solution containing sugar or salt [7]. Air-drying and osmodehydrofreezing are also used to remove a portion of water from vegetables prior to freezing [8]. Alternatively, so-called "chemical" blanching (using chemicals) and microwave-assisted blanching can be effectively applied prior to freezing to retain nutritional compounds, such as vitamin C [9]. Magnetic-field-assisted freezing helps enhance the freezing rate and improve the quality of frozen foods in terms of sensory properties compared to other freezing methods [10]. Freezing time can be shortened by 15–26%, and the quality of frozen products can be improved when combined electric- and magnetic-field-assisted freezing is applied to the freezing and thawing of vegetables [11].

Sweet pepper (*Capsicum annum* L.) is one of the most important fruit crops, containing biologically active compounds such as antioxidants, ascorbic acid, carotenoids, and vitamin E [12]. The shelf life of fresh sweet pepper fruits varies from 4 to 21 days and is limited by decay and microbial growth. It is also influenced by fruit cultivar, physiological maturity, and storage conditions [13]. Freezing is an important method for extending the shelf life of sweet peppers, which helps preserve the nutritional and sensory qualities of the fruits [14]. Storage temperature and humidity have a significant influence on the quality of sweet pepper fruits, including water loss rate and texture [15]. Pepper fruits are highly susceptible to chilling injury at temperatures below 7–10°C; therefore, different methods are used to alleviate chilling injury, including modified atmosphere, hot water, and chemical treatments [16]. The combined treatment (hot water at 45°C for 15 min + methyl salicylate at 0.05 mmol/L) is also used, as it helps reduce chilling injury and the loss of ascorbic acid and total phenolics [17].

Conventional pickling is a widely used method for enhancing the texture, color, and flavor of foods [18]. For sweet pepper pickling, an acidic marinade is used, consisting of vinegar, salt, sugar, bay leaves, allspice berries, and black pepper [19]. Advanced vegetable pickling techniques promote probiotic growth, enhancing the activity and stability of bioactive compounds, and improving product quality and health benefits [20]. To improve the quality and nutritional value of pickled peppers, they are fermented with dairy ingredients or by-products of the cheese-making process [21]. One novel technique for processing vegetables is pickling them using lactic acid bacteria (*Levilactobacillus brevis*, *Limosilactobacillus fermentum*, and *Lactiplantibacillus plantarum*), which results in increased vitamin C and total polyphenols [22].

For consumption or culinary use, frozen vegetables are thawed. If this process is performed incorrectly, it can significantly reduce the quality and nutritional value of vegetables. Conventional vegetable thawing techniques include ambient-temperature thawing and water thawing. In addition, a novel method has been developed, namely combined microwave- and infrared-assisted thawing, which helps shorten the thawing time by 65–75% and reduces thawing losses by 10–40% [23]. To reduce drip loss and maintain the sensory quality of foods, high-pressure, ultrasound-assisted, ohmic, radiofrequency, and vacuum-assisted thawing techniques have also been developed [24]. Defrosting at room temperature is a very slow process, and if the temperature is above 5°C, it creates favorable conditions for the rapid growth of harmful microorganisms [25]. Therefore, rapid thawing techniques are preferred over slower ones to obtain a safe product.

The intensive lifestyle of most urban populations has increased the popularity of frozen semi-finished products, which permit quick meal preparation. Consequently, the food industry continues to expand the range of such products. The production of plant-based semi-finished products with maximal preservation of nutrients and sensory properties is particularly promising. In this context, the development of technology for producing frozen pickled sweet peppers that ensures rapid meal preparation and extended product shelf life is of considerable interest.

The main problem is that freezing, low-temperature storage, and subsequent thawing of vegetables can cause damage to cellular structures, leading to moisture loss, texture deterioration, and a reduction in the content of biologically active compounds. Therefore, the technology for producing frozen pickled vegetables, particularly sweet peppers, requires further investigation into the effects of freezing, storage conditions, and thawing methods on product quality and safety.

The aim of the study is to investigate the nutritional value, sensory attributes, and safety properties of frozen pickled sweet peppers during low-temperature storage and after thawing using different techniques.

9.2 Materials and methods

For the study, ripe fresh red sweet peppers without visible damage were used. The peppers were washed with water, the seeds and stalks were removed, and the pulp was cut into slices along the fruit. The peppers were blanched in hot water for 1–2 min.

