

Impact of Long-Term Storage on Polyphenol Oxidase Activity, Ascorbic Acid, and Chlorophyll Content in Blanched and Frozen Green Bell Peppers (*C. annuum* L. var. *grossum*)*

Haşlanmış ve Dondurulmuş Yeşil Dolmalık Biberlerde (*C. annuum* L. var. *grossum*) Uzun Süreli Depolamanın Polifenol Oksidaz Aktivitesi, Askorbik Asit ve Klorofil İçeriği Üzerine Etkisi

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Abstract

Green bell peppers (California Wonder-*C. annuum* L., var. *grossum*) are widely used in various processed products in the food industry. Therefore, it is important to store it for a long time without losing its quality characteristics so that it can be used not only in its season but throughout the year. To extend the shelf life of peppers and preserve their quality attributes, different methods such as blanching, freezing, and frozen storage are used in the industry. Previous studies have examined the effects of blanching and short-term freezing; however, the impact of blanching followed by long-term frozen storage on the quality of California Wonder peppers has not been investigated. This study aimed to examine the quality changes in California Wonder (*C. annuum* L., var. *grossum*) green bell peppers subjected to water blanching under different conditions (control (C), 75°C-2 min, 75°C-3 min, 90°C-65 s) during a 12-month long-term frozen storage period at -18°C. For this purpose, protein content, polyphenol oxidase (PPO) enzyme activity, ascorbic acid, and chlorophyll content were determined in the samples. It was found that the protein content in blanched samples significantly decreased compared to the control. PPO enzyme activity increased during the storage period, while ascorbic acid and chlorophyll content showed a significant decrease ($p<0.05$). At the end of the storage period, PPO enzyme activity was highest in the control sample, followed by samples blanched at 75°C-3 min, 75°C-2 min, and 90°C-65 s, respectively. High temperature and short blanching time were found to be effective in reducing PPO enzyme activity. Compared to blanched samples, the control sample exhibited a significant decrease in ascorbic acid content ($p<0.05$). Chlorophyll degradation during storage led to the loss of green color, but chlorophyll content was preserved in samples blanched at 75°C-2 min and 90°C-65 s ($p<0.05$). Among all processing conditions, the sample blanched at 90°C-65 s was determined to exhibit the best product quality.

Keywords: California Wonder, Green bell pepper, Water blanching, Polyphenol oxidase enzyme, Ascorbic acid, Chlorophyll

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Atıf: Yılmaz Harputlu, L., Elmacı, Y. (2026). Haşlanmış ve dondurulmuş yeşil dolmalık biberlerde (*C. annuum* L. var. *grossum*) uzun süreli depolamanın polifenol oksidaz aktivitesi, askorbik asit ve klorofil içeriği üzerine etkisi. *Tekirdağ Ziraat Fakültesi Dergisi*, 23(2): 508-519.

Citation: Yılmaz Harputlu, L., Elmacı, Y. (2026). Impact of long-term storage on polyphenol oxidase activity, ascorbic acid, and chlorophyll content in blanched and frozen green bell peppers (*C. annuum* L. var. *grossum*). *Journal of Tekirdag Agricultural Faculty*, 23(2): 508-519.

*This study was summarized from the Leman Yılmaz's PhD thesis.

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Öz

Yeşil dolmalık biberler (California Wonder-*C. annuum* L. var. *grossum*) gıda endüstrisinde birçok işlenmiş üründe yaygın olarak kullanılmaktadır. Bu nedenle sadece mevsiminde değil tüm yıl boyunca kullanılabilmesi amacıyla kalite özelliklerini kaybetmeden uzun süre saklanması önem taşımaktadır. Biberlerin raf ömrünü uzatmak ve kalite özelliklerini korumak amacıyla endüstride haşlama, dondurma ve dondurarak saklama gibi farklı yöntemler kullanılmaktadır. Gerçekleştirilen çalışmalarda haşlamanın ve kısa süreli dondurmanın etkileri incelenmiş ancak haşlanarak uzun süre dondurarak depolamanın California Wonder biber kalitesi üzerine etkilerinin incelenmediği belirlenmiştir. Bu çalışmada farklı koşullarda (kontrol (C), 75°C-2 dk, 75°C-3 dk, 90°C-65 sn) su ile haşlama işlemi uygulanmış California Wonder (*C. annuum* L. var. *grossum*) yeşil dolmalık biberlerin kalite değişimleri, -18°C'de 12 ay boyunca dondurulmuş depolama süresi boyunca incelenmesi amaçlanmıştır. Bu amaçla örneklerde protein miktarı, polifenol oksidaz (PPO) enzim aktivitesi, askorbik asit ve klorofil içeriği belirlenmiştir. Haşlanmış örneklerde protein içeriğinin kontrole göre önemli ölçüde azaldığı belirlenmiştir. PPO enzim aktivitesi depolama süresi boyunca artmış, askorbik asit içeriği ve klorofil içeriğinde belirgin bir azalma gözlenmiştir ($p<0.05$). Depolama süresinin sonunda PPO enzim aktivitesi en yüksek kontrol örneğinde, ardından sırasıyla, 75°C-3 dk, 75°C-2 dk ve 90°C-65 sn haşlanmış örneklerde gözlenmiştir. Yüksek sıcaklık ve kısa haşlama süresi uygulaması, PPO enzim aktivitesini azaltmada etkili bir yaklaşım olarak bulunmuştur. Kontrol örneği, haşlanmış örneklerle karşılaştırıldığında, askorbik asit içeriğinde önemli ölçüde azalma göstermiştir ($p<0.05$). Depolama sırasında klorofil bozulması, yeşil rengin kaybına neden olmuş, 75°C-2 dk ve 90°C-65 sn haşlama işlemi uygulanmış örneklerde klorofil içeriği korunmuştur ($p<0.05$). Tüm işlem koşulları arasında, 90°C-65 sn haşlanma işlemi uygulanmış örneğin, en iyi ürün kalitesini sergilediği belirlenmiştir.

