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Chlorophylls Reductions in Fresh-Cut Chard (*Beta vulgaris* var. *cicla*) with Various Sanitizing Agents

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ABSTRACT

Safety of fresh-cut products is a widespread health concern and can be achieved by washing treatments with various agents. However, use of these agents can adversely affect the product quality depending on the processing and subsequent storage conditions. The effects of washing treatments with chlorine (50-200 mg L^{-1}), hydrogen peroxide (5.00-15.0%) and ozone (6.50 and 10.0 mg L^{-1}) followed by a cold storage (15 days/4 °C) period on chlorophylls contents of fresh-cut *Beta vulgaris* var. *cicla* (chard) were investigated by HPLC-DAD. In this study, treating samples with the sanitizing agents resulted in reductions in both chlorophyll a and chlorophyll b contents. These reductions generally increased with increasing the agent concentration. Chlorophyll a was found to be more sensitive than chlorophyll b to oxidation reactions with the agents used. Chlorophyll reductions of samples treated with ozone were at the higher level than samples treated by using other agents. Since the differences between chlorophylls contents of the samples treated with chlorine and hydrogen peroxide are very small, hydrogen peroxide can be suggested as an alternative to chlorine for sanitizing chard ($P<0.05$).

Keywords: *Beta vulgaris* var. *cicla;* Chard; Chlorophyll; Chlorine; Hydrogen peroxide; Ozone

Farklı Sanitasyon Ajanları Kullanımı ile Taze, Yıkanmış ve Doğranmış Pazılarda (*Beta vulgaris* var. *cicla*) Klorofil Düzeyinin Azalması

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ÖZET

Taze, yıkanmış ve doğranmış (fresh-cut) ürünlerde gıda güvenliği yaygın bir sorun olup, farklı yıkama ajanları kullanımı ile bu sorunun giderilmesi mümkün olabilir. Bununla birlikte, bu ajanların kullanımının proses ve daha sonra saklama

koşullarına bağlı olarak, ürün kalitesini olumsuz etkileyebilir. Bu çalışmada, farklı düzeylerde klor (50-200 mg L^{-1}), hidrojen peroksit (% 5.00-15.0) ve ozon (6.50 ve 10.0 mg L^{-1}) yıkama ajanı olarak kullanımının, soğuk depolama süresince (15 gün/4 °C) taze, yıkamış ve doğranmış *Beta vulgaris var. cicla* (pazı) klorofil içeriğine etkileri HPLC-DAD kullanılarak incelenmiştir. Çalışma sonucunda, sanitasyon (yıkama) ajanlarının kullanımı ile örneklerin klorofil a ve klorofil b içeriğinde düşüş belirlenmiştir. Bu düşüş genel olarak kullanılan ajan konsantrasyonu değişim düzeyi ile aynı yönde olmuştur. Klorofil a'nın, klorofil b'ye göre kullanılan ajanlardan kaynaklanan oksidasyona daha hassas olduğu tespit edilmiştir. Ozonla muamele edilen örneklerdeki klorofil kaybı, diğer ajanların kullanıldığı örneklere göre daha yüksek olarak belirlenmiştir. Klor ve hidrojen peroksit ile muamele edilmiş örneklerin klorofil içeriği arasındaki farklar çok küçük olduğu için, hidrojen peroksit pazı sanitasyonunda klora alternatif olarak önerilebilir ($P<0.05$).

Anahtar Kelimeler: *Beta vulgaris* var. *cicla;* Pazı; Klorofil; Klor; Hidrojen peroksit; Ozon

1. Introduction

Washing fruits and vegetables with sanitizing agents, like chlorine (Cl), ozone (O_3), hydrogen peroxide (H_2O_2), is a common practice to reduce the number of microorganisms and to extend the shelf life of the product. There are many scientific studies dealing with the effects of these washing processes most of which have focused on the microbial inactivation efficacy (Achen & Yousef 2001; Singh et al 2002; Garcia et al 2003; Selma et al 2007; Zorlugenc et al 2008; Olmez 2010; Zhou et al 2012; Elizaquivel et al 2012; Luo et al 2012). However, very little is known about the effects of these agents on the physical and chemical characteristics of the produce. Use of these agents at high concentrations in order to achieve higher microbial inactivation can cause deleterious effects on product quality such as losses in color, aroma, and nutritional value.