The marinade contained (per 1 kg of sweet pepper slices): water (400 g), sunflower oil (80 g), sugar (50 g), natural honey (40 g), salt (15 g), and spices (dried dill and parsley (2 g), bay leaves (1 g), fresh garlic (4 g), and allspice (1 g). In the marinade, milder citric acid (4 g), which has no pungent odor, was used instead of traditional acetic acid. To prepare the marinade, water was boiled and sugar, salt, and spices were added and heated until the sugar and salt were dissolved. Citric acid and honey were then added, the mixture was boiled for 2–3 min, and the pepper slices were poured with the hot marinade. The pH of the marinade was 3.9–4.0. The peppers were pickled in the marinade for 12 h at room temperature (20°C). After pickling, the pepper slices with marinade were placed in plastic containers, and frozen at –20°C, at which temperature the product was stored.

Samples of pickled sweet pepper were analyzed before freezing and after freezing during storage (10, 90, 180, and 270 days) at a temperature of –20°C. For the analysis of sensory and physicochemical properties, as well as bioactive compounds, frozen pickled sweet pepper samples were thawed in air at 20°C.

The sensory parameters of pickled sweet pepper, dry matter content, sugar content, titratable acidity, ascorbic acid content, carotenoid content, flavonoid content, pectin content, and microbiological parameters were determined. In addition, the sensory parameters of pickled sweet pepper were evaluated after thawing using different techniques (in air at room temperature, in water, and in a microwave oven).

A panel of seven trained experts evaluated the sensory attributes (appearance, color, aroma, consistency, and taste) of pickled sweet pepper samples using a 5-point scale according to method described in [26].

The dry matter content of pickled sweet peppers was determined according to method [27]. About 5 g of homogenized pickled peppers or frozen pickled pepper samples were weighed and evaporated to dryness in a SESH-3MU drying oven at 105°C for 5 h. The samples were then cooled in a desiccator and weighed. The dry matter content was expressed as a percentage of the initial weight of the sample. The dry matter content of the marinade was determined in a similar manner; however, the samples were dried until a constant weight was achieved.

The total sugar content of pickled and frozen pickled sweet peppers was determined according to the modified phenol-sulfuric acid method [28]. Briefly, 1 g of finely chopped pepper pulp was homogenized and extracted with 10 mL of 80% ethanol at 80°C for 30 min. The extract was filtered, and 1 mL of the filtrate was mixed with 1 mL of 5% phenol solution, followed by the rapid addition of 5 mL of concentrated H_2SO_4. The mixture was incubated at room temperature for 20 min, and the absorbance was measured at 490 nm using a ULAB 108UV spectrophotometer. Total sugar concentration was calculated from a calibration curve constructed with a glucose standard.

Titratable acidity, expressed as a percentage of citric acid, of pickled and frozen pickled sweet peppers was determined according to the method [29]. For the determination of titratable acidity, the samples were homogenized with distilled water and titrated with 0.1 N NaOH using phenolphthalein as an indicator. The titration endpoint was defined as the appearance of a persistent pink color. The results were expressed as a percentage of citric acid equivalents.

Ascorbic acid was extracted from the samples using meta-phosphoric acid with the addition of a small amount of acid-washed quartz sand. The resulting supernatant was then titrated with standard 2,6-dichlorophenolindophenol [27].

Carotenoids in pickled sweet peppers was determined according to [30]. Dried samples were extracted with acetone using a homogenizer until the filtrates were completely decolorized. The extract was washed with diethyl ether and separated with 10% NaCl. The organic phase was dried over anhydrous Na_2SO_4 and saponified with 20% KOH in methanol for 1 h at 20°C. The pigments were re-extracted with diethyl ether, evaporated, and dissolved in acetone (10 mL). Aliquots (1 mL) were centrifuged at 12,000 rpm and stored at –20°C until analysis. The carotenoid profile of the extract was quantified using β-apo-8′-carotenal as an internal standard. The carotenoid profile was determined by HPLC using a Shimadzu LC-20AT Prominence system. The mobile phases used were 81:15:4 methanol/methyl tert-butyl ether (MTBE)/H_2O (solution A) and 91:9 MTBE/methanol (solution B). Gradient elution was performed from 100% A to 50% A and 50% B in 45 min, followed by 100% B in the next 10 min and 100% A in the next 5 min at a flow rate of 0.8 mL/min. Carotenoids were monitored at 450 nm.