Anahtar Kelimeler: California Wonder, Yeşil dolmalık biber, Suda haşlama, Polifenol oksidaz enzimi, Askorbik asit, Klorofil

1. Introduction

Bell pepper (*Capsicum annuum* L.) is classified as a vegetable commonly consumed fresh or utilized in various processed food products. In recent years, the need for sliced and cubed frozen peppers has significantly increased, driven by growing consumer preferences for safe food, healthy eating habits and the desire to consume raw, minimally processed vegetables (Castro et al., 2008; Niyaz and Demirbaş, 2018). Since it is one of the common vegetables grown in Türkiye (Canan and Uluşık, 2024), to prolong the shelf life and preserve the quality attributes of green bell peppers, it is essential to employ blanching, freezing, and frozen storage techniques. Blanching is a crucial heat treatment before many preservation processes, having a significant impact on the overall quality of the product, such as drying, canning, and freezing. The primary objectives of the blanching process include inactivating enzymes like polyphenol oxidase (PPO) and peroxidase (POD), preventing undesirable changes in flavor, color, and texture, extending shelf life by reducing microbial load, and inhibiting oxidation by limiting air exposure in the intracellular space (Wang et al., 2017; Xiao et al., 2017). Freezing and frozen storage effectively preserve the original appearance, taste, and nutritional content of fruits and vegetables. The freezing process reduces water activity and enzymatic activity, which are factors that negatively affect the shelf life of these products (Alexandre et al., 2013; Alhamdan et al., 2018).

During blanching, freezing, and frozen storage of green bell peppers, changes occur in protein content, PPO activity, ascorbic acid (AsA), and chlorophyll content. It has been reported that 25-35% of total production of sweet peppers occur as post-harvest loss (Cheema et al., 2018). Bell peppers have a short shelf life and require proper treatment and maintenance to preserve their post-harvest quality. Enzymatic browning occurs due to the catalytic effect of the PPO enzyme during the industrial processing of fruits and vegetables. This impairs the appearance and taste of the product, reduces its nutritional value, decreases consumer acceptance, and leads to significant economic losses in the food industry (Pekyardımcı, 1992; Nuñez-Delicado, et al, 2005; Nguyen et al., 2019, Gil and Tudela, 2020). POD enzyme causes issues in the processing and storage of fruits and vegetables, including color deterioration, the formation of off-flavors, and a loss of nutritional value (Wu et al., 2024). Since the thermal resistance of the PPO enzyme in green bell peppers (*Capsicum annuum* L.) is higher than that of the POD enzyme, PPO was determined to be the blanching indicator enzyme for green bell peppers (Castro et al., 2008). Generally, exposure of PPO to temperatures between 70-90°C eliminates its catalytic activity, with the required inactivation time depending on the product (Kaur and Kaushal, 2019; Nguyen et al., 2019).

Generally, AsA is selected as the primary component to assess nutrient loss during the blanching process. The retention of AsA after blanching is considered as an indicator that other nutrients are preserved (Xiao et al., 2017). The catabolism of AsA occurs during the freezing process and frozen storage, and this parameter is used to determine the optimal frozen storage time for vegetables and fruits. AsA content serves as a reliable index for frozen fruits and vegetables, allowing the estimation of product degradation at any stage, from the market to the consumer (Turmanidze et al., 2017). It has been reported that AsA or its derivatives directly inactivate the PPO enzyme (Derardja et al., 2019).

Intense green color is considered a key quality criterion in green vegetables. The color of fresh green vegetables fades and deteriorates, often turning to a dull brownish-yellow color when processed. Chlorophylls, which are unstable compounds, can degrade into various derivatives during blanching, a crucial step before freezing, drying, or canning green vegetables (Nguyen et al., 2023; Durgawati et al., 2025).

In studies conducted on green bell pepper, many researchers have investigated the total protein content (Kahlon et al., 2007; Castro et al., 2008; Dorantes-Alvarez, et al., 2011) examined PPO activity of green bell pepper at different process parameters. Barbagallo et al. (2012) studied the PPO activity of California Wonder green bell pepper during a 30 day storage period at 4°C. The effects of blanching on AsA content in green peppers (Howard, et al, 1994; Castro et al., 2008) and chlorophyll content in Jalapeño peppers (Cervantes-Paz et al., 2014) were also evaluated. Wang et al. (2012; 2016) and Chen et al. (2018) observed that as the storage time of peppers increased chlorophyll content declined at temperatures ranging from 3°C-7°C temperature.

Although numerous studies have been published on the impact of blanching and short-term cold storage on the quality characteristics of green bell peppers, no research has investigated the impact of blanching and long-term frozen storage of green bell peppers. Hence, the aim of our study was to evaluate the effects of water blanching

and individual quick freezing (IQF) under varying process conditions (C, 75°C-2 min, 75°C-3 min and 90°C-65 s) on California Wonder (*C. annuum* L., var. *grossum*) green bell peppers during 12 months of frozen storage at -18°C, focusing on protein, PPO enzyme activity, ascorbic acid (AsA), and chlorophyll content.

2. Materials and Methods

2.1. Materials

Green bell peppers (California Wonder genus - *C. annuum* L. var. *grossum*) were supplied from Bursa province, Turkey. The samples were washed, cut into 1×1 cm cubes, and subjected to water blanching before freezing. The blanching time and temperature were selected based on industrial processing conditions for frozen green bell peppers. The samples were blanched at 75°C for 2 minutes, 75°C for 3 minutes, and 90°C for 65 seconds. Both the C and blanched samples were frozen using the IQF method (Advanced I.Q.F Spiral Freezer, Advanced Equipment Inc., Richmond, BC, Canada) and stored at -18°C. Analyses were conducted at 0, 2, 4, 6, 8, 10, and 12 months of storage. All chemicals used in this study were of analytical grade.

2.2. Protein content

The protein content of the green bell peppers was measured using the Dumas method (AOAC, 2007). The thawed samples were dried at 75°C until constant weight before the analysis. Factor of 6.25 was used to calculate the protein content of peppers.

2.3. PPO enzyme activity

The spectrophotometric assay outlined by Cemeroglu (2018) was modified to determine PPO enzyme activity of the samples.

Acetone powder (AP): A Waring blender was used to homogenize the frozen bell peppers (50 g) for 2 min with 125 mL cold acetone (-40°C) and 0.25 g polyvinylpyrrolidone (PVPP). After filtering, the residue was subjected to a second extraction with 100 mL of cold acetone and filtered. The resulting AP was allowed to air-dry overnight at ambient temperature.

PPO extraction: A total of 2.0 g of AP was dissolved in 150 mL of 0.25 molL⁻¹ Na-phosphate buffer (pH 6.8, 4°C) and 0.25 g PVPP was added. The suspension was agitated with a magnetic stirrer for 20 min at a temperature of 4°C. After filtration and centrifugation (1400 g, 10 min), the filtrate was stored at 4°C until spectrophotometric measurement.