Chlorine (Cl), usually as hypochlorous acid HOCl, formed by dissociation of sodium hypochlorite (NaClO) in water, is the most commonly used disinfectant agent in the food industry. It is quite effective in inactivation food-borne microorganisms. However, it leads to the formation of toxic compounds on food contact surfaces and in wash water. For instance, trihalomethane compounds formed by the reaction of free-Cl with soluble organic compounds are proved to be carcinogenic (Kim et al 1999). For this reason, some restrictions in the use of Cl for washing agricultural products are implemented (Beltran et al 2005). Researchers and food processors investigate alternative applications to chlorination.

Ozone (O_3) and H_2O_2 appear to be promising alternatives with great potential applications in the food industry. After being used for years to disinfect water for drinking purposes, O_3 was approved for use as a disinfectant or sanitizer in food processing (FR 2001). Due to its quick decomposition to oxygen with no safety concerns about residues, it could be an acceptable technology to use with commodities marketed under "organic" classification (Gabler et al 2010). H_2O_2 is another chemical that can be used for disinfection of food (Kim et al 2007) and food contact surfaces (Khadre & Yousef 2001). Both O_3 and H_2O_2 are GRAS (generally recognized as safe) substances with high oxidation-reduction potentials, 2.1 and 1.8 mV, respectively (Kim et al 2003). Probably, because of these strong oxidizing activities, oxidations of color pigments such as carotenoids (Henry et al 2000) and anthocyanins (Simmons et al 1997) were reported.

Chlorophylls, principal color pigments in green vegetables, have two main types, namely chlorophyll a and chlorophyll b. Chlorophyll a is usually present at a concentration of 2-3 times higher than chlorophyll b in agricultural products (Kirca et al 2006). Minimizing chlorophyll degradation is an industrial challenge since chlorophylls are susceptible to chemical and physical changes during processing of vegetables. For instance, during thermal processing, the natural cellular structures disintegrate resulting in amenability of the pigment to various reactions

such as conversion of chlorophyll a and chlorophyll b to their corresponding pheophytins (Turkmen et al 2006). Also, reactions can occur through the removal of phytol group from chlorophylls and pheophytins by the action of enzyme chlorophyllase, resulting in the less stable chlorophyllides and pheophorbides, respectively (Kirca et al 2006). Bleaching of chlorophylls by oxidative reactions is another means of chlorophyll degradation. Procedures, in which strong oxidizing agents take place, may adversely affect nutritional and chemical product quality (e.g. may cause discoloration) depending on the concentration and time of exposure to the sanitizing solution.

The aim of this study was to investigate the effects of washing treatments with Cl, O_3, and H_2O_2 solutions on chlorophylls contents of fresh-cut chard throughout cold storage for 15 days and to compare these agents and their different doses in terms of chlorophyll degradation.

2. Material and Methods

2.1. Materials

Chard (*Beta vulgaris* var. *cicla*) used in the study was obtained from a local farmers market in Ankara and used immediately in the experiments. Chlorophyll a (Sigma C-5753) and chlorophyll b (Sigma C-5878) standards were purchased from Sigma Co (St. Louis, MO, USA). Methanol and chloroform were obtained from Riedel-de Haen (Seelze, Germany) whereas hexane was obtained from Sigma Aldrich (Steinheim, Germany). All solvents were either analytical or high performance liquid chromatography (HPLC) grade.

2.2. Preparation of chard samples for the treatments

Leaves that are uniform in size and color were selected and washed under running tap water to remove dirt, soil, etc. Midribs (white sections) were excised with a sharp stainless-steel knife and discarded. The rest of the leaves (leaflets) were cut into 1.00-1.50 cm wide strips with the knife. Cut leaf pieces were blended (mixed) for uniformity and separated into four lots for different treatments. One of the lots was directly used for chlorophyll analyses (controls) whereas the others were immediately treated with Cl, O_3 or H_2O_2 solutions.