Water-soluble pectin content in pickled sweet peppers was determined according to [31], with minor modification. Briefly, 25 g minced peppers were homogenized in 95% ethanol for 3 min, filtered, and the filtrate was washed twice with 75% ethanol. The solid residue was treated with 15 mL EDTA-Na_2, and the pH was adjusted to 11.5 with 0.1 M NaOH and incubated for 30 min. The solution was then adjusted to pH 5.5 with 2 N acetic acid, 15 mL of water was added, and the mixture was incubated at 50°C for 30 min. After filtration, the filtrate was made up to 50 mL and used as the water-soluble pectin extract. For analysis, 1 mL of extract was mixed with 6 mL H_2SO_4, boiled for 20 min, cooled, and then 0.2 mL of 0.15% carbazole was added, followed by incubation at 20°C for 2 h. Absorbance was measured at 520 nm using a ULAB 108UV spectrophotometer, and pectin content was calculated from a calibration curve prepared with a standard pectin solution.

Anthocyanin content in pickled sweet pepper was determined using the pH differential method [32]. The pepper pulp was ultrasonically extracted with methanol containing 1% HCl, and the supernatant was collected. Absorbance was measured at 530 nm and 700 nm using a ULAB 108UV spectrophotometer. Anthocyanin concentration was calculated according to the equation described in [32].

Catechin content in pickled sweet pepper was determined by HPLC [33] using a Shimadzu LC-20AT Prominence system equipped with a C18 column (250 × 4.6 mm, 5 μm) and UV detection at 280 nm. Samples were extracted with a methanol-water solution (70:30, v/v) containing 0.1% formic acid and filtered through a 0.22 μm membrane. Separation was performed using a gradient of water with 0.1% formic acid and acetonitrile at a flow rate of 1.0 ml/min. Catechin was identified by comparison with a catechin standard and quantified using a calibration curve.

The total flavonoid content in pickled sweet pepper samples was determined using a spectrophotometric method [34] based on the reaction with aluminum chloride ($AlCl_3$). A calibration curve was constructed using quercetin-3-rhamnoglucoside as a standard. About 0.5 mL of diluted extract was mixed with 0.4 mL of 25% ethanol and 0.5 mL of 10% sodium acetate. The mixture was kept in the dark at 20°C for 30 min, and the absorbance was measured at 430 nm using a ULAB 108UV spectrophotometer.

Microbiological analyses were performed according to the method [35]. About 25 g of pickled sweet peppers were homogenized in 225 mL of peptone water. Decimal dilutions were prepared and inoculated onto the media to enumerate aerobic mesophilic bacteria, yeasts, and molds. Bacteria were cultured on plate count agar, while yeasts and molds were cultured on yeast extract glucose chloramphenicol agar.

Polyphenol oxidase activity in pickled sweet pepper samples was determined spectrophotometrically according to a modified method described in [36]. Briefly, 1 g of pepper pulp was homogenized in 10 mL of 0.1 M sodium phosphate buffer (pH 7.0) containing 2 mM ascorbic acid. The homogenate was centrifuged at medium speed for 15 min at 4°C, and the supernatant was collected as the enzyme extract. The reaction mixture consisted of 2.8 mL of 0.1 M phosphate buffer containing 20 mM catechol as the substrate and 0.2 mL of enzyme extract. The increase in absorbance at 420 nm due to quinone formation during substrate oxidation was monitored using a ULAB 108UV spectrophotometer. Polyphenol oxidase activity was calculated from the rate of absorbance increase and expressed as μmol of substrate oxidized per minute per gram of fresh weight.

All analyses were carried out in triplicate. Statistical analysis was performed using Mathcad software to calculate mean values and standard deviations.

9.3 Frozen pickled sweet peppers

9.3.1 Sensory properties of frozen pickled sweet peppers

Table 9.1 presents the results of the sensory evaluation of pickled sweet peppers before freezing and during low-temperature storage after freezing.