Determination of PPO activity: The obtained extract was filtered using a 0.45 µm PTFE filter (Isolab). The spectrophotometer cuvette was preheated to 30°C in a heated spectrophotometer (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer) and the temperature was kept constant throughout the measurement process. A total of 2.8 mL of 0.05 molL⁻¹ Na-phosphate buffer (30°C, pH 6.8) was placed in the quartz spectrophotometer cuvette followed by the addition of 0.1 mL of liquid enzyme extract. The mixture was incubated in the spectrophotometer for 3 min. After incubation, 0.1 mL of 0.5 molL⁻¹ pyrocatechol solution was added and PPO enzyme activity was measured at 410 nm for 3 min. The reaction rate was determined by calculating the slope of the initial linear segment of the absorbance versus the time curve (Eq. 1).

$$\text{Activity [U g}^{-1} \text{ fresh weight (fw)]} = [(E/0,001) \times (1/He) \times (Hrk) \times (Sf) \times (V) \times (A/H)] / T \quad (\text{Eq. 1})$$

E: Absorbance (Abs/min)

H_e : Volume of the enzyme extract in the reaction mixture

H_{rk} : Total volume of the reaction mixture

S_f : Dilution factor

V: Total volume of the enzyme extract (mL)

A: Amount of obtained acetone powder (g)

H: Amount of used raw material (g)

T: Amount of used acetone powder in the analysis (g)

2.4. AsA content

For the determination of AsA content, 5 g of frozen bell pepper was used. The measurement was performed at 518 nm using UV-visible spectrophotometer (Varian Cary 60 Scan) (ISO, 1984) (Eq. 2).

$$\text{AsA content (mg/100 g fw)} = [(100 \times (A - b)) / (10 \times a)] \quad (\text{Eq. 2})$$

A: Absorbance

b: b constant in the equation $y = ax + b$

a: Slope constant in the equation $y = ax + b$

The concentration corresponding to the absorbance value ($A_{\text{dye}} - A_{\text{sample}}$) of the sample was determined using the standard curve. Since the sample was diluted twenty-fold, the calculated concentration was multiplied by 20 to obtain the final value.

2.5. Chlorophyll content

Chlorophyll content was determined according to Özkan and Bilek (2015) using 5 g of frozen pepper sample. The absorbance values of the blank sample (80% acetone solution) and the filtrate were measured at 663 and 645 nm using a UV-visible (Varian Cary 60 Scan) spectrophotometer (Eq. 3).

$$\text{Total chlorophyll (mg g}^{-1}\text{fw)} = [(20.02 \times A_{645} + 8.02 \times A_{663}) \times \text{Dilution factor}] / 1000 \quad (\text{Eq. 3})$$

A: Absorbance

2.6. Statistical analysis

Analyses were conducted in triplicate and repeated three times. Statistical evaluation of the data was conducted using the IBM SPSS software package (IBM SPSS version 20). The differences between means were evaluated using ANOVA (Analyses of Variance) and Duncan multiple comparison tests. Correlation tests were performed using the Pearson r test. ANOVA and Duncan multiple comparison tests were statistically evaluated at a 95% confidence level, while the Pearson r test was assessed at a 99% confidence level.

3. Results and Discussion

3.1. Protein content

The protein content of the C, 75°C-2 min, 75°C-3 min and 90°C-65 s blanched green bell peppers were determined as 13.07%, 12.41%, 12.43% and 12.29% dry matter (dm), respectively (Figure 1). A significant decrease in protein content was observed for the blanched samples in comparison to the C ($p < 0.05$). However, the difference was not significant among the blanched samples ($p > 0.05$). Similarly, in research carried out by Castro et al. (2008), the total protein content of green bell peppers (*Capsicum annuum* L.) was found to be 13.8 g kg⁻¹ protein (fw) with a significant decrease of about 15-60% were observed after blanching. This effect increased progressively with higher blanching temperatures and longer times. Kahlon et al. (2007) reported the protein content of green bell peppers (*Capsicum annuum* L.) as 16.9% (dm).

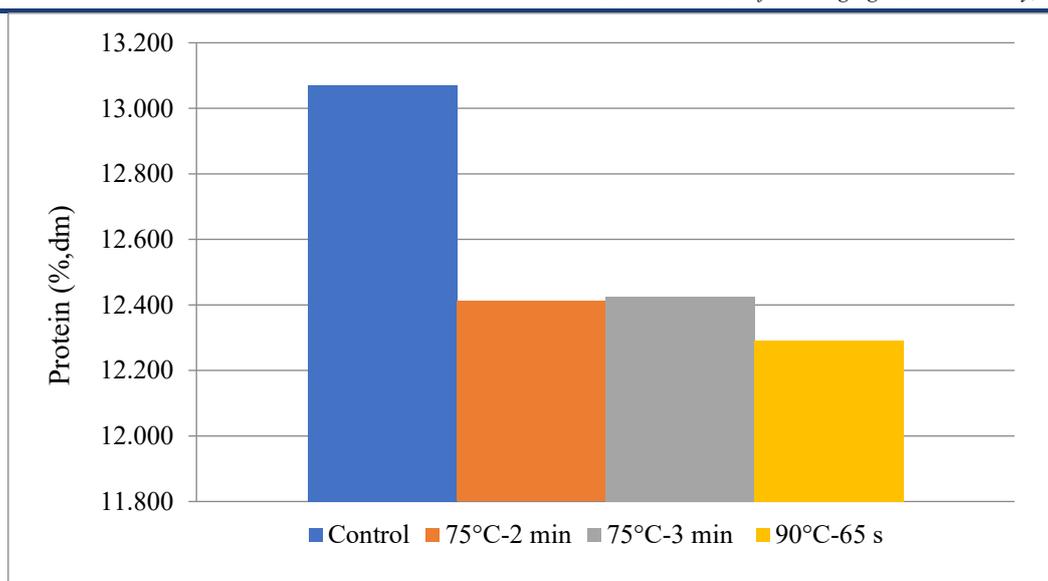


Figure 1. Effects of blanching parameters on residual protein content of green bell peppers

Table 1. PPO enzyme activity, AsA and chlorophyll content of the green bell peppers during frozen storage

