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Research Article

CONTROL OF ENZYMATIC BROWNING IN POTATO WITH CALCIUM CHLORIDE AND ASCORBIC ACID COATINGS

Gülçin Yıldız

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Iğdır University, Faculty of Engineering, Food Engineering Department, Iğdır, Turkey

ORCID IDs of the authors:

G.Y. 0000-0001-6229-7338

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Correspondence:

Gülçin YILDIZ

E-mail: gulcn86@gmail.com

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ABSTRACT

This study deals with the inactivation of browning enzymes in potato by using various chemical agents. Potato purchased from a supermarket was treated by ascorbic acid and calcium chloride solutions alone and in combination. The effects of different chemical treatments on the surface appearance and selected quality indexes of potato slices during 2-weeks of storage at refrigeration conditions (0-4°C) were evaluated. Potato slices were immersed directly to the distilled water at room temperature for the Control samples to evaluate the browning process. The combined chemical treatments showed less browning in the potato slices compared to the applied solutions alone. Beside the inactivation of the enzyme, the combined treatment also showed a better success to prevent color degradation of potatoes. This study showed that the combining chemicals rather than using them alone is important in terms of processing of cut potato slices with a less browning and acceptable colors.

Keywords: Ascorbic acid, Color, Calcium chloride, Potato, Polyphenol oxidase enzyme

Introduction

Enzymatic browning is a process which occurs in several plants and is in charge of important loss of food product. Because of enzymatic browning almost half of tropical fruits are discarded because of the quality defects (Whitaker *et al.,* 1995). The browning is mainly catalyzed by the enzyme called as polyphenol oxidase (PPO) (Marshall *et al.,* 2000). When plant tissues are damaged by physical effects such as cutting, peeling or slicing, PPO is activated by releasing into the cytosol. In the presence of O_2 and PPO, monophenol is hydrooxylated to o-diphenol and diphenol can be oxidized to o-quinones, which then undergoes polymerization to produce dark brown polymers (Caodi, 2007).

Potatoes are one of the most famous product which is consumed in many countries, and are exposed to enzymatic browning and microbial spoilage. The browning reaction could be reduced or prevented by enzyme inactivation with some parameters such as reducing the pH (i.e. addition of lemon juice or other acids), reducing the amount of available oxygen (i.e. vacuum packing of cut food), and adding several preservative chemicals (such as sulfur dioxide) (Annese *et al.,* 1997; Sapers *et al.,* 1990). Among these chemical, citric, acetic, malic, and ascorbic acids are used frequently in order to prevent or reduce enzymatic browning (Whitaker *et al,* 1995)**.** It was concluded that the differences in browning reduction efficiency could be arise from the food products used in the research and/or the concentrations of the chemicals (Sapers *et al.,* 1987).

Ascorbic acid is an isomer which have been used commonly in the food industry due to its antioxidant properties. The role of this acid in browning is to reduce the *o*-quinones to diphenols (Golan-Goldhirsh *et al.,* 1984), and its effectiveness is determined by redox potential, rate of oxidation, and stability during processing. According to Tortoe *et al.* (2007), ascorbic acid (AA) was found to be the most effective treatment, whereas calcium chloride (CC) was most efficient in limiting the decay of fruits. By taking care of this statement, the combination of those two could be the best compounds for minimally processed foods because of their minimized browning. Therefore, the objective of this study was investigate the effects of different chemical treatments alone or in combination on the surface appearance and selected quality parameters of potato slices during 2-week of storage at refrigeration conditions (0- 4°C). This will provide a significant information to prevent enzymatic browning in potato with the most effective and efficient chemical treatment with the best concentration.

Materials and Methods

Potato Samples and Chemical Treatments

Russet potatoes were supplied from a supermarket were stored at 4°C. Potatoes were washed with running tap water during 2 min, and followed by cutting with a sharp stainless steel knife for the production of potato slices (50 mm thickness). And then, these potato slices were washed by running tap water during 1 min and instantly dipped into 3.0% (w/w) chemicals during 3 min (Figure 1-Flow chart). On the other hand, control potato samples were prepared by dipping them in distilled water with no chemical treatments. After dipping, slices were drained with a paper towel for 2 min and immediately packed in polyethylene bags, labeled and stored at 4°C for during 2 weeks for the shelf life study. All dipping solutions (ascorbic acid and calcium chloride) were prepared at 3 % (w/w) concentration using distilled water, in addition to the combination of AA and CC in various rates (i.e. 1% AA-2% CC; 1.5% AA-1.5% CC; and 2% AA-1% CC). Experiments were done in triplicate. Analysis were done at days 0, 7, and 14.