Table 9.1 Sensory evaluation of pickled sweet peppers before freezing and during low-temperature storage after freezing (points)

Storage (days)	Sensory evaluation (mean)					
	Appearance	Color	Aroma	Taste	Consistency	Average score
0	4.9	5.0	5.0	5.0	5.0	4.98
10	4.8	5.0	5.0	4.6	4.3	4.74
90	4.8	5.0	5.0	4.7	4.4	4.78
180	4.9	5.0	5.0	4.9	4.7	4.90
270	4.9	5.0	5.0	4.9	4.8	4.92

Storage conditions did not affect the color or aroma of the peppers. The color of the peppers remained bright red throughout storage, and the aroma was typical of pickled peppers, with notes of the spices used. The appearance was rated 4.8 points on days 10 and 90 of storage, slightly lower than at the beginning and end of the storage period. According to expert assessments, the taste of the peppers deteriorated at the beginning of storage but improved by the end of the storage period. A similar trend was observed for changes in pepper consistency. During storage, the pulp of the pepper slices softened due to low temperatures; yet they retained their shape and elasticity. The taste of the product was typical of pickled peppers. The average sensory score was highest before storage (4.98), lowest on day 10 of storage (4.74), and increased to 4.92 points by day 270.

9.3.2 Physicochemical properties of frozen pickled sweet peppers

The dry matter content of pickled sweet peppers before storage was 5.92% (**Table 9.2**). During storage, it decreased to 95.1% of the initial value (5.63%) by day 270. Similarly, the dry matter content of the marinade decreased from 3.50% before low-temperature storage to 3.38% by day 180 and 3.40% by day 270. For comparison, the dry matter content of fresh sweet peppers ranges from 8.4 to 13.3% [27]. Thus, the dry matter content decreases during the pre-treatment and pickling stages.

During 270 days of storage, the total sugar content of frozen pickled peppers decreased to 93.9% of its initial value, from 5.43% to 5.10% (**Table 9.2**). Fresh sweet pepper fruits have a total sugar content ranging from 2.40 to 5.86% [29]. The increased sugar content in pickled sweet peppers is due to the addition of sugar to the marinade.

Table 9.2 Physicochemical parameters of pickled sweet peppers before freezing and during low-temperature storage after freezing

Storage (days)	Dry matter content (%)			Sugar content (%)		Titratable acidity (%)
	in pepper fruits	in marinade	preserved in peppers (%)	total	preserved in peppers (%)	
0	5.92 ± 0.08	3.50 ± 0.22	–	5.43 ± 0.17	–	0.31 ± 0.02
10	5.88 ± 0.34	3.47 ± 0.08	99.3	5.40 ± 0.28	99.4	0.30 ± 0.01
180	5.82 ± 0.11	3.38 ± 0.15	98.3	5.21 ± 0.23	95.9	0.30 ± 0.01
270	5.63 ± 0.15	3.40 ± 0.14	95.1	5.10 ± 0.24	93.9	0.32 ± 0.02

The total acidity of the frozen product did not change significantly during storage, remaining between 0.30% and 0.32% (**Table 9.2**). The titratable acidity of fresh sweet pepper ranges from 0.16 to 0.23% [29]. The acidity of pickled pepper was higher due to the addition of citric acid to the marinade.

9.3.3 Bioactive compounds of frozen pickled sweet peppers

The content of bioactive compounds in pickled sweet peppers before freezing and during low temperature storage after freezing is presented in **Table 9.3**. The ascorbic acid content of pickled sweet peppers was 226.7 mg/100 g. During storage, it decreased to 181.8 mg/100 g by day 270. Thus, at the end of long-term storage (day 270), the ascorbic acid content was 80.2% of the initial value, indicating that slow degradation occurs even at low temperatures. The vitamin C content in fresh sweet peppers, depending on the variety and growing conditions, ranges from 115.5 to 239.8 mg/100 g [27]. Thus, frozen pickled pepper maintain a relatively high level of ascorbic acid. A decrease in its content is also observed during the pre-treatment stage, particularly during blanching, when part of the water-soluble vitamin C passes into the water.

Table 9.3 Content of bioactive compounds in pickled sweet peppers before freezing and during low-temperature storage after freezing (mg/100 g)

Bioactive compounds	Storage (days)				
	0	10	90	180	270
Ascorbic acid	226.7 ± 0.6	199.6 ± 0.4	192.4 ± 0.2	187.3 ± 0.1	181.8 ± 0.6
Carotenoids	22.5 ± 0.3	21.2 ± 0.5	20.1 ± 0.2	19.4 ± 0.8	19.1 ± 0.4
Water-soluble pectin	417.4 ± 0.6	635.5 ± 0.3	796.4 ± 0.4	607.8 ± 0.3	513.1 ± 0.4
Anthocyanin	0.87 ± 0.07	1.04 ± 0.09	2.02 ± 0.07	1.56 ± 0.11	1.48 ± 0.22
Catechin	63.2 ± 0.3	66.2 ± 0.1	129.6 ± 0.5	131.5 ± 0.2	133.1 ± 0.2
Total flavonoids	144.6 ± 0.3	156.6 ± 0.3	315.7 ± 0.4	312.7 ± 0.1	347.7 ± 0.4