	Frozen storage time (month)	C	75°C-2 min	75°C-3 min	90°C-65 s
PPO enzyme activity (U g ⁻¹ fw)	0	19.83 ± 0.12 ^{A, a}	13.09 ± 1.75 ^{A, b}	15.20 ± 0.17 ^{A, c}	8.51 ± 0.33 ^{A, d}
	2	22.25 ± 1.89 ^{AB, a}	15.91 ± 1.25 ^{AB, b}	17.47 ± 1.92 ^{A, b}	9.87 ± 0.21 ^{A, c}
	4	24.45 ± 1.71 ^{B, a}	17.62 ± 2.14 ^{B, b}	21.05 ± 2.31 ^{B, c}	12.89 ± 0.17 ^{B, d}
	6	28.52 ± 2.27 ^{C, a}	18.63 ± 1.69 ^{BC, b}	22.29 ± 2.07 ^{BC, c}	14.26 ± 1.12 ^{B, d}
	8	33.81 ± 1.96 ^{D, a}	21.51 ± 1.45 ^{CD, b}	23.90 ± 1.81 ^{BC, b}	16.60 ± 1.02 ^{C, c}
	10	35.36 ± 1.26 ^{D, a}	22.18 ± 2.40 ^{DE, b}	25.37 ± 2.37 ^{C, b}	17.59 ± 1.28 ^{C, c}
AsA content (mg100 g ⁻¹ fw)	0	92.79 ± 6.96 ^{A, a}	79.86 ± 9.85 ^{A, a}	73.43 ± 8.20 ^{A, a}	81.54 ± 8.93 ^{A, a}
	2	72.96 ± 6.41 ^{B, a}	75.74 ± 7.50 ^{AB, a}	69.84 ± 6.94 ^{A, a}	75.83 ± 5.98 ^{AB, a}
	4	50.87 ± 8.80 ^{C, a}	72.04 ± 7.26 ^{ABC, b}	68.20 ± 8.03 ^{A, b}	66.24 ± 6.91 ^{BC, b}
	6	45.70 ± 7.30 ^{CD, a}	65.04 ± 5.05 ^{BCD, b}	63.44 ± 4.54 ^{AB, b}	62.85 ± 1.20 ^{CD, b}
	8	42.80 ± 6.95 ^{CD, a}	59.71 ± 4.32 ^{CD, b}	62.97 ± 4.01 ^{AB, b}	61.67 ± 2.01 ^{CD, b}
	10	37.16 ± 6.11 ^{D, a}	54.06 ± 6.01 ^{D, b}	55.40 ± 6.71 ^{BC, b}	58.48 ± 6.12 ^{CD, b}
Chlorophyll content (mg g ⁻¹ fw)	0	0.0415 ± 0.0006 ^{A, a}	0.0395 ± 0.0010 ^{A, bc}	0.0388 ± 0.0007 ^{A, c}	0.0403 ± 0.0004 ^{A, ab}
	2	0.0405 ± 0.0014 ^{AB, a}	0.0396 ± 0.0005 ^{A, a}	0.0378 ± 0.0017 ^{AB, a}	0.0397 ± 0.0006 ^{AB, a}
	4	0.0387 ± 0.0010 ^{B, a}	0.0389 ± 0.0016 ^{AB, a}	0.0371 ± 0.0010 ^{AB, a}	0.0385 ± 0.0016 ^{ABC, a}
	6	0.0363 ± 0.0011 ^{C, a}	0.0378 ± 0.0006 ^{ABC, a}	0.0362 ± 0.0010 ^{BC, a}	0.0383 ± 0.0014 ^{BC, a}
	8	0.0350 ± 0.0016 ^{CD, a}	0.0372 ± 0.0011 ^{BC, a}	0.0350 ± 0.0015 ^{C, a}	0.0379 ± 0.0009 ^{BC, a}
	10	0.0336 ± 0.0009 ^{DE, a}	0.0367 ± 0.0010 ^{C, b}	0.0342 ± 0.0005 ^{C, a}	0.0375 ± 0.0010 ^{C, b}
12	0.0324 ± 0.0011 ^{E, a}	0.0368 ± 0.0009 ^{C, b}	0.0345 ± 0.0010 ^{C, c}	0.0373 ± 0.0011 ^{C, b}	

Each value is expressed as mean ± SD (n = 4).

A, B, C, D, E refers to the differences of PPO enzyme activity, AsA and chlorophyll content among the treatments for each month at 0.05 level

a, b, c, d refers to the differences of PPO enzyme activity, AsA and chlorophyll content by month for each process parameter on column basis at 0.05 level

3.2. PPO enzyme activity

PPO activity of peppers was determined as 19.83, 13.09, 15.20 and 8.51 U g⁻¹ (fw), for the C, 75°C-2 min, 75°C-3 min and 90°C-65 s blanched samples, respectively, at the beginning of storage (Table 1). The PPO activity of the C sample significantly increased at the end of the 4th and 6th months (p<0.05) and the increase after 8 months was not significant (p>0.05). The PPO activity of 75°C-2 min and 75°C-3 min blanched samples increased significantly by the 4th month compared to 0th month, with both samples showing a gradual increase in PPO activity

throughout the storage period. However, the PPO activity of 75°C-3 min blanched sample increased significantly at the 12th month of storage, with this change being different from other months ($p < 0.05$). Significant increases in PPO activity were observed at the 4, 8 and 12th months for the 90°C-65 s blanched sample ($p < 0.05$).

The residual activity of PPO in green bell peppers during 12 months of frozen storage are illustrated in *Figure 2*. The residual PPO activities of the 75°C-2 min, 75°C-3 min and 90°C-65 s blanched samples were determined as 66.01%, 76.65% and 42.91%, respectively (*Figure 2*). Schweiggert et al. (2005) reported complete inactivation of PPO when peppers and ground (powdered) peppers were boiled at 80°C-10 min. Similarly, Castro et al. (2008) determined the residual PPO activity of green bell peppers blanched at various temperatures and times (70°C-1 min, 70°C-2.5 min, 80°C-1 min, 80°C-2.5 min, 98°C-1 min and 98°C-2.5 min) finding values of approximately 66%, 61%, 57%, 66%, 50% and 24%, respectively.