2.3. Preparation of washing solutions and treatment procedures

High purity water (better than ASTM Type 2) obtained from a TKA Pacific UP/UPW water purification system (TKA Water Purification Systems GmbH, Niederelbert, Germany) was used for preparing all aqueous washing solutions. Washing solutions of Cl (50, 100 and 200 mg L^{-1}) and H_2O_2 (5.00, 10.0 and 15.0%) were prepared by appropriate dilutions of sodium hypochlorite solution (Sigma Aldrich, available Cl 10.0-13.0%) and H_2O_2 solution (Riedel de Haen, 30%, v v^{-1}), respectively. Cl levels in the treatment solutions were determined by Cl test strips (Quantofix, 1-100 mg L^{-1}, Macherey Nagel, Duren, Germany). The most effective ozonation method mentioned in the literature (Kim et al 1999; Achen & Yousef 2001; Olmez 2010), bubbling, was used for O_3 treatments of chard samples. O_3 was produced by a corona discharge generator (OG 20, Opal, Ankara, Turkey) with a production capacity of 20 g h^{-1}. The generator had an oxygen concentrator inside and used oxygen gas concentrated from the air for O_3 production. The generator could be run at two different levels, 10 and 20 g h^{-1}. Gaseous O_3, passing through silicone hose, was bubbled into the water by the help of a stainless-steel sparger with 10 µm pore size (Solvent inlet filter, Fisher Scientific, Fair Lawn, NJ, USA). The gas flow was controlled at 827 mL min^{-1} by a Riteflow flowmeter (150 mm, Size 2, Bel-Art Products, Pequannock, NJ, USA). O_3 concentrations in water were determined by using indigo blue dye, which is based upon Standard Methods (APHA, 1992). For this method, a stock indigo solution was prepared with potassium indigo trisulfonate (234087, Sigma Aldrich) and phosphoric acid (Riedel-de Haen). Indigo trisulfonate was decolorized by O_3 and the color changes were measured in spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) at 600 nm. At the end of ozonation processes, the O_3 concentrations in

water were 6.50±0.12 and 10.0±0.14 mg L^{-1} in case of running the O_3 generator at low (10 g h^{-1}) and high (20 g h^{-1}) levels, respectively.

Treatments were conducted in 1-L borosilicate glass jars containing the sanitizing solutions. For O_3 treatments, gaseous O_3 was bubbled into the water present in the jar. Jars containing 20 g of cut chard sample and 800 mL of sanitizing solution were shaken with an orbital shaker (Biosan OS-10, Riga, Latvia) at a speed of 200 rpm. All treatments were conducted at room temperature (22 °C) for 15 min. This treatment time is quite long and maybe impractical for the industry. However, it was necessary to use an extended treatment time to observe any detrimental effects of the agents -if they have- to chlorophylls contents of chard.

Treated chard samples were soaked in distilled water (1 L) for 1 min for rinsing. After removing the excessive water with a manual salad spinner, samples were placed in plastic zip-lock freezer bags and stored at 4±1 °C for 15 days.

2.4. Extraction of chlorophylls and HPLC analysis

Chlorophyll extractions were carried out according to the method of Teng & Chen (1999) with modifications. Extraction of chlorophylls was performed under dim light and at low temperatures to minimize photo degradation of the pigments. All the leaves from each treatment were homogenized using a lab blender (Waring blender) for 1 min. Some of the homogenized sample (~5 g) was put into a mortar and the tissue was mashed with a pestle. Mashed sample (0.20±0.001 g) was weighed in a test tube, to which 3.00 mL of methanol were added. After vortexing for 1 min at high speed, the methanol-phase containing the chlorophylls was transferred to a 25 mL volumetric flask. The residue in the test tube was re-extracted with 3.00 mL of methanol. This extraction procedure was repeated several (6-7) times until the residue became colorless. Then, all the extracts were pooled and brought to volume with methanol. This crude extract was centrifuged (Sigma, Model 2-16, Osterode, Germany) at 4000 rpm for 10 min and filtered through a hydrophilic PTFE Millex-LCR membrane filter (Millipore, Bedford, MA, USA) with 0.45 µm pores into an amber flask and immediately injected to HPLC.

2.5. HPLC analysis

HPLC analysis was carried out using a Shimadzu system (Kyoto, Japan) consisting of a LC-20AD pump, a DGU-20A5-E degasser and a UV-VIS photo diode array detector (SPD-M20). The chromatograms were recorded at 430 and 460 nm for chlorophyll a and chlorophyll b, respectively (Teng & Chen 1999). A Phenomenex (Torrance, CA, USA) analytical column (C18, 5 µm, 250 mm x 4.6 mm i.d.) was used in the experiments. A mixture of methanol:chloroform:n-hexan (85:7.5:7.5) at a flow rate of 1 mL min^{-1} under isocratic conditions was used as the mobile phase.

2.6. Standard solutions

Stock solutions of chlorophyll a (40 mg L^{-1}) and chlorophyll b (20 mg L^{-1}) were prepared by dissolving chlorophyll a and chlorophyll b standards, respectively, in methanol. Standard solutions of chlorophyll a (1.00, 5.00, 10.0, 15.0 and 20.0 mg L^{-1}) and chlorophyll b (1.00, 5.00, 7.00, 10.0 and 15.0 mg L^{-1}), prepared by appropriate dilutions of stock solutions with methanol, were injected in to HPLC and calibration curves were prepared.

