CHLOROPHYLL COLOR RETENTION IN GREEN PEPPER PRESERVED IN NATURAL LEMON JUICE

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ABSTRACT

The effect that dipping green pepper (*Capsicum annuum*, L.) in natural lemon juice followed by blanching had on the rate of the pepper chlorophyll color retention was studied. Dipping green pepper in natural lemon juice immediately after blanched with chloride salts of copper, zinc and magnesium decreased the degradation of chlorophyll after 2 weeks of storage by 13.6, 31.7 and 74.3%, respectively. The minimal loss in chlorophyll of green pepper treated with copper chloride is attributed to the rapid chelating of the copper complexes and higher heat and acid stability compared to zinc that improved the green color of blanched peeper during storage. Pepper samples that blanched with different chloride salts of copper, zinc and magnesium (100 ppm) and coated with carboxymethyl cellulose (CMC) were not significantly ($P < 0.05$) different in chlorophyll content from uncoated samples on any given days.

PRACTICAL APPLICATIONS

In order to preserve the green color (i.e., chlorophyll) of pepper from degrading into an olive green pigment (pheophytin) in acid solutions, peppers should be blanched with copper and zinc chlorides. Treatments were able to reduce chlorophyll degradation by 71.7 and 65.0%, respectively. The results were attributed to the formation of stable color complexes with stable copper and zinc cation color complexes. Coating application had no effect on chlorophyll degradation while drying of blanched green pepper can increase chlorophyll degradation. For preservation of pepper green color in natural lemon juice for long period, it is recommended to blanch the pepper first with copper chloride before dipping in lemon juice.

INTRODUCTION

The consumption of fresh foods, particularly fruits and vegetables, has a great value due to increasing demand for fresh, healthy and convenient foods while drying of blanched green pepper increased chlorophyll degradation. Green pepper (*Capsicum annuum*, L.) whether fresh, cooked or ready to eat salad is among the vegetables highly sold in markets not only for its nutritional value including vitamins C, E and A, minerals and fibers, but also for its low caloric content (Daood *et al.* 1996; Oboh *et al.* 2007) and content of antioxidants compounds (i.e., phenols, chlorophyll, carotenoides and ascorbic acid) (Krinsky 2001; Oboh *et al.* 2007; Rico *et al.* 2007).

Chlorophylls are highly susceptible to degradation during processing resulting in color shift of chlorophylls from brilliant green to olive brown compounds such as pheophytin and pheophorbide in senescent tissues (Schwartz and von Elbe 1983; Koca *et al.* 2006). Chlorophyll degradation was significantly mediated by factors such as enzyme chlorophyllase, heat, light, oxygen, chemicals and acids (Gunawan and Barringer 2000; Koca *et al.* 2006).

Green pepper dipped in dressing (i.e., lemon juice or vinegar) usually lost its prominent brilliant green color due to acidic condition. The change in appearance is the fundamental factor affecting the quality of green pepper preferences, acceptability, and pleasantness of such dressing.

Chlorophyll degradation in low pH dressing is mainly related to the replacement of magnesium ion in the porphyrin ring by hydrogen ions and subsequent formation of pheophytin and pheophorbide that result in a change in color from bright green to dull olive green or yellow green (Heaton and Marangoni 1996; Toivonen and Brummell 2008).

Chlorophyll breakdown and the ability of chlorophyll retention and its effect on color was studied by Brown *et al.* (1991) and Yamauchi and Watada (1991) using parsley leaves. The authors reported that color degradation was induced by oxidation. Funamoto *et al.* (2002, 2003) and Costa *et al.* (2005, 2006), on the other hand, indicated that several enzymes including peroxidase, lipoxygenase and chlorophyll oxidase played the mediator of chlorophyll breakdown in broccoli, spinach leaves and cabbage.