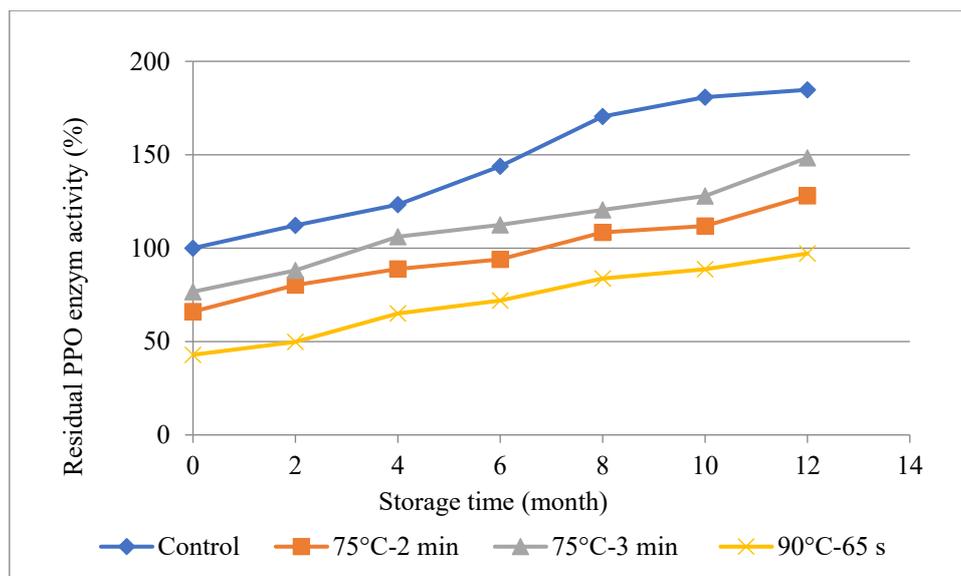


Figure 2. Effects of blanching parameters on residual PPO enzyme activity of green bell peppers during frozen storage

Residual PPO activity increased for each process parameter as the storage time progressed, with blanching treatments causing significant decreases in activity ($p < 0.05$) (*Figure 2*). Similarly, when minimally processed California Wonder peppers were kept at 4°C, PPO activity showed an increase as the storage time extended (Barbagallo et al., 2012). Upon assessing each process parameter over the months, the PPO activities of the green bell peppers were ranked from highest to lowest as follows; C, 75°C-3 min, 75°C-2 min and 90°C-65 s.

3.3. AsA content

The AsA content of green bell peppers was determined as 92.79 mg100 g⁻¹ (fw) at the beginning (0th month) of storage (*Table 1*). In research carried out by Yahia et al. (2001), the AsA content of green peppers ranged from 12-180 mg100 g⁻¹ (fw). AsA content varies depending on the degree of ripeness and pepper variety. For example, AsA content in different types of unripe and ripe peppers ranged from 19.55-253.56 mg100 g⁻¹ (fw) (Bae et al., 2014). Barbagallo et al. (2012) reported the AsA content of fresh green bell pepper as 124 mg100 g⁻¹, while Kaur et al. (2020) reported 120 mg100 g⁻¹ for the *Indira* variety. Negative effect of freezing on AsA content have been observed in both red bell peppers (Castro et al., 2008; Alexandre et al., 2013; Wang et al., 2017) and green bell peppers (*Capsicum annuum* L.) (Castro et al., 2008). In Castro et al.'s (2008) study, the AsA content for C sample after freezing was reported as 88.5 mg100 g⁻¹ (fw).

The AsA content of peppers throughout 12 months frozen storage is shown in *Table 1* and *Figure 3*. When examining the differences between the process parameters, the difference was not significant for the AsA content of the C and the blanched samples at the beginning of storage ($p > 0.05$). A significant difference was observed

between the C sample and the blanched samples starting from the 4th month of storage ($p < 0.05$). However, the differences were not significant among the blanched samples at the 4, 6, 8, 10 and 12 months ($p > 0.05$).

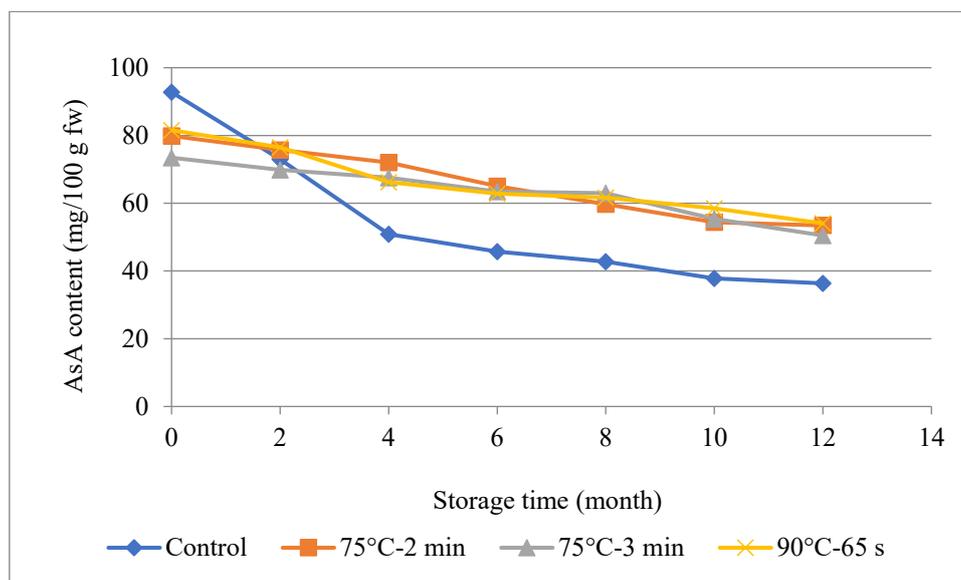


Figure 3. Effects of blanching parameters on AsA content of green bell peppers during frozen storage

Xiao et al. (2017) stated that the loss of AsA depends on blanching temperature and time, with higher temperatures and shorter processing times leading to higher retention of AsA. Howard et al. (1994) reported that Jalapeño peppers lost 75% of their AsA content when blanched before pasteurization. Castro et al. (2008) observed that 70°C-1 min blanching followed by freezing did not cause a significant change in AsA content in comparison with frozen C sample (*C. annuum* L.), but significant changes were noted with increased blanching temperature and time. Similar results regarding the decrease in AsA have been documented in research on California Wonder (*Capsicum annuum* L. var. *grossum*) green peppers, red bell peppers (Alexandre et al., 2013), peas (Gökmen et al., 2005), and chicory (Francis et al., 2014) as the duration of frozen storage increased. The presence of ascorbic acid oxidase in the C samples contributed to the lower AsA content compared to the blanched samples as storage time increased. Blanching can inactivate ascorbic acid oxidase and remove residual oxygen from vegetable tissue, thereby promoting higher retention of ascorbic acid (Turmanidze et al., 2017; Wang et al., 2020).

According to the Pierson r test, a negative correlation was found between AsA content and PPO activity ($r = -0.739$). Similar results were observed in sugar cane (Mao et al., 2007), mung bean sprouts (Sikora and Świeca, 2018) and apricot (Derardja et al., 2019).