In pickled sweet peppers, the carotenoid content was 22.5 mg/100 g and decreased during low-temperature storage. After 270 days of storage, the carotenoid content decreased by 15.1%, reaching 19.1 mg/100 g (**Table 9.3**). The carotenoid content in fresh sweet peppers depends on the variety and stage of maturity, ranging widely from 14.1 mg/100 g (*Red Lamuyo* peppers) to 323.1 mg/100 g (organic peppers) [30].

The content of water-soluble pectin in frozen pickled peppers increased during storage for up to 90 days, from 417.4 mg/100 g (unfrozen pickled peppers) to 796.4 mg/100 g (frozen pickled peppers), and then decreased to 513 mg/100 g after 270 days of storage (**Table 9.3**). However, on day 270 of storage, the water-soluble pectin content in the peppers was still 22.9% higher than its content in unfrozen pickled peppers. This may be due to cell structure destruction and partial conversion of protopectin into water-soluble pectin. The content of water-soluble pectin in fresh peppers ranges from 1.291 to 1.761 mg/100 g DW [31], while the content of insoluble pectin ranges from 237 to 830 mg/100 g DW.

The anthocyanin content in pickled peppers increased during low-temperature storage and exceeded that in unfrozen pickled peppers by 70.1% at day 270 (**Table 9.3**). The anthocyanin content in fresh pepper fruits depends on the stage of ripeness and, in ripe fruits (purple to red), does not exceed 10 mg/100 g [32].

The catechin content in pickled peppers was 63.2 mg/100 g (**Table 9.3**). During the first 10 days of storage after freezing, it increased slightly to 66.2 mg/100 g. During the subsequent storage period, the catechin content more than doubled. After 270 days of storage, the catechin content of frozen pickled peppers reached 133.1 mg/100 g. For comparison, the catechin content in fresh peppers ranges from 74.5 to 79.4 mg/100 g [33].

The total flavonoid content during storage followed a pattern similar to that of catechin: an initial slight increase followed by a doubling after 90 days (**Table 9.3**). Prior to freezing, the flavonoid content in pickled peppers was 144.6 mg/100 g, and after 270 days of low-temperature storage, it reached 347.7 mg/100 g. For comparison, the total flavonoid content in fresh peppers ranges from 80.0 to 112.6 mg/100 g [34].

9.3.4 Microbiological indicators of frozen pickled sweet peppers

The microorganisms most commonly responsible for food spoilage include *Pseudomonas*, *Enterobacteriaceae*, and *Brochothrix thermosphacta* [37]. These organisms cause slime formation, deterioration of food texture, and the development of off-flavors. During storage, low-temperature conditions inhibit the growth of microorganisms [38]. The quantity of microorganisms in frozen products depends on several factors, including pH, temperature, product type, pre-treatment, storage time before freezing. Due to the risk of microbial growth, pre-treatment operations should be performed as quickly as possible. The spores of many bacteria do not die during prolonged exposure to an acidic environment, although they do not develop under such conditions. A neutral or slightly alkaline environment promotes their longer survival. The presence of sugar in the solution also helps to preserve their resistance during freezing. Pathogenic microorganisms that cause food poisoning do not develop in quick-frozen products, as they are not psychrophilic and have a minimum growth temperature of 5°C. Some microorganisms are inactivated during freezing, while others are inactivated during storage in the frozen state. The destruction of microflora at low temperatures is slow and depends on the composition and type of microorganisms. Gram-negative bacteria, including coliforms, are more sensitive to low temperatures than Gram-positive bacteria.

Microbiological analysis of fresh sweet pepper samples demonstrated contamination with microorganisms, including molds and bacteria, while no yeasts were detected on the pepper fruit surface (**Table 9.4**).