To stabilize chlorophyll in green vegetables during processing various treatments have been applied. Applications of alkaline in blanching water or brine solution (Gilpin *et al.* 1959; Koca *et al.* 2006), high temperature short time processing (Schwartz and Lorenzo 1991), edible coatings (Olivas and Barbosa-Canovas 2005; Moreira *et al.* 2011) and modified atmospheric packaging (Gorny *et al.* 2002; Aguayo *et al.* 2006) and the use of zinc and copper chlorides (Guzman *et al.* 2002) are examples of techniques used to stable chlorophyllides in vegetable tissues.

Green pepper color is considered as one of the most important sensory attributes that determine several products acceptability. Although several techniques (i.e., low pH in the presence of zinc, magnesium and copper chlorides and/or carboxymethylcellulose) are used to aiming to limit color changes in vegetables, the effect of such techniques on chlorophyll degradation has not been studied extensively. Therefore, the objectives of this study were to evaluate the effectiveness of a combination of techniques on minimizing color change (i.e., chlorophyll degradation) on green pepper during storage.

MATERIALS AND METHODS

Sample Preparation

Fresh green peppers (*Capsicum annuum*, L.) were obtained from a local market in Amman, Jordan. Samples were washed, destalked and chopped to approximately 7 mm pieces using a household kitchen Aid mixer (Model KSM150PSER) at speed 4 for 2 min and then stored immediately at −18C in polyethylene bags until blanching.

Blanching With Zinc, Magnesium and Copper Chloride

Approximately 300 g of chopped green peppers were blanched separately in for 3 min at 90C in (1) water (2) copper chloride solution ($CuCl_2 \cdot 2H_2O$, Fisher Scientific Co., UK), (3) zinc chloride solution ($ZnCl_2$, Sigma–Aldrich Co., St. Louis, MO) and (4) magnesium chlorides solution ($MgCl_2 \cdot 6H_2O$, Sigma–Aldrich Co., St. Louis, MO) at concentrations of 100 ppm. After blanching, chopped peppers from each treatment were left to cool at room temperature (23.3C), and then dried at 40C for 6 h in a natural convection oven (Memmert, GmbH Co., Germany). A batch of fresh chopped pepper was pre-dried at 40C for 6 h to evaluate the effect of pre-drying process on the color retention of pepper.

Coating Formulations

Edible coating was prepared using carboxymethylcellulose (CMC) at a concentration of 2% (w/v) by dissolving 2 g of powdered CMC in 50 mL boiled distilled water followed by heating at 90C for 8 min using a water bath. The film was applied by pouring coating solution over the treated chopped peppers. After edible coating application, chopped peppers were dried by air using a fan (i.e., 30C for 2 h) and films were allowed to set. A control sample without coating film was used to comparison.

Coated and noncoated samples were placed in 200 mL plastic cups filled with natural lemon juice in a ratio of 1:2 of chopped pepper to lemon juice, covered and stored in a refrigerator (4C) during the entire storage duration.

pH Measurements

The pH values of chopped peppers and natural lemon juice was determined separately, using the method of Martins and Silva (2002). In brief, 50 g of chopped pepper was vortexed for 3 min followed by pH determinations using pH meter (model 211, Hanna Instruments, NC) previously calibrated with pH 4.00 and 7.00 buffers and adjusted to operate at room temperature for fresh, blanched and chopped peppers in three replicates.

Chlorophylls Extraction and Measurements

Chlorophylls from chopped treated and untreated green peppers were extracted during storage (i.e., 7 days intervals) using the method of Koca *et al.* (2006) with slight modification. In summary, 25 mL of 90% acetone was added to 3 g of pepper samples in a centrifuge tubes. The mixture was then sonicated for 5 min using Elmasonic S ultrasonic (Elma GmbH & Co KG, Kolpingstr, Germany). Samples were then left to steep in refrigerator for 24 h. The extract of each sample was clarified by centrifugation at 4,000 × g for 5 min. Extracts were then was brought to initial volume of 25 mL with 90% acetone. Chlorophyll and pheophytin levels were evaluated using ESS method 150.1 (1991) spectrophotometric method.