3.4. Chlorophyll content

Chlorophyll content of each process parameter significantly decreased as the storage time increased (Table 1). The decrease in chlorophyll content of the C sample after the 10th month was not significant ($p > 0.05$). Chlorophyll content of the 75°C-2 min, 75°C-3 min and 90°C-65 s blanched samples significantly decreased on the 8th, 6th and 6th month, respectively. By the end of 12 months, the chlorophyll content of 75°C-2 min, 75°C-3 min and 90°C-65 s blanched samples had decreased by 11.33%, 16.87% and 10.12%, respectively, in comparison with the C sample (Table 1). Chen et al. (2018) reported the chlorophyll content of green bell peppers (*C. annuum* var. *grossum*) as 0.72 ± 0.05 mg g⁻¹ (fw), Wang et al. (2016) for *Capsicum annuum* L. cv. Mutianqiushuo as 0.1544 g kg⁻¹ (fw), and Wang et al. (2012) for *C. annuum* L. cv. “Zhongjiao 7” as 0.12 g kg⁻¹ (fw).

The chlorophyll content of peppers throughout 12 months frozen storage is shown in Figure 4. The blanching process caused a significant decrease in chlorophyll content for the 75°C-2 min (4.82%) and 75°C-3 min (6.51%) blanched samples ($p < 0.05$). The difference was not significant between the chlorophyll content of the 75°C-2 min and 90°C-65 s blanched sample ($p > 0.05$). However, the chlorophyll content of these two samples was significantly higher than that of the C and the 75°C-3 min blanched sample at 12th month ($p < 0.05$). Consistent with the findings

of our study, Cervantes-Paz et al. (2014) reported that boiling medium ripe brown 50% red Jalapeño peppers at 92°C-12.48 min, reduced chlorophyll content. Lisiewska et al. (2004) and Murcia et al. (2000) stated that chlorophyll content decreased between 12-66% during blanching, while Lisiewska et al. (2004) explained that chlorophyll losses could range from 3-9% in blanched leafy vegetables. In general, high temperature and short time applications provide more chlorophyll protection than low temperature and long time applications (Martínez, et al., 2020).

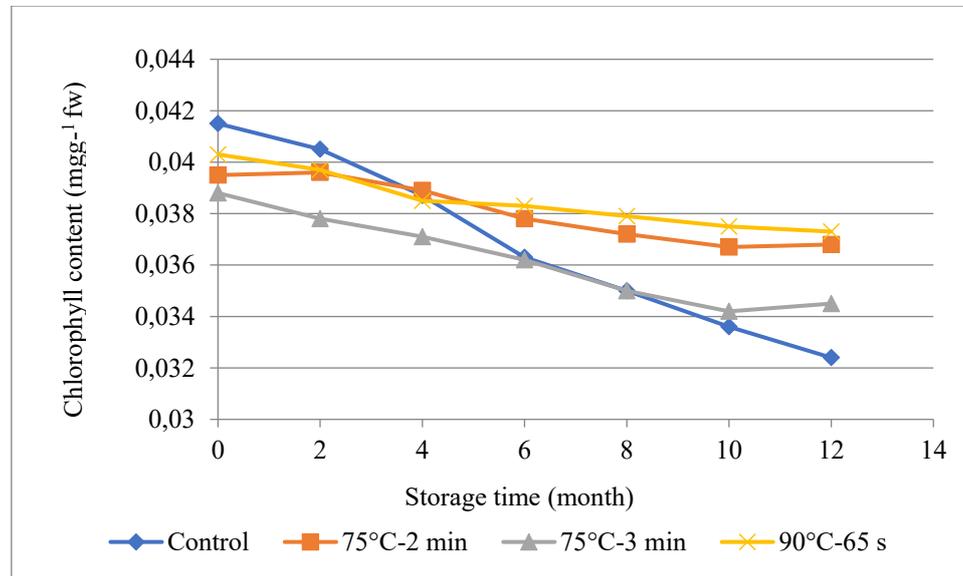


Figure 4. Effects of blanching parameters on chlorophyll content of green bell peppers during frozen storage

Some publications have reported that chlorophyll content decreases as the storage time increases (from 9 days to 18 days) for green bell pepper stored in the refrigerator (at 3°C as opposed to 7°C) (Wang et al., 2012; Wang et al., 2016; Chen et al., 2018). However, there is no consensus on the extent of chlorophyll loss depending on the storage time. In their study, Mondragón-Portocarreño et al. (2006) reported that chlorophyll content in turnip greens did not show a significant decline during frozen storage. Grzeszczuk et al. (2007) reported minimal chlorophyll losses during frozen storage of spinach, while Olivera et al. (2008) found greater chlorophyll losses in frozen unboiled spinach. Kmicik, et al. (2008) stated that chlorophyll preservation depends on species, pretreatment before freezing and storage temperature during frozen storage. Lisiewska et al. (2004) found that dill could be stored for 6 months at -20°C without blanching. Thermal treatments increased chlorophyll content for boiled (10 min), water blanched (90°C-60 s) chicory (*Cichorium intybus* L.) (Francis et al., 2014). Petzold et al. (2014) and Ferreira et al. (2020) pointed out that the blanching process stimulates the disintegration of chlorophyll containing organelles, making chlorophyll pigments more prone to degradation. The blanching and frozen storage period may cause the conversion of chlorophylls into pheophytins, thereby leading to chlorophyll losses.

4. Conclusions

The blanching treatments applied to California Wonder genus (*C. annuum* L., var. *grossum*) green bell peppers significantly reduced their protein content. Both blanching and frozen storage of green bell peppers had clear effects on PPO activity, AsA, and chlorophyll content. As storage time increased, PPO activity increased for each processing parameter, leading to a decline in the quality of peppers. This increase in PPO activity, along with a reduction in chlorophyll content, caused a decrease in the characteristic dark green color and brightness of the bell peppers. Given the importance of PPO enzyme activity for peppers, the 90°C-65 s blanching treatment resulted in the lowest PPO enzyme activity by the end of the 12th month of storage. This long-term frozen storage research on green bell peppers marks a significant contribution to the field. The findings will serve as a valuable guide for producers, helping them optimize the long-term use of green bell peppers, an essential ingredient in the food industry.

Acknowledgment

This study was supported by Ege University Scientific Research Projects Coordination Unit (Project No:17/MUH/017), Turkey. The authors would also like to thank Dr. Oetker Food Industry and Trade Inc for supplying the green bell pepper.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Yılmaz Harputlu, L., Elmacı, Y.; Design: Yılmaz Harputlu, L., Elmacı, Y.; Data Collection or Processing: Yılmaz Harputlu, L., Elmacı, Y.; Statistical Analyses: Yılmaz Harputlu, L.; Literature Search: Yılmaz Harputlu, L., Elmacı, Y.; Writing, Review and Editing: Yılmaz Harputlu, L., Elmacı, Y.