Table 9.4 Analysis of epiphytic microflora in frozen pickled sweet peppers

Storage (days)	Number of microorganisms (CFU/mm^2)		
	Molds	Yeasts	Bacteria
0 (fresh pepper)	483	n.d.	16,918
10	2.1	n.d.	5,212
90	1.6	n.d.	6,213
180	3.0	n.d.	6,850
270	5.1	n.d.	11,257

Note: n.d. – not detected

After 10 days of storage, the number of microorganisms in frozen pickled peppers decreased sharply compared to fresh peppers. In particular, the number of molds decreased from 483.0 to 2.1 CFU/mm^2, and the number of bacteria decreased from 16,918 to 5,212 CFU/mm^2. No yeasts were detected throughout the entire storage period. These results indicate that molds and certain types of bacteria are resistant to the acidic environment of the marinade (**Table 9.4**). After 90 days of storage, the number of molds further decreased to 1.6 CFU/mm^2, while bacteria increased to 6,213 CFU/mm^2. During prolonged storage, the number of molds and bacteria gradually increased, indicating that they retained the ability to grow. After 270 days of storage, the number of molds reached 5.1 CFU/mm^2 and bacteria 11,257 CFU/mm^2. However, these values did not exceed those observed in fresh peppers, although the number of bacteria increased sharply during the final three months of storage.

Low microorganism content is a key factor in ensuring the microbiological safety of frozen pickled peppers. The microbiological indicators fully comply with sanitary standards and regulations, confirming that the product is safe for consumption.

9.4 Thawed pickled sweet peppers

9.4.1 Effect of different thawing techniques on the sensory properties of pickled sweet peppers

Frozen pickled sweet peppers were thawed using different techniques, including conventional air-thawing at room temperature (20°C), water thawing, and thawing in a microwave oven. All samples of frozen pickled peppers were stored at −20°C for 90 days prior to thawing.

In terms of appearance, the pickled pepper sample thawed using microwave-assisted thawing received the highest score of 5.0 (**Table 9.5**), while the lowest score (4.4) was observed for the air-thawed sample. A similar trend was observed for color. The aroma of peppers thawed using water thawing and microwave-assisted thawing was rated highly, with scores of 4.9 and 5.0, respectively. All thawed samples received high scores for taste, with water-thawed and microwave-thawed samples achieving the maximum score of 5.0. Regarding consistency, the air-thawed and water-thawed samples received lower scores (4.2 and 4.1, respectively) compared to the microwave-thawed sample (4.7). The highest average sensory score (4.94) was observed for peppers thawed using microwave-assisted thawing, indicating that this technique is the most effective for maintaining the sensory quality of pickled sweet peppers.

Table 9.5 Sensory evaluation of thawed pickled sweet peppers (points)

Thawing techniques	Sensory evaluation (mean)					
	Appearance	Color	Aroma	Taste	Consistency	Average score
Air-thawing	4.4	4.6	4.5	4.8	4.2	4.50
Water thawing	4.7	4.0	4.9	5.0	4.1	4.54
Microwave-assisted thawing	5.0	5.0	5.0	5.0	4.7	4.94

9.4.2 Ascorbic acid content of thawed pickled sweet peppers

Table 9.6 presents the ascorbic acid content in microwave-thawed pickled pepper samples after storage in air at room temperature (20°C) for 2, 4, 6, and 10 hours. These measurements were performed on frozen pickled peppers after 10, 90, 180, and 270 days of low-temperature storage.

The ascorbic acid content in thawed pickled peppers after two hours of storage at room temperature decreased by 5.6–11.1% compared to its level immediately after thawing (**Table 9.6**). Samples stored in a frozen state for a longer period exhibited a higher percentage of ascorbic acid loss. A similar trend was observed in samples stored for 4, 6, and 10 hours after thawing. Compared to the content immediately after thawing, the ascorbic acid level decreased by 9.2–13.1% after 4 hours of storage, by 11.6–16.1% after 6 hours, and by 22.6–24.8% after 10 hours. In absolute terms, the ascorbic acid content decreased both with increasing duration of frozen storage and with prolonged storage in the thawed state. The lowest ascorbic acid content (136.8 mg/100 g) was observed in the sample stored at low temperatures for 270 days and subsequently held at room temperature for 10 hours after thawing.