2.7. Dry matter determination

To eliminate the variations in water content occurring due to respiration, transpiration, etc. during storage, all calculations were made on dry matter (DM) basis. DM contents of chard samples were determined according to Gornicki & Kaleta (2007), in triplicate.

2.8. Statistical analysis

The data were subjected to analysis of variance (ANOVA) to determine the significant differences between means by using Minitab statistical package (v.13, MINITAB Inc., USA). Values (n= 3) were reported as mean degradation rate ± standard error. Duncan's multiple range test, at a significance level of P= 0.05, was conducted for the separation

of means by using MSTAT-C statistical software (MSTAT 1991, Michigan State University, MI, USA).

3. Results and Discussion

Retention times for chlorophyll a and chlorophyll b were determined as 8.1 and 5.8 min, respectively. Chromatograms of chlorophyll a and chlorophyll b in untreated chard samples are shown as an example in Figure 1.

The chlorophyll a contents in chard samples treated with various doses of different sanitizing agents are shown in Table 1. Chlorophyll a levels significantly decreased just after treatments with the agents in all samples. Statistical analysis revealed that disinfectant doses and storage time significantly affected the levels of chlorophyll a in chard samples. Moreover, these factors showed a significant interaction for Cl and H_2O_2 treatments ($P<0.05$), but not for O_3 ($P>0.05$).

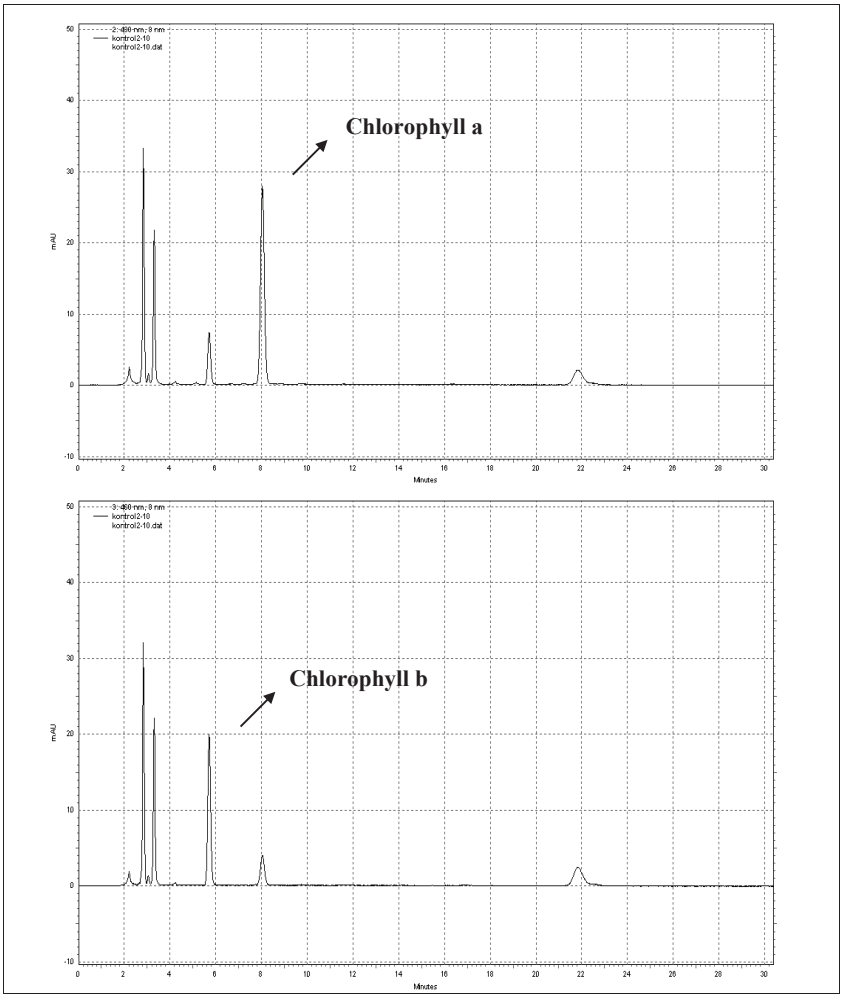

Figure 1- Chlorophyll a (at 430 nm) and chlorophyll b (at 460 nm) peaks in untreated chard

Şekil 1- İşlem görmemiş pazıda klorofil a (430 nm) ve klorofil b (460 nm) pikleri

Table 1- Chlorophyll a contents (mg kg^{-1}, DM) in chards throughout storage after treating with various doses of sanitizers (mean ± standard error, n= 3)