TABLE 1. EFFECTS OF TREATED (i.e., BLANCHED, BLANCHED/DRIED AND BLANCHED/DRIED/COATED) CHOPPED GREEN PEPPER WITH DIFFERENT CHLORIDE SALTS ($CuCl_2$, $ZnCl_2$ and $MgCl_2$ AT (100 ppm) ON CHLOROPHYLL CONTENT (μg/g FW) WHEN DIPPED IN NATURAL LEMON JUICE (i.e., pH 2.7) DURING STORAGE AT 4C

	Blanching treatment			
Blanched/dried/coated	Blanched/dried	Blanched	No treatment	Storage (days)
No chemical treatment				
22.3 ± 0.35a	22.4 ± 0.22a	23.4 ± 0.57a	33.7 ± 0.35a	0
12.8 ± 0.07b	10.5 ± 0.42b	11.4 ± 0.21b	9.7 ± 0.35b	1
3.2 ± 0.28c	2.3 ± 0.28c	3.4 ± 0.21c	2.1 ± 0.14c	8
2.1 ± 0.14d	1.0 ± 0.04d	<0.1 ± 0.00d	<0.1 ± 0.00d	15
0.3 ± 0.01e	<0.1 ± 0.00e	<0.1 ± 0.00d	<0.1 ± 0.00d	22
<0.1 ± 0.00f	<0.1 ± 0.00e	<0.1 ± 0.00d	<0.1 ± 0.00d	29
$ZnCl_2$			No treatment	
27.2 ± 0.35a	26.9 ± 0.21a	35.6 ± 0.35a	33.7 ± 0.35a	0
24.8 ± 0.14b	23.6 ± 0.56b	32.1 ± 0.14b	9.7 ± 0.35b	1
22.7 ± 0.98c	18.2 ± 0.49c	21.5 ± 0.70c	2.1 ± 0.14c	8
19.1 ± 0.84d	16.8 ± 0.35d	23.0 ± 0.28d	<0.1 ± 0.00d	15
18.0 ± 0.70d	16.6 ± 0.14d	21.8 ± 0.35e	<0.1 ± 0.00d	22
12.9 ± 0.28e	11.4 ± 0.42e	17.3 ± 0.42f	<0.1 ± 0.00d	29
$MgCl_2$			No treatment	
19.8 ± 1.13a	19.8 ± 0.35a	28.5 ± 0.70a	33.7 ± 0.35a	0
8.8 ± 0.70b	8.2 ± 0.56b	13.6 ± 0.49b	9.7 ± 0.35b	1
7.1 ± 0.35c	4.6 ± 0.63c	8.8 ± 1.06c	2.1 ± 0.14c	8
4.9 ± 0.49d	2.3 ± 0.42d	5.3 ± 0.42d	<0.1 ± 0.00d	15
2.3 ± 0.42e	<0.1 ± 0.00e	<0.1 ± 0.00e	<0.1 ± 0.00d	22
<0.1 ± 0.00f	<0.1 ± 0.00e	<0.1 ± 0.00e	<0.1 ± 0.00d	29
$CuCl_2$			No treatment	
35.8 ± 0.84a	35.8 ± 0.35a	40.6 ± 0.84a	33.7 ± 0.35a	0
31.3 ± 0.70b	30.2 ± 0.84b	33.2 ± 0.49b	9.7 ± 0.35b	1
27.9 ± 1.27c	27.7 ± 0.84c	29.8 ± 0.98c	2.1 ± 0.14c	8
27.8 ± 1.20c	28.0 ± 0.70c	29.1 ± 0.84c	<0.1 ± 0.00d	15
28.6 ± 0.42c	27.7 ± 0.28c	27.4 ± 0.84d	<0.1 ± 0.00d	22
22.2 ± 0.77d	21.9 ± 0.91d	21.1 ± 0.98e	<0.1 ± 0.00d	29

For same blanching and chemical treatment, means of chlorophyll content (μg/g FW) during storage (same column) having different letters are significantly different ($P < 0.05$) according to the LSD.