Table 9.6 Ascorbic acid content of thawed pickled sweet peppers during short-term storage (mg/100 g)

Storage of thawed-samples (hours)	Frozen storage (days)			
	10	**90**	**180**	**270**
0	199.6	192.4	187.3	181.8
2	187.8	175.6	169.2	161.6
preserved in peppers (%)	94.1	91.3	90.3	88.9
4	181.2	170.3	163.7	157.9
preserved in peppers (%)	90.8	88.5	87.4	86.9
6	176.5	163.5	157.9	152.6
preserved in peppers (%)	88.4	85.0	84.3	83.9
10	154.5	148.5	141.1	136.8
preserved in peppers (%)	77.4	77.2	75.3	75.2

9.4.3 Enzyme activity in thawed pickled sweet peppers

The dynamics of enzymatic changes in frozen foods are determined by their chemical composition, properties, and storage conditions. Subzero temperatures do not cause complete or long-term enzyme inactivation, but rather lead to temporary and partial inhibition of enzyme activity due to changes in environmental conditions, including a reduction in the liquid phase resulting from water crystallization, an increase in ion concentration, and changes in the pH of the medium. The quality of sweet peppers may be affected by enzymatic activities. In particular, polyphenol oxidase catalyzes the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones [39]. Further reactions of these quinones result in the accumulation of melanin, which is responsible for browning in plant tissues.

Polyphenol oxidase activity in pepper fruits was determined during the storage period, beginning immediately after thawing and continuing after 2, 6, 12, and 24 hours of storage in the thawed state. The results of the polyphenol oxidase activity determination are presented in **Table 9.7**. In the sample of pickled pepper thawed immediately after freezing, polyphenol oxidase activity was 5.0 μmol/min. During storage of the thawed sample at room temperature (20°C), enzyme activity increased, reaching 17.58 μmol/min after 12 h and then slightly decreased to 15.98 μmol/min after 24 h. Thus, after 24 h of storage in the thawed state, polyphenol oxidase activity was approximately three times higher than immediately after thawing. A similar pattern of changes was observed in samples thawed on days 90, 180, and 270 of low-temperature storage. However, the initial enzyme activity decreased with increasing duration of frozen storage. In particular, the polyphenol oxidase activity was 4.75 μmol/min in the sample thawed on day 90, 4.12 μmol/min in the sample thawed on day 180, and 4.01 μmol/min in the sample thawed on day 270. The highest polyphenol oxidase activity in all samples was observed 12 h after thawing: 16.31 μmol/min after 90 days of frozen storage, 14.98 μmol/min after 180 days, and 13.65 μmol/min after 270 days.

Table 9.7 Polyphenol oxidase activity in thawed pickled sweet peppers during short-term storage (μmol/min)

Frozen storage (days)	Storage of thawed-samples (hours)				
	0	2	6	12	24
0 (immediately after freezing)	5.00	13.68	16.67	17.58	15.98
90	4.75	12.03	15.11	16.31	13.72
180	4.12	10.87	13.02	14.98	11.23
270	4.01	10.11	12.18	13.65	10.98

9.4.4 Microbiological indicators of thawed pickled sweet peppers

Freezing does not completely eliminate microorganisms, and a large number of bacterial spores remain viable. After thawing, these spores may germinate and cause rapid spoilage of the product. Thawed products also provide a favorable nutrient medium for the growth of various microorganisms.

During the thawing of quick-frozen products, psychrophilic microorganisms are the first to multiply. Their metabolic activity begins long before the temperature becomes sufficiently high for the growth of pathogenic microorganisms. Therefore, even after a relatively long period following thawing, products frozen without prior heat treatment are generally not a source of infection by pathogenic microorganisms. However, microorganisms that are harmless to human health but cause food spoilage may proliferate, leading to product deterioration and making the food unfit for consumption. Foods manufactured in accordance with technological requirements for pre-treatment, freezing, storage, and thawing are considered safe according to hygienic standards.

Immediately after thawing, the frozen pickled pepper sample stored at low temperature for 10 days contained 5,212 CFU/mm^2 of bacteria and 2.1 CFU/mm^2 of mold (**Table 9.8**). No yeast was detected in the sample. During the first 12 h of storage at room temperature (20°C), bacterial and mold counts increased to 12,380 CFU/mm^2 and 10.4 CFU/mm^2, respectively. This increase can be explained by the rise in ambient temperature and product moisture after thawing, which creates favorable conditions for the intensive growth of microorganisms. During the following 12 h, microbial counts decreased, with bacteria dropping to 7,430 CFU/mm^2 and molds to 8.3 CFU/mm^2. This decline is likely due to microbial competition, the accumulation of metabolic by-products, and environmental changes that inhibit microbial activity. Yeasts remained undetected throughout the entire storage period at room temperature.