Çizelge 1- Farklı sanitasyon ajanları ile işlem sonrası depolama süresince pazıların klorofil a içeriklerindeki (mg kg^{-1}, KM) değişimler (ortalama ± standard hata, n= 3)

Disinfectant	*Disinfectant dose*	*Chlorophyll a*			
		Storage time (days)			
		0	*5*	*10*	*15*
Control	-	15650±250^{Aa*}	13330±87^{Ab}	12370±114^{Ac}	11730±149^{Ac}
Chlorine	50 mg L^{-1}	12810±287^{Ba}	12490±82^{Bab}	11820±139^{ABb}	10740±411^{Bc}
	100 mg L^{-1}	12690±326^{Ba}	11770±83^{Cb}	11250±90^{BCb}	10520±196^{Bc}
	200 mg L^{-1}	12140±283^{Ba}	11710±54^{Ca}	10770±370^{Cb}	9837±238^{Cc}
Ozone	6.50 mg L^{-1}	12980±177^{Ba}	12412±190^{Ba}	11567±139^{Bb}	10022±201^{Bc}
	10.0 mg L^{-1}	12138±205^{Ba}	10895±178^{Cb}	9369±88^{Cbc}	8705±99^{Cc}
Hydrogen peroxide	5.00%	13260±126^{Ba}	12820±93^{Aa}	11740±329^{ABb}	10700±411^{Bc}
	10.0%	11990±472^{Ca}	11680±332^{Bab}	11120±278^{BCb}	9981±196^{Bc}
	15.0%	11280±296^{Ca}	11060±227^{Ba}	10630±422^{Ca}	9105±238^{Cb}

*, means with different letters shown with lower case (a-c) show significant differences among sampling days (P<0.05) and means with different letters shown with upper case (A-C) show significant differences among doses and types of the agents (P<0.05)

Chlorophyll a content decreased in all samples including control throughout storage (Table 1). In control samples, 15.0, 21.0 and 25.0% reductions of chlorophyll a were observed at day 5, 10 and 15, respectively. Chlorophyll b contents of the samples treated by various doses of different sanitizers during 15-day storage are given in Table 2.

Similar to the results observed for chlorophyll a, chlorophyll b content decreased in all samples including control throughout storage. Reduction rates of chlorophyll b were 12.0, 17.0 and 23.0% at day 5, 10 and 15, respectively, in control samples. Disinfectant dose and storage time did not show any significant interaction on chlorophyll content of

Table 2- Chlorophyll b contents (mg kg^{-1} DM) in chards throughout storage after treating with various doses of sanitizers (mean ± standard error, n= 3)

Çizelge 2- Farklı sanitasyon ajanları ile işlem sonrası depolama süresince pazıların klorofil b içeriklerindeki (mg kg^{-1}, KM) değişimler (ortalama ± standard hata, n= 3)

Disinfectant	*Disinfectant dose*	*Chlorophyll b*			
		Storage time (days)			
		0	*5*	*10*	*15*
Control	-	5641±79^{Aa*}	4964±66^{Ab}	4682±65^{Abc}	4344±62^{Ac}
Chlorine	50 mg L^{-1}	5026±101^{Ba}	4200±68^{Bb}	4102±92^{Bb}	4062±117^{Bb}
	100 mg L^{-1}	4654±68^{BCa}	4117±105^{BCb}	4030±88^{BCbc}	3781±49^{Cc}
	200 mg L^{-1}	4297±77^{Ca}	4052±66^{Cb}	3802±89^{Cb}	3778±65^{Cb}
Ozone	6.50 mg L^{-1}	4970±103^{Ba}	4570±82^{Bb}	4290±101^{Bc}	3892±95^{BCd}
	10.0 mg L^{-1}	4443±111^{Ca}	3825±92^{Db}	3628±93^{Db}	3328±90^{Dc}
Hydrogen peroxide	5.00%	4617±75^{BCa}	4296±95^{Bb}	4233±88^{Bb}	4118±114^{ABb}
	10.0%	4519±77^{Ca}	4093±109^{Cb}	3811±86^{Cbc}	3610±82^{Cc}
	15.0%	4065±89Da	3830±79^{Db}	3779±64^{Cb}	3497±69^{Dc}

*, means with different letters shown with lower case (a-d) show significant differences among sampling days (P<0.05) and means with different letters shown with upper case (A-D) show significant differences among doses and types of the agents (P<0.05)

chard (P>0.05). Chlorophyll loss in leafy vegetables during storage is an ordinary consequence of senescence due to the disintegration of the plant tissues. Reductions in green colors of lettuce (Bolin & Huxsoll 1991), chard (Roura et al 2000), chicory and rocket (Ferrante et al 2004) during storage were also reported in previous studies.