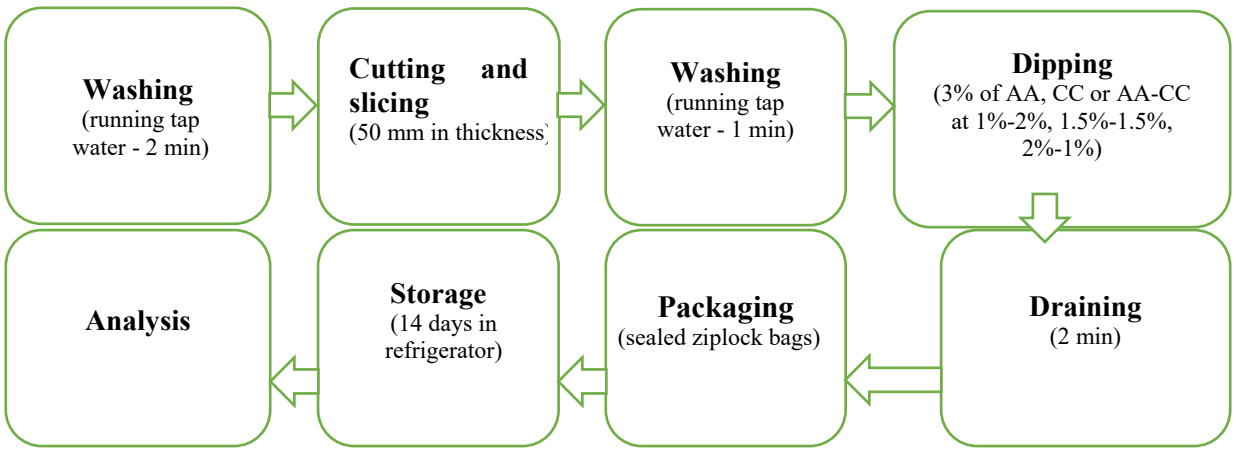


Figure 1. Processing diagram for fresh cut potato slices

Color Analysis

Colors of the potatoes wwbere analyzed by a Minolta Chroma Meter CR-300 from the surface of the samples (Yildiz *et al.,* 2016). White standard plate was used for the calibration of the machine. For each samples color values (L, a, and b) were read 5 times at different locations on the sample surface (one reading was from the center, one from the left-top side, one from the right-top side, one from the left-bottom, and one from right-bottom of the sample) at room temperature (RT). And finally, just one number for averaged L, a, and b values was reported. 3 replications were used for the color measurement, and the analysis was done at 0, seventh and fourteenth days.

PPO Activity Analysis

According to the method stated by Montgomery *et al.* (1975) was followed for the PPO activity measurement in the potato samples using a spectrophotometer. Thirty grams of tissue were homogenized using a blender in 0.2 g polyvinylpolypirrolidone and 70 mL of 0.5 M phosphate buffer for a minute. The clear supernatant after centrifugation (1200 g, 15 min, 20°C) was obtained as enzyme extracts.

2 mL of phosphate buffer (0.05 M) stored in a refrigerator at 4°C and 0.5 mL enzyme extract were added to a glass tube. Before the reading, fresh 0.5 mL catechol was put into the mix. The mixture was poured into cuvettes, and the increase in absorbance was read over 3 min in every 15 s at 420 nm. The PPO activity analysis was done at the 0, 7th and 14th days.

The absorbance of the assay solution was plotted against the reaction time to show the enzyme kinetics. Slope of the absorbance vs. time curve was calculated, and the result was expressed as a "U/g FW". The data was divided by 0.001 and PPO activity was described as "U/mL" in agreement with Cemeroglu (2007).

$$Activity\left(\frac{Unite}{mL}enzyme\ extract\right) = \frac{E}{0.001}\frac{1}{H_e}(H_{rk})(S_f)$$

where E: the slope of the absorbance vs. time curve,

0.001: a constant value,

H_e: the volume of the enzyme extract

$H_{rk:}$ the total volume of the reaction mixture (mL), and

S_f: a dilution factor.

Statistics

The differences were determined by using the General Linear Models procedure in SAS program. Significant differences among the means were identified with Fisher's least significant difference (LSD) test at alpha = 0.05.