References

- Alexandre, E. M. C., Brandão, T. R. S. and Silva, C. L. M. (2013). Impact of nonthermal technologies and sanitizer solutions on microbial load reduction and quality factor retention of frozen red bellpeppers. *Innovative Food Science and Emerging Technologies*, 17: 99-105.
- Alhamdan, A., Hassan, B., Alkahtani, H., Aldelkarim, D. and Younis, M. (2018). Freezing of fresh Barhi dates for quality preservation during frozen storage. *Saudi Journal of Biological Sciences*, 25(8):1552-1561.
- AOAC (2007). Protein (Crude) in Animal Feed. AOAC Official Method 968.06. Official Methods of Analysis. (18th ed.). Washington D.C., U.S.A.
- Bae, H., Jayaprakasha, G. K., Crosby, K., Yoo, K. S., Leskovar, D. I., Jifon, J. and Patil, B. S. (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grownpeppers. *Journal of Food Composition and Analysis*, 33(2): 195-202.
- Barbagallo, R. N., Chisari, M. and Patané, C. (2012). Polyphenoloxidase, total phenolics and ascorbic acid changes during storage of minimally processed California Wonder and "Quadratod'Asti" sweetpeppers. *LWT -Food Science and Technology*, 49(2): 192-196.
- Canan, S., Uluışık, E. N. (2024). Vegetable losses and waste along the supply chain and farmers' willingness to pay for recycling: towards to green supply chain. *Journal of Tekirdag Agricultural Faculty*, 21(1): 148-165.
- Castro, S. M., Saraiva, J. A., Lopes-da-Silva, J.A., Delgadillo, I., Loey, A.V., Smout, C. and Hendrickx, M. (2008). Effect of thermal blanching and of high pressure treatments on sweet green and red bellpepper fruits (*Capsicum annuum* L.). *Food Chemistry*, 107(4): 1436-1449.
- Cemeroğlu, B. S. (2018). Gıda Analizleri. (4. baskı) Büro Basımevi, Ankara, Türkiye.
- Cervantes-Paz, B., Yahia, E. M., Ornelas-Paz, J. J., Victoria-Campos, C. I., Ibarra-Junquera, V., Pérez-Martínez, J. D. and Escalante-Minakata, P. (2014). Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeño peppers at intermediate stages of ripening. *Food Chemistry*, 146: 188-196.
- Cheema, A., Padmanabhan, P., Amer, A. J., Parry, M., Lim, T. K., Subramanian, J. and Paliyath, G. (2018). Postharvest hexanal vapor treatment delays ripening and enhances shelf life of greenhouse grown sweet bellpepper (*Capsicum annum* L.). *Postharvest Biology and Technology*, 136: 80-89.
- Chen, H. Z., Zhang, M., Bhandari, B. and Guo, Z. (2018). Evaluation of the freshness of fresh-cut green bellpepper (*Capsicum annuum* var. *grossum*) using electronic nose. *LWT -Food Science and Technology*, 87: 77-84.
- Derardja, A. E., Pretzler, M., Kampatsikas, I., Barkat, M. and Rompel, A. (2019). Inhibition of apricot polyphenoloxidase by combinations of plant proteases and ascorbic acid. *Food Chemistry*, X(4): 100053.
- Dorantes-Alvarez, L., Jaramillo-Flores, E., González, K., Martínez, R. and Paradab, L. (2011). Blanching peppers using microwaves. *Procedia Food Science*, 1: 178-183.
- Durgawati, P. B., Balasubramanian, P., Xiao, H.W. and Sutar, P.P. (2025). Effect of cyclic vacuum-steam blanching on the quality characteristics and functional properties of Malabar spinach (*Basella alba*) dried by non-water infrared refractance window drying. *Food Chemistry*, 465: 141901.
- Francis, F., Talhouk, S., Batal, M. and Olabi, A. (2014). Sensory and quality parameters of raw and processed Chicory-Hindbeh, a commonly consumed dark leafy green in Lebanon (*Cichorium intybus* L.) during frozen storage. *LWT -Food Science and Technology*, 58(1): 230-238.
- Ferreira, S. S., Monteiro, F., Passos, C. P., Silva, A. M. S., Ferreira Wessel, D., Coimbra, M. A. and Cardoso, S. M. (2020). Blanching impact on pigments, glucosinolates, and phenolics of dehydrated broccoli by-products. *Food Research International*, 132: 109055.
- Gil, M. I. and Tudela, J. A. (2020). Fresh and Fresh-cut Fruit Vegetables: Peppers. In: Controlled and Modified Atmospheres for Fresh and Fresh-Cut Produce, Ed(s): Gil, M. I., Beaudry, R., Academic Press.
- Gökmen, V., Bahçeci, K. S., Serpen, A. and Acar, J. (2005). Study of lipoxygenase and peroxidase as blanching indicator enzymes in peas: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *LWT -Food Science and Technology*, 38(8): 903-908.
- Grzeszczuk, M., Jadczyk, D. and Podsiadlo, C. (2007). The effect of blanching, freezing and freeze-storage on changes of some chemical compounds content in New Zealand spinach. *Vegetable Crops Research Bulletin*, 66: 95-103.
- Howard, L. R., Smith, R. T., Wagner, A. B., Villalon, B. and Burns, E. E. (1994). Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed Jalapeños. *Journal of Food Science*, 59: 362-365.
- ISO (1984). Fruits, Vegetables and Derived Products – Determination of Ascorbic Acid Content. Part 2:12th Methods. International Organization for Standardization, Ref. No-150 6557/2, Switzerland.
- Kahlon, T. S., Chapman, M. H. and Smith, G. E. (2007). In vitro binding of bile acids by spinach, kale, brussels sprouts, broccoli, mustard greens, green bell pepper, cabbage and collards. *Food Chemistry*, 100(4): 1531-1536.
- Kaur, K. and Kaushal, P. (2019). Enzymes as Analytical Tools for the Assessment of Food Quality and Food Safety. In: Advances in Enzyme Technology, Ed(s): Singh, R. S., Singhania, R. R., Pandey, A., Larroche, C. Elsevier Publishing.