3.1. Effect of Cl treatment on chlorophyll content in chard samples throughout storage

Treating chard samples with Cl solutions at various concentrations (50.0-200 mg L^{-1}) resulted in reductions in both chlorophyll a and chlorophyll b contents (Table 1 and Table 2). These reductions increased with increasing Cl concentration. At the end of storage period (15 days), reduction rates were 31.0, 33.0 and 37.0% for chlorophyll a and 28.0, 33.0 and 33.0% for chlorophyll b in samples treated with Cl solutions of 50.0, 100 and 200 mg L^{-1}, respectively. Reducing chlorophylls levels after Cl treatments in green bell peppers were also reported by Nunes & Emond (1999). They dipped green bell peppers into Cl solutions (0.00-200 mg L^{-1}) for varying time (0-45 min) and observed that total chlorophyll contents decreased with increasing time of dipping and Cl concentration.

In our study, the differences between Cl doses on chlorophyll a degradation were not significant at the beginning of storage, but became significant from day 5 on (P<0.01). Likewise, chlorophyll a and chlorophyll b levels in parsley samples treated with chlorinated (100 mg L^{-1}) and ozonated (12 mg L^{-1}) water significantly decreased compared to control samples beginning from the fifth day of storage (Karaca 2010). The author claimed that cellular fluids released due to cutting or vigorous washing were removed by water rinse. Kenny & O'Beirne (2009) reported that color loss was more pronounced in water-dipped and Cl-dipped lettuce than the samples subjected to a milder treatment (tap-rinsing). In intact cell tissues, chlorophyll is separated spatially from chlorophyllase, a key enzyme in chlorophyll metabolism. When cells of fresh produce are ruptured, as occurs during cutting or vigorous washing, chemical reactions are initiated that shorten storage life (Bolin & Huxsoll 1991). Chlorophyllase and its substrate, chlorophyll, come into contact and chlorophyll degradation reactions occur particularly in tissues adjacent to those that are damaged by cutting action, when acids and hydrolyzing enzymes of the vacuoles are released (Roura et al 2000).

In all samples including control, chlorophyll b levels determined at day 5 were significantly lower than those determined at the beginning of storage. It shows that chlorophyll b degradation takes place very rapidly in chard tissues. There were no significant differences between chlorophyll a contents of the samples treated with 50.0 and 100 mg L^{-1} Cl and between chlorophyll b contents of the samples treated with 100 and 200 mg L^{-1} Cl (P>0.05). Hence, it can be said that using higher concentrations of Cl would not result in any additional chlorophyll degradation, in other words, would not cause color loss in chard. Enhancing Cl concentration can be useful for achieving higher microbial inactivation levels. However, excessive use of Cl can also result in higher formation of toxic residues on produce surface and in wash water.

3.2. Effect of O_3 treatment on chlorophyll content in chard samples throughout storage

Similar to the results of Cl treatments, treating chard samples with O_3 solutions resulted in reductions in chlorophyll a and chlorophyll b contents (Table 1 and Table 2). At the end of storage period, reduction rates were 36.0 and 44.0% for chlorophyll a and 31.0 and 41.0% for chlorophyll b in samples treated with O_3 concentrations of 6.50 and 10.0 mg L^{-1}, respectively. These results show the susceptibility of chlorophyll a and chlorophyll b in chard to O_3. Philosoph-Hadas et al (1994) also claimed that chlorophylls are extremely sensitive to oxidative compounds such as O_3 and free radicals. In addition, recognizable discolorations were reported in many products such as broccoli (Skog & Chu 2001), lettuce (Singh et al 2002; Olmez & Akbas 2009), spinach (Klockow & Keener 2009; Vurma et al 2009) and *Arabidopsis thaliana* (Kubo et al 1995) after O_3 treatments.

The chlorophyll a contents of chard samples treated with high O_3 dose (10.0 mg L^{-1}) were significantly lower at day 5 than that at day zero. On the other hand; when treated with low O_3 dose (6.50

mg L^{-1}), the chlorophyll a level was maintained on the fifth day of storage and a decline was observed at day 10. In all samples, decreases were determined in chlorophyll b content on each day of sampling. Reduction in chlorophyll a content was slightly higher than that in chlorophyll b (3.00-5.00%) after treating with both O_3 doses.