Results and Discussion

Color is an important quality parameter which is very effective on consumer decision and inform us related to many other properties of food products, such as ripeness degree in fruit and vegetables (Dorante-Alvarez *et al.,* 2000). Fresh-cut potatoes are expected to have a bright surface color and be free of decay. The color readings of control and treated potato samples over storage at 4°C are shown in Tables 1. The L (lightness) values of all treated potatoes samples significantly higher than the control at all storage times (Table 1). The highest L values were observed for the samples treated with combined ascorbic acid (AA, 2%)-calcium chloride (CC, 1%) application. The L values decreased significantly with the storage time in all treated samples except AA (1.5 %) + CC (1.5 %) and AA (2 %) + CC (1 %) samples. The similar results were observed in the work of Yildiz (2018). It was observed that the L values of the banana slices treated with different chemical agents decreased with the storage time. The a (redness) values of the potatoes significantly increased during storage (Table 1). While the lowest a values were found at day 0 (for all the treatments), the highest a values were observed on day 14. Similarly, the b (yellowness) values of the potatoes significantly increased with the storage time in all samples (Table 1). An increase in redness (a) and yellowness (b) values, and a decrease in lightness (L) values indicated an increased enzymatic browning in all cut potatoes during 2 weeks of storage. Pérez-Gago *et al.* (2005) also stated the findings where color changes of apples were announced with a decrease in L (lightness) values, and increase in a (redness) and b (yellowness) values during the storage. It was also reported that sweet potatoes samples treated with different chemical preservatives showed lower L values with the storage (Sgroppo *et al.,* 2010). Moreover, browning measured by a (yellowness) value showed a significant increase mostly from seventh to fourteenth day of storage in apple slices treated with different chemicals including ascorbic acid and calcium chloride (Tortoe *et al.,* 2007).

The processing of products such as cutting caused removal of skins in the case of fruits and this leads to water loss and deterioration (Altekruse *et al.,* 1997; Agar *et al.,* 1999). It also causes increase in respiration rate and ethylene production (Saltveit, 1997). In addition, reaction between phenols and PPO causes browning discoloration (Martinez *et al.,*

1995; Heaton *et al.,* 1996). In this study, different chemical agents including ascorbic acid and calcium chloride alone and in combination were used to reduce the browning of potatoes slices. The PPO activity changes of potato slices during a two-week period at 4°C are listed in Table 2. The PPO activities of potatoes treated with AA (2%) + CC (1%) were in the range of 78 to 110 U/mL during 2 weeks of storage, and that for the Control 185 to 292 U/mL. The PPO activities of all treatments including control significantly increased during storage, namely in untreated (control) potatoes samples. It has been frequently reported that the most effective browning in fresh-cut products is achieved by using a combination of treatments (Ahvenainen, 2000). In this study, even though all chemical treatments decreased the PPO activity significantly compared to the control samples, the combination of chemicals rather than applying them alone was resulted with less browning. In addition, especially AA (2%) + CC (1%) treated potato samples showed lowest PPO activity compared to the other treatments, which is the indication of less browning. This is supported by the color values (Table 1). AA (2%) + CC (1%) treated samples showed less browning by having a higher L value, and lower a and b values compared to the other treated samples. In overall, the AA (2%) + CC (1%) treated potato samples yielded the best result showing less browning and optimum quality of fresh-cut potatoes. In the study of Tortoe *et al.* (2007) the moderate browning was observed in Golden Delicious apples treated with combined ascorbic acid-sodium chloride solution after 14 days while the other chemicals were resulted with severe browning. It was reported that as the concentration of ascorbic acid increased with different samples, the inhibitory effect on the initial rate of reaction improved (Dziezak, 1986). This statement is in a good agreement with the findings of this study. The less browning activity was observed when the concentration of ascorbic acid changed from 1% to 2% (Table 2). For example, the PPO activity was found as 98 U/mL for the first day samples treated with ascorbic acid at 1% concentration with calcium chloride. On the other hand, significantly less browning was achieved with ascorbic acid at 2% concentration (78 U/mL). Finally, Lee *et al.* (1999) found that the combining the ascorbic acid, erythorbic acid, citric acid and acetic acid is more effective compared to when applying them alone in precut lotus roots.