- Kaur, R., Kaur, K. and Ahluwalia, P. (2020). Effect of drying temperatures and storage on chemical and bioactive attributes of dried tomato and sweet pepper. *LWT- Food Science and Technology*, 117: 108604.
- Kmiecik, W., Lisiewska, Z., Słupski, J. and Gębczyński, P. (2008). The effect of pretreatment, temperature and length of frozen storage on the retention of chlorophylls in frozen Brassicas. *Acta Scientiarum Polonorum Technologia Alimentaria*, 7: 21-34.
- Lisiewska, Z., Kmiecik, W. and Słupski, J. (2004). Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pretreatment on the content of chlorophylls and carotenoids in frozen dill (*Anethum graveolens* L.), depending on the time and temperature of storage. *Food Chemistry*, 84: 511-518.
- Mao, L.C., Xu, Q. and Que, F. (2007). Maintaining the quality of sugarcane juice with blanching and ascorbic acid. *Food Chemistry*, 104(2): 740-745.
- Martínez, S., Armesto, J., Gómez-Limia, L. and Carballo, J. (2020). Impact of processing and storage on the nutritional and sensory properties and bioactive components of *Brassica* spp. A review. *Food Chemistry*, 313: 126065.
- Mondragón-Portocarrero, A.C., Pena-Martínez, B., Fernández-Fernández, E., Romero-Rodríguez, A. and Vázquez-Odériz, L. (2006). Effects of different pre-freezing blanching procedures on the physicochemical properties of *Brassica rapa* leaves (Turnip Greens, *Grelos*). *International Journal of Food Science and Technology*, 41(9): 1067-1072.
- Murcia, M. A., López-Ayerra, B., Martínez-Tomé, M. and García-Carmona, F. (2000). Effect of industrial processing on chlorophyll content of broccoli. *Journal of the Science of Food and Agriculture*, 80(10): 1447-1451.
- Nguyen, T. D. L. H., Vo, T. T., Lam, T. D. and Bach, L. G. (2019). Water blanching conditions on the quality of green asparagus butt segment (*Asparagus officinalis* L.). *Materials Today: Proceedings*, 18(7): 4799-4809.
- Nguyen, T. D. L. H., Ta, T. M. N. and Dong, T. A. D. (2023). Effect of blanching, freezing and microwave-assisted pretreatment methods on chlorophyll extraction from *Sauropus androgynus* leaves. *Journal of Agriculture and Food Research*, 14: 100766.
- Niyaz, Ö. C., Demirbaş, N. (2018). Food safety perceptions of fresh fruits and vegetables consumers. *Journal of Tekirdag Agricultural Faculty*, 15(2): 36-44.
- Núñez-Delgado, E., Serrano-Megías, M., Pérez-López, A. J. and López-Nicolás, J. M. (2005). Polyphenol oxidase from dominga table grape. *Journal of Agricultural and Food Chemistry*, 53(15): 6087-6093.
- Olivera, D. F., Viña, S. Z., Marani, C. M., Ferreyra, R. M., Mugridge, A., Chaves, A. R. and Mascheroni, R. H. (2008). Effect of blanching on the quality of Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) after frozen storage. *Journal of Food Engineering*, 84(1): 148-155.
- Özkan, G. and Bilek, S. (2015). Enzyme-assisted extraction of stabilized chlorophyll from spinach. *Food Chemistry*, 176: 152-157.
- Pekyardımcı, Ş. (1992). Polyphenol oxidase and browning reactions. *Food Journal*, 17(3): 181-186.
- Petzold, G., Caro, M. and Moreno, J. (2014). Influence of blanching, freezing and frozen storage on physicochemical properties of broad beans (*Vicia faba* L.). *International Journal of Refrigeration*, 40: 429-434.
- Schweiggert, U., Schieber, A. and Carle, R. (2005). Inactivation of peroxidase, polyphenoloxidase, and lipoxygenase in paprika and chili powder after immediate thermal treatment of the plant material. *Innovative Food Science & Emerging Technologies*, 6(4): 403-411.
- Sikora, M. and Świeca, M. (2018). Effect of ascorbic acid postharvest treatment on enzymatic browning, phenolics and antioxidant capacity of stored mung bean sprouts. *Food Chemistry*, 239: 1160-1166.
- Turmanidze, T., Jgenti, M., Gulua, L. and Shaiashvili, V. (2017). Effect of ascorbic acid treatment on some quality parameters of frozen strawberry and raspberry fruits. *Annals of Agrarian Science*, 15(3): 370-374.
- Wang, Q., Ding, T., Gao, L., Pang, J. and Yang, N. (2012). Effect of brassinolide on chilling injury of green bell pepper in storage. *Scientia Horticulturae*, 144: 195-200.
- Wang, Q., Ding, T., Zuo, J., Gao, L. and Fan, L. (2016). Amelioration of postharvest chilling injury in sweet pepper by glycine betaine. *Postharvest Biology and Technology*, 112: 114-120.
- Wang, J., Yang, X. H., Mujumdar, A. S., Wang, D., Zhao, J. H., Fang, X. M., Zhang, Q., Xie, L., Gao, Z. J. and Xiao, H. W. (2017). Effects of various blanching methods on weight loss, enzymes inactivation, phytochemical contents, antioxidant capacity, ultrastructure and drying kinetics of red bell pepper (*Capsicum annuum* L.). *LWT - Food Science and Technology*, 77: 337-347.
- Wang, H., Zhang, Q., Mujumdar, A. S., Fang, X. M., Wang, J., Pei, Y. P., Wu, W., Zielinska, M. and Xiao, H. W. (2020). High-humidity hot air impingement blanching (HHAIB) efficiently inactivates enzymes, enhances extraction of phytochemicals and mitigates brown actions of Chili pepper. *Food Control*, 111: 107050.
- Wu, Y., Liu, Y., Jia, Y., Feng, C.H., Zhang, H., Ren, F. and Zhao, G., 2024. Effects of thermal processing on natural antioxidants in fruits and vegetables. *Food Research International*, 192: 114797.
- Xiao, H. W., Pan, Z., Deng, L. Z., El-Mashad, H. M., Yang, X. H., Mujumdar, A. S., Gao, Z. J. and Zhang, Q. (2017). Recent developments and trends in thermal blanching – A comprehensive review. *Information Processing in Agriculture*, 4(2): 101-127.
- Yahia, E. M., Contreras-Padilla, M. and Gonzalez-Aguilar, G. (2001). Ascorbic acid content in relation to ascorbic acid oxidase activity and polyamine content in tomato and bell pepper fruits during development, maturation and senescence. *LWT - Food Science and Technology*, 34(7): 452-457.