3.3. Effect of H_2O_2 treatment on chlorophyll content in chard samples throughout storage

Treating chard samples with H_2O_2 solutions at various concentrations (5.00-15.0%) also resulted in reductions in both chlorophyll a and chlorophyll b contents (Table 1 and Table 2). In samples treated with 5.00, 10.0 and 15.0% of H_2O_2 solutions 32.0, 37.0 and 42.0% reductions of chlorophyll a and 27.0, 36.0 and 38.0% reductions of chlorophyll b were observed, respectively, at the end of storage.

Chlorophyll a levels determined just after washing treatments with H_2O_2 were maintained at day 5 after treatments with the solutions of 5.00 and 10.0% and at day 10 after treatments with the solutions of 15.0%. There were no significant differences between chlorophyll a contents of the samples treated with 10.0 and 15.0% of H_2O_2 at day zero, 5 and 10 ($P>0.05$). Moreover, no significant differences were observed between the chlorophyll b contents of samples treated with the solution of 5.00% H_2O_2 and that of control at the end of storage period ($P>0.05$). This means that the solution of H_2O_2 at 5.00% concentration did not cause any additional loss of chlorophyll b in chard samples stored for 15 days. The reductions in chlorophylls content in chard samples increased with increasing the concentration of H_2O_2 as well as other agents. Likewise, many other researchers (Simmons et al 1997) reported that when used as a surface disinfectant, H_2O_2 caused degradation of pigments (chlorophylls, anthocyanins, etc.) and this detrimental effect increased with increasing the agent concentration. In addition, H_2O_2 is claimed to be involved in a system (phenolic-peroxidase-H_2O_2 system) in *in vitro* bleaching of chlorophylls (Kato & Shimizu 1987). By the function of this system, chlorophyll is oxidized to colorless, low-molecular weight compounds (Yamauchi et al 2004).

3.4. Comparison of susceptibilities of chlorophylls against different sanitizing agents

For both chlorophyll a and chlorophyll b, the differences between treatments were less pronounced in the beginning of storage, but became more evident on the last sampling day, especially in the treatments with high O_3 dose. Chlorophyll a and chlorophyll b retentions in chards treated with various agents after 15-day storage are presented in Figure 2 and Figure 3, respectively. Chlorophyll a and chlorophyll b retentions in control samples were 75.0 and 77.0%, respectively, at the end of storage period. Chlorophyll a retention rates in Cl, O_3 and H_2O_2 treated samples were 63.0-69.0, 56.0-64.0, and 58.0-68.0%, respectively, at the end of storage period. Corresponding rates for chlorophyll b were 67.0-72.0, 59.0-69.0, and 62.0-73.0%. It shows that the decrease of chlorophyll a is slightly more marked than that of chlorophyll b.

At the end of storage period, chlorophyll a and chlorophyll b reductions were 32.0 and 27.0%, respectively, in samples treated with the solution of H_2O_2 at 5.00%. In samples treated with 10.0 and 15.0% H_2O_2 solutions 37.0 and 42.0% chlorophyll a reduction and 36.0 and 38.0% chlorophyll b reduction were observed, respectively. Similar results were obtained for Cl and O_3 treatments. For instance, in samples treated with 200 mg L^{-1} Cl, 6.50 mg L^{-1} and 10.0 mg L^{-1} O_3, chlorophyll a and chlorophyll b reductions were 37.0 and 33.0%, 35.0 and 31.0%, and 43.0 and 41.0%, respectively, at the end of storage. Overall, it can be said that chlorophyll a is more intensely degraded than chlorophyll b after all treatments, suggesting that chlorophyll a is more susceptible to oxidation with these agents. In previous studies, many authors suggested that chlorophyll a is more sensitive than chlorophyll b to heat (Weemaes et al 1999), sulphur dioxide and ethylene (Zhou et al 2010) treatments. Although nearly one-third of chlorophyll a and one-fourth of chlorophyll b reduced at the end of storage period, formation of pheophytins was not observed after any treatments. Pheophytins are the main degradation products of chlorophylls that form mainly during thermal processes (Turkmen et al 2006). Probably, in the degradation of chlorophylls with oxidizing agents such as Cl, O_3 and H_2O_2,