Table 1. Color changes in control and treated potato samples over storage at 4°C

Treatments	Storage (days)	L	a	b
Control	0	68.3 ± 0.8^{c}	-0.5 ± 0.5^{e}	12.3 ± 0.4^{c}
	7	63.3 ± 0.6^{d}	1.2 ± 0.3^{b}	15.5 ± 0.4^{b}
	14	54.2 ± 0.8^{e}	1.8 ± 0.2^{a}	19.2 ± 0.6^{a}
Ascorbic acid	0	70.4 ± 0.3^{b}	-0.3 ± 0.4^{e}	9.6 ± 0.3^{d}
	7	67.3 ± 0.5^{c}	0.1 ± 0.2^{d}	12.3 ± 0.2^{c}
	14	64.2 ± 0.6^{d}	1.3 ± 0.7^{b}	12.8 ± 0.4^{c}
Calcium chloride	0	73.8 ± 0.7^{a}	-0.4 ± 0.1^{e}	9.3 ± 0.3^{d}
	7	71.2 ± 0.2^{b}	-0.1 ± 0.3^{d}	12.5 ± 0.5^{c}
	14	67.7 ± 0.1^{c}	1.2 ± 0.5^{b}	12.8 ± 0.5^{c}
Ascorbic Acid (1%) -Calcium chloride (2%)	0	75.1 ± 0.1^{a}	-0.6 ± 0.2^{e}	9.9 ± 0.1^{d}
	7	74.2 ± 0.3^{a}	0.6 ± 0.7^{c}	12.4 ± 0.2^{c}
	14	71.9 ± 0.3^{b}	1.3 ± 0.2^{b}	12.9 ± 0.3^{c}
Ascorbic Acid (1.5%) -Calcium chloride (1.5%)	0	72.3 ± 0.2^{b}	-0.6 ± 0.3^{e}	9.1 ± 0.1^{d}
	7	71.7 ± 0.5^{b}	0.7 ± 0.3^{c}	12.3 ± 0.3^{c}
	14	71.2 ± 0.6^{b}	1.3 ± 0.2^{b}	12.5 ± 0.5^{c}
Ascorbic Acid (2%) -Calcium chloride (1%)	0	75.4 ± 0.3^{a}	-0.7 ± 0.3^{e}	9.8 ± 0.5^{d}
	7	74.8 ± 0.5^{a}	0.8 ± 0.1^{c}	10.1 ± 0.3^{d}
	14	74.3 ± 0.5^{a}	1.1 ± 0.7^{b}	10.6 ± 0.1^{d}

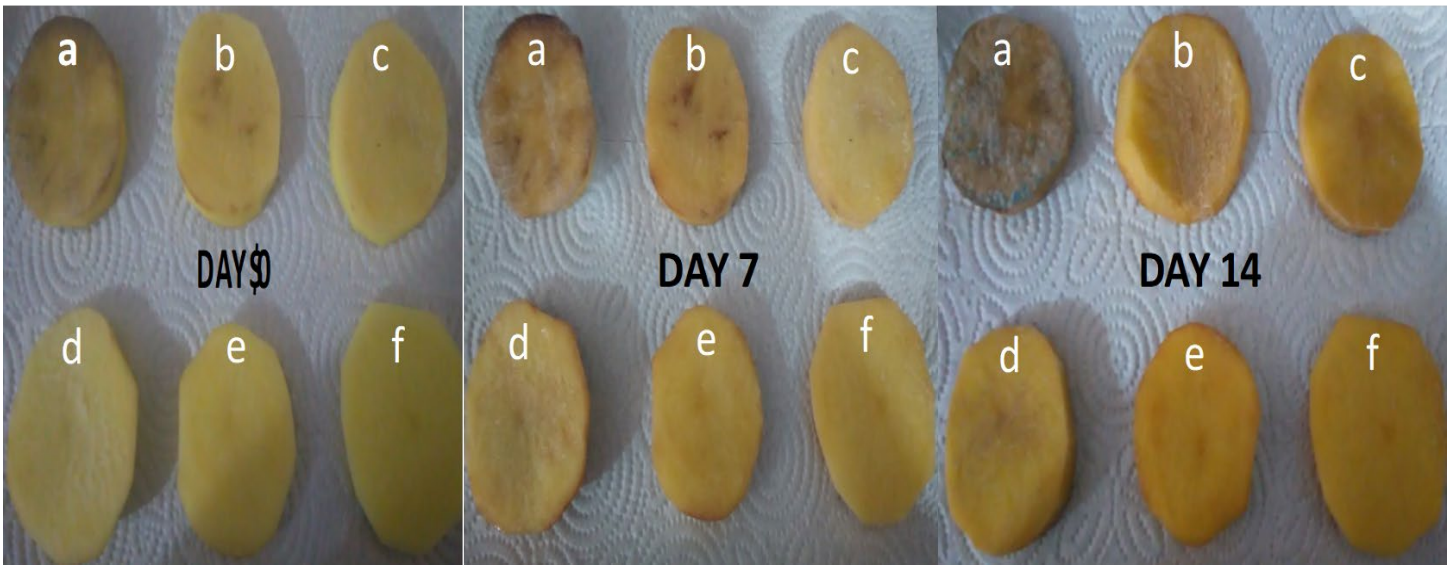
[a-e] Treatment means within storage times (columns) with the same letter in each sample are not significantly different ($p<0.05$).