Statistical Analysis

All measurements were performed in duplicate and mean values were reported. Analysis of variance (ANOVA) using JMP (release 10, SAS institute, Cary, NC) was performed to determine any significant differences among treatment parameters. Least significant differences (LSD) were used to determine significant differences in properties among treatments.

RESULTS AND DISCUSSION

Effects of Blanching and Drying on Chlorophyll Retention

Adequate blanching was performed for all chopped pepper treatments to inactivate enzymatic activity so not to contribute to color degradation during storage and to inactivate enzymes and to enhance the green color by expelling air pockets within the peppers. However, undesirable visual color changes from green to dull green and olive brown were observed within the first day of the experiment in fresh, blanched and dried pepper samples when soaked in acidic lemon juice. These undesirable color changes, which increased with storage duration, reflected the significant reduction ($P < 0.05$) in chlorophyll content of fresh pepper from an initial value of 33.7 μg/g fresh weight (FW) to 9.8 μg/g in fresh pepper dipped in natural lemon juice after 1 day storage (Tables 1 and 2).

Blanching with tap water did not improve the green pepper color, i.e., the initial chlorophyll content decreased from 33.7 to 23.6 μg/g after blanching and to 10.5 μg/g after blanching and drying at 40C for 6 h (Table 1). After one day of storage, chlorophyll content decreased significantly ($P > 0.05$) to 11.2, 10.5 and 12.7 μg/g for blanched, blanched/dried and blanched/dried/coated treatments, respectively.

TABLE 2. EFFECTS OF STORAGE DURATION OF CHOPPED GREEN PEPPER TREATED (i.e., BLANCHED, BLANCHED/DRIED AND BLANCHED/DRIED/COATED) CHOPPED GREEN PEPPER WITH DIFFERENT CHLORIDE SALTS ($CuCl_2$, $ZnCl_2$ and $MgCl_2$ AT (100 ppm) ON CHLOROPHYLL CONTENT (μg/g FW) WHEN DIPPED IN NATURAL LEMON JUICE AT pH 2.7 AND STORED AT 4C