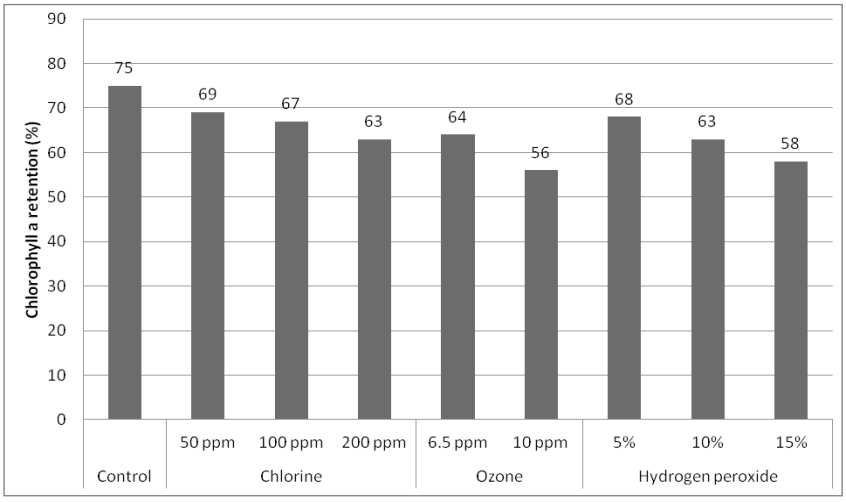

Figure 2- Chlorophyll a retention in chards treated with various agents after 15-day storage [The chlorophyll a content in untreated samples at the beginning of storage (15650 mg kg^{-1} DM was assumed as 100%, ppm= mg kg^{-1})

Şekil 2- 15 günlük depolama sonrası farklı ajanlarla muamele edilen pazılarda belirlenen klorofil a düzeyleri [İşlem uygulanmamış örneklerde depolama başlangıcındaki klorofil a içeriği (kuru maddede 15650 mg kg^{-1} % 100 olarak kabul edilmiştir, ppm= mg kg^{-1})

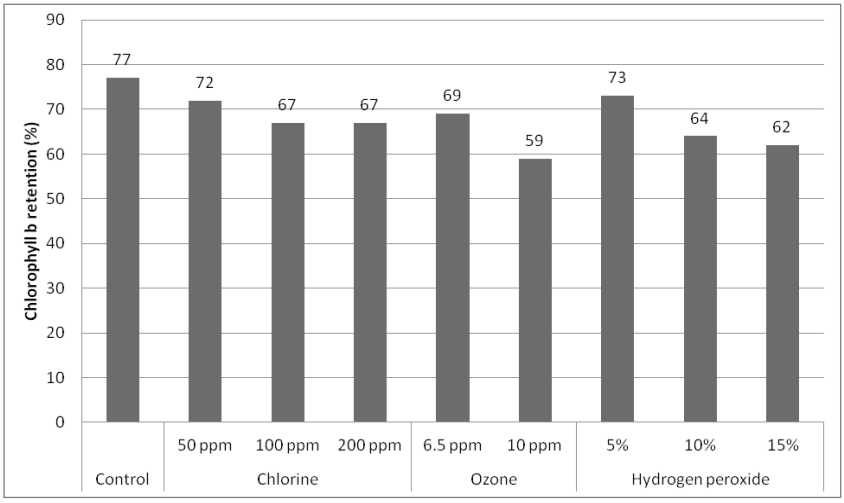

Figure 3- Chlorophyll b retention in chards treated with various agents after 15-day storage [The chlorophyll b content in untreated samples at the beginning of storage (5641 mg kg^{-1} DM was assumed as 100%, ppm= mg kg^{-1})

Şekil 3- 15 günlük depolama sonrası farklı ajanlarla muamele edilen pazılarda belirlenen klorofil b düzeyleri [İşlem uygulanmamış örneklerde depolama başlangıcındaki klorofil a içeriği (kuru maddede 5641 mg kg^{-1} % 100 olarak kabul edilmiştir, ppm= mg kg^{-1})

different pathways dominate the oxidative degradation mechanisms. Beltran et al (2005) and Lopez-Galvez et al (2010) did not determine any significant differences between chlorophyll contents of lettuce treated with different agents like O_3, Cl and Cl-dioxide. According to our results, since the chlorophyll contents of the samples treated with Cl and H_2O_2 are so close (1.00-5.00% difference), H_2O_2 can be suggested as an alternative of Cl.

4. Conclusions

In conclusion, both chlorophyll a and chlorophyll b contents decreased in all samples including controls during storage. At the end of 15-day storage, 25.0% of chlorophyll a and 23.0% of chlorophyll b reductions were observed in control (untreated) samples. Chlorophyll a reductions in Cl, O_3, and H_2O_2 treated samples were 31.0-37.0, 36.0-44.0, and 32.0-42.0%, respectively, at the end of storage. These rates were 28.0-33.0, 31.0-41.0, and 27.0-38.0% for chlorophyll b. Results revealed that chlorophyll a is more sensitive than chlorophyll b to oxidation reactions with the sanitizers used. H_2O_2 appears to be a good alternative of Cl in terms of color retention in chard. In addition, O_3 use can be appropriate at low dose and for short storage times.

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