The surface pictures of potatoes sliced with different chemical treatments are shown in Figure 2. All treated and control samples showed darker colors compared to the combined treatments (Figure 2–d, e, f), but especially for AA (2%) + CC (1%) treated potatoes (Fig.2-h). This is in agreement with the Hunter L (lightness) values where AA (2%) + CC (1%) treated potato slices had significantly higher L values compared to the control. All samples showed a color degradation starting from first day to last day. Specifically, 14th days-samples showed a severe color degradation, namely in control samples. Appearance plays an important role which influence the consumer's perception and final acceptance of a food product (Lund *et al.,* 2000). It is possible to predict that people will be favor of purchasing potatoes treated by AA (2%) + CC (1%) since they have less browning and preferable color.

Table 2. Polyphenol oxidase (PPO) activity changes of potato slices during a two-week period at 4°C

Treatments	Storage (days)	PPO activity (U/mL) at 4°C
Control	0	185 ± 1.1^{c}
	7	235 ± 1.7^{b}
	14	292 ± 1.5^{a}
Ascorbic acid	0	104 ± 1.8^{g}
	7	143 ± 2.1^{e}
	14	167 ± 1.5^{d}
Calcium chloride	0	102 ± 1.7^{g}
	7	141 ± 1.2^{e}
	14	165 ± 1.3^{d}
Ascorbic Acid (1%) - Calcium chloride (2%)	0	98 ± 2.5^{g}
	7	123 ± 3.7^{f}
	14	138 ± 3.8^{e}
Ascorbic Acid (1.5%) - Calcium chloride (1.5%)	0	95 ± 3.2^{g}
	7	120 ± 2.2^{f}
	14	136 ± 1.7^{e}
Ascorbic Acid (2%) - Calcium chloride (1%)	0	78 ± 1.7^{h}
	7	96 ± 1.3^{g}
	14	110 ± 2.1^{f}

$^{a-h}$ Treatment means within storage times (columns) with the same letter in each sample are not significantly different ($p<0.05$).



(a: no treatment; b: calcium chloride c: ascorbic acid d: ascorbic acid (1%)-calcium chloride (2%); e: ascorbic acid (1.5 %)-calcium chloride (1.5%); f: ascorbic acid (2%)-calcium chloride (1%)).

Figure 2. Appearance of potato slices with different treatments during a two-week period at 4°C

Conclusion

The quality of potato samples treated with different chemical agents was evaluated for a period of two weeks. A significant difference in color and PPO activity was observed among the different treatments. It was observed that the inactivation of polyphenol oxidase (PPO) enzyme was achieved for the combined ascorbic acid (2%)-calcium chloride (1%) application at most. Browning measured by yellowness (a) value showed a significant increase from first to last day of storage, especially in fresh potato samples. The ascorbic acid (2%)-calcium chloride (1%) treated potato samples showed significantly higher L (lightness) values than that control and other treated samples. In addition, this combination showed relatively less browning activity. Overall, potato samples treated with ascorbic acid-calcium chloride combination showed a better properties during two weeks of storage compared to the control and other treatments as evidenced by the measurements with instruments, and the appearance of the samples. This study is important in terms of applying chemical treatments with the best and effective concentration to inhibit browning activity in fresh-cut potato samples. This piece of work will be beneficial to produce processed raw potato products with a longer shelf life and higher consumer acceptability by preserving the color and inhibiting the enzymatic browning. A practical implication is that applying combined chemical agents with a best ratio can be used to get a high-quality browning-free products.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

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