Storage (days)						Chemical	Blanching
29	22	15	8	1	0	Treatment	Treatment
<0.1 ± 0.00a	<0.1 ± 0.00b	<0.1 ± 0.00c	2.1 ± 0.14b	9.7 ± 0.35c	33.7 ± 0.35a	No treatment	Fresh
<0.1 ± 0.00a	<0.1 ± 0.00b	<0.1 ± 0.00c	3.4 ± 0.21a	11.4 ± 0.21b	23.4 ± 0.57b		Blanched
<0.1 ± 0.00a	<0.1 ± 0.00b	1.0 ± 0.04b	2.3 ± 0.28b	10.5 ± 0.42c	22.4 ± 0.22b		Blanched/dried
<0.1 ± 0.00a	0.3 ± 0.01a	2.1 ± 0.14a	3.2 ± 0.28a	12.8 ± 0.07a	22.3 ± 0.35b		Blanched/dried/coated
<0.1 ± 0.00d	<0.1 ± 0.00d	<0.1 ± 0.00d	2.1 ± 0.14c	9.7 ± 0.35d	33.7 ± 0.35b	$ZnCl_2$	Fresh
17.3 ± 0.42a	21.8 ± 0.35a	23.0 ± 0.28a	21.5 ± 0.70a	32.1 ± 0.14a	35.6 ± 0.35a		Blanched
11.4 ± 0.42c	16.6 ± 0.14c	16.8 ± 0.35c	18.2 ± 0.49b	23.6 ± 0.56c	26.9 ± 0.21c		Blanched/dried
12.9 ± 0.28b	18.0 ± 0.70b	19.1 ± 0.84b	22.7 ± 0.98a	24.8 ± 0.14b	27.2 ± 0.35c		Blanched/dried/coated
<0.1 ± 0.00a	<0.1 ± 0.00b	<0.1 ± 0.00c	2.1 ± 0.14d	9.7 ± 0.35b	33.7 ± 0.35a	$MgCl_2$	Fresh
<0.1 ± 0.00a	<0.1 ± 0.00b	5.3 ± 0.42a	8.8 ± 1.06a	13.6 ± 0.49a	28.5 ± 0.70b		Blanched
<0.1 ± 0.00a	<0.1 ± 0.00b	2.3 ± 0.42b	4.6 ± 0.63c	8.2 ± 0.56c	19.8 ± 0.35c		Blanched/dried
<0.1 ± 0.00a	2.3 ± 0.42a	4.9 ± 0.49a	7.1 ± 0.35b	8.8 ± 0.70c	19.8 ± 1.13c		Blanched/dried/coated
<0.1 ± 0.00b	<0.1 ± 0.00c	<0.1 ± 0.00b	2.1 ± 0.14c	9.7 ± 0.35c	33.7 ± 0.35c	$CuCl_2$	Fresh
21.1 ± 0.98a	27.4 ± 0.84b	29.1 ± 0.84a	29.8 ± 0.98a	33.2 ± 0.49a	40.6 ± 0.84a		Blanched
21.9 ± 0.91a	27.7 ± 0.28b	28.0 ± 0.70a	27.7 ± 0.84b	30.2 ± 0.84b	35.8 ± 0.35b		Blanched/dried
22.2 ± 0.77a	28.6 ± 0.42a	27.8 ± 1.20a	27.9 ± 1.27b	31.3 ± 0.70b	35.8 ± 0.84b		Blanched/dried/coated

For same storage duration, means of chlorophyll content (μg/g FW) of various blanching and same chemical treatment having different letters are significantly different ($P < 0.05$) according to the LSD.

Effects of Blanching With Copper, Zinc and Magnesium Chlorides and Drying on Chlorophyll Retention of Green Peppers in Natural Lemon Juice

Effect of blanching with copper, zinc and magnesium chlorides and drying on chlorophyll retention of green peppers dipped in acidic lemon juice are presented in Fig. 1. Results indicated green color retention and increased stability throughout the storage duration for pepper treated with copper chloride and coated with CMC. However, acidic treatment resulting from addition of natural lemon juice (pH 2.67) to the blanched chopped peppers (pH 5.50) resulted in significant ($P < 0.05$) degradation of chlorophyll to pheophytin. The effect of pH on the rate of chlorophyll retention and conversion to pheophytin is well documented (Robertson 1985; Mahraja and Sankat 1996; Guzman *et al.* 2002; Min *et al.* 2004; Koca *et al.* 2006; Toivonnen and Brummell 2008). Color changes were attributed to the loss of the magnesium ion from the chlorophyll molecule due to the low pH, which activated chlorophyll degradation to pheophytin and transformed green tissue to an olive-brown color (Toivonnen and Brummell 2008).

Figure 1 presents treatment of blanched pepper samples with added $CuCl_2$, $ZnCl_2$ and $MgCl_2$ (i.e., 100 ppm each). $CuCl_2$ showed better green color retention than samples blanched with $ZnCl_2$ or $MgCl_2$. Chlorophyll degradation was most pronounced in untreated samples and samples blanched with $MgCl_2$. Moreover, untreated samples or samples blanched with magnesium chloride or coated with CMC could not reduce the degradation of chlorophyll into pheophytin during the storage duration. These results are in agreement with Guzman *et al.* (2002) who reported that the addition of zinc and copper chlorides reduced the degradation of chlorophyll into pheophytin in avocado puree; also in agreement with Von Elbe and Schwartz (1986) who reported that processed beans with zinc chloride showed better color retention than sample without zinc chloride. The retention of chlorophyll color upon addition of zinc or copper was attributed to the formation of stable zinc and copper complexes of chlorophyll derivatives.