Table 9.8 Analysis of the number of microorganisms in thawed pickled sweet peppers (CFU/mm^2)

Frozen storage (days)	Microorganisms	Storage of thawed-samples (hours)				
		0	2	6	12	24
10	Bacteria	5,212	6,920	10,815	12,380	7,430
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	2.1	3.8	7.5	10.4	8.3
90	Bacteria	6,213	9,200	13,500	15,980	11,300
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	1.6	2.0	4.1	8.0	6.0
180	Bacteria	6,850	18,308	25,660	48,400	23,170
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	3.0	5.0	8.0	12.0	9.0
270	Bacteria	11,257	13,100	18,600	21,430	14,123
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	5.1	9.0	15.0	19.0	14.0

Note: n.d. – not detected

A similar pattern of microbial growth was observed in pickled sweet pepper samples thawed after 90, 180, and 270 days of frozen storage. In the sample frozen for 90 days, bacterial and mold counts immediately after thawing were 6,213 CFU/mm^2 and 1.6 CFU/mm^2, respectively. After 12 h at room temperature, these counts increased to 15,980 CFU/mm^2 for bacteria and 8.0 CFU/mm^2 for molds. After 24 h of thawed storage, the counts decreased to 11,300 CFU/mm^2 for bacteria and 6.0 CFU/mm^2 for molds.

Analysis of the microbiological state of thawed pickled pepper samples indicated that longer frozen storage led to higher microbial counts after thawing. Under favorable conditions, microorganism multiplied actively during the first 12 h of thawed storage. The highest counts of bacteria and molds were observed in samples thawed after 180 and 270 days of low-temperature storage and kept at room temperature for 12 h. In particular, the highest bacterial count (48,400 CFU/mm^2) was recorded after 12 h of room-temperature storage of a sample previously frozen for 180 days. The maximum mold count (19.0 CFU/mm^2) was observed after 12 h of room-temperature storage of a sample frozen for 270 days.

Thus, after thawing, the bacterial and mold counts in pickled peppers, even after 24 h of storage at 20°C, remain below the permissible levels for quick-frozen vegetables: bacteria – $7 \cdot 10^4$ CFU/g, molds – $1 \cdot 10^2$ CFU/g, and yeasts – $5 \cdot 10^2$ CFU/g. However, storing thawed pickled peppers at 20°C is not recommended, even in the absence of toxigenic or pathogenic microorganisms. Thawed pickled peppers should be stored only for the time required for thawing and are best consumed immediately after thawing.

9.5 Conclusions

The scientific novelty of this study lies in the comprehensive assessment of the quality and safety of frozen pickled sweet peppers during low-temperature storage and in determining the effects of different thawing techniques and short-term storage of the thawed product on its quality attributes.

Quick freezing of sweet peppers ensures the preservation of their sensory and nutritional properties for a long period. Pickling sweet peppers before freezing makes it possible to obtain a product that is ready for consumption immediately after thawing. During low-temperature storage of pickled peppers, the content of ascorbic acid is maintained at 80.2–88.0% and carotenoids at 84.9–94.2% of the initial values, while the contents of water-soluble pectin, anthocyanins, catechins, and total flavonoids increases. The contents of dry matter and total sugars in frozen peppers decrease slightly during storage by 0.7–4.9% and 0.6–6.1%, respectively. The amount of epiphytic microflora in frozen pickled sweet peppers is lower compared to that in fresh sweet peppers. As a result, the sensory properties of the product remain highly rated even after 270 days of frozen storage.

The most appropriate technique for thawing pickled sweet peppers is microwave thawing, as it ensures high sensory quality of the product, particularly in terms of consistency. This technique is also considerably faster than thawing in water or air. When thawed pickled pepper are stored at 20°C for up to 10 h, the ascorbic acid content decreases by up to 25%, and polyphenol oxidase activity increases, although the microbiological safety of the product remains ensured. However, it is recommended that thawed sweet peppers be consumed immediately after microwave thawing.

Further studies are needed to determine optimal packaging materials and conditions for frozen pickled peppers in order to preserve their quality during storage and transportation and ensure the product's safety for consumers.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Manuscript has no associated data.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Nadiia Zahorko: Conceptualization, Supervision, Methodology, Empirical research design, Data analysis, Writing – original draft.

Igor Dudarev: Conceptualization, Literature review, Methodology, Data analysis, Writing – original draft.

Valentyna Tkachuk: Literature review, Interpretation of results, Validation, Language editing, Academic writing support, Writing – review & editing.

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