Chopped pepper samples previously blanched with zinc chloride or copper chloride or magnesium chlorides followed by drying at 40C for 6 h showed remarkable degradation in green color (Fig. 2). The severe color changes were a reflection of how tissue damage can enhance the degradation of chlorophyll by both blanching and drying (Tiikens *et al.* 2001; Koca *et al.* 2006).

Effect of CMC Coating of Green Pepper on Chlorophyll Retention

Edible coatings are expected to preserve green color by reducing the conversion of chlorophyll into pheophytin in acidic conditions. However, results of this study showed that pepper treatments (i.e., blanched with different chloride salts and dried before CMC coatings) did not differ

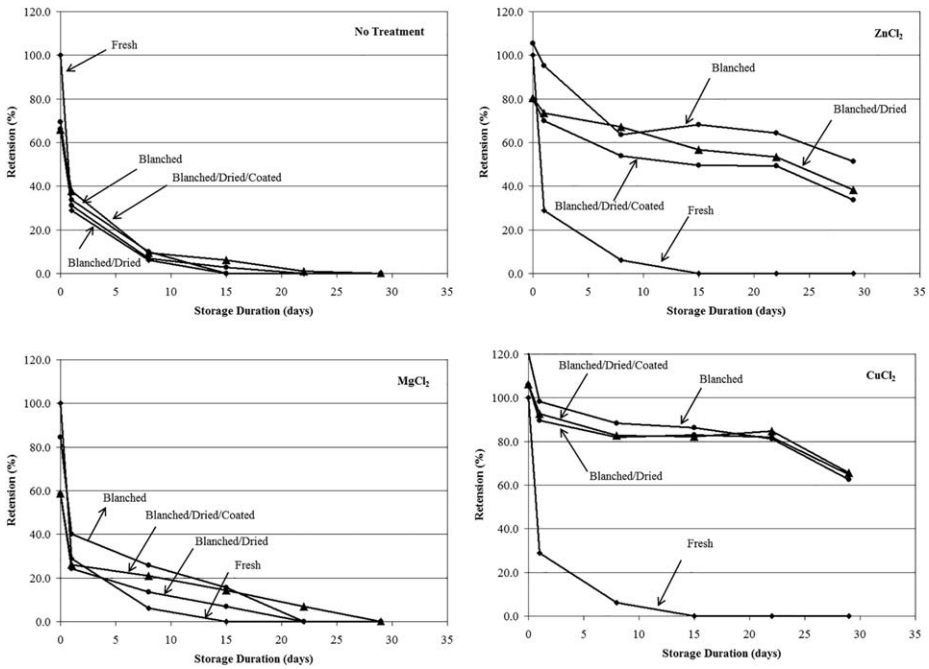

FIG. 1. CHLOROPHYLL RETENTION (%) OF BLANCHING TREATMENT (i.e., BLANCHED, BLANCHED/DRIED AND BLANCHED/DRIED/COATED) CHOPPED GREEN PEPPER WITH DIFFERENT CHLORIDE SALTS (i.e., $CuCl_2$, $ZnCl_2$ and $MgCl_2$ AT (100 ppm)) WHEN DIPPED IN NATURAL LEMON JUICE (i.e., pH 2.7) DURING STORAGE AT 4C

significantly ($P < 0.05$) in chlorophyll content from coated groups on any given storage duration (Table 2 and Fig. 1). The results confirm that coating application had no effect on chlorophyll degradation. The results agree with Lerdthanangkul and Krochta (1996) that edible coatings had no effect on color preservation of green peppers.

FIG. 2. GREEN PEPPER IN LEMON JUICE BASED DRESSING; (a) TREATED WITH $CuCl_2$ AND CMC; (b) TREATED WITH $ZnCl_2$ AND CMC; (c) NO TREATMENT (CONTROL); (d) TREATED WITH $MgCl_2$ AND CMC

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