Effect of antibrowning agents on banana slices and vacuum-fried slices

Muanmai Apintanapong*, Kuluma Cheachumluang, Punnarai Suansawan and Noppawan Thongprasert

Department of Food Science and Technology, School of Science, University of the Thai Chamber of Commerce, Bangkok 10400, Thailand. *e-mail: a_muanmai@yahoo.com

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Abstract

Enzymatic browning of banana slices is occurred practically in vacuum-fried banana slices during processing. Control of enzymatic browning can be achieved by various methods, and chemical treatment is one of them. However, most of the reported inhibitory activities were measured individually at different conditions in different commodities. Therefore, the aim of this study was to evaluate the most effective antibrowning agents for banana slices by using individual 12 compounds and combination treatments of the best selected three compounds with three widely used agents (ascorbic acid, citric acid and lactic acid). Mixed antibrowning agents were selected and applied in vacuum-fried banana slices. Sensory discrimination using bipolar R-index was done in comparison with untreated sample. Among the compounds tested, calcium chloride and cysteine showed the highest inhibitory activity on banana slice browning due to least change in lightness (P ≤ 0.05). Tartaric acid, formic acid, oxalic acid, ascorbic acid and citric acid belonged to a medium inhibitor group followed by the weak inhibitor group of lactic acid, acetic acid, succinic acid and sodium chloride. According to the change in lightness and blueness (DL* and Db*), calcium chloride, cysteine and tartaric acid were selected to use in combination with three widely used agents. Synergistic effect was found, it appeared that 0.5% tartaric acid and 1% ascorbic acid (TA), 0.5% calcium chloride and 1% ascorbic acid (CA) and 0.5% cysteine and 1% citric acid (CC) have strong potential for browning inhibition. In sensory discrimination, treated samples had lower colour intensity than control, whereas their appearances were better and they had significantly (α ≤ 0.05) more sour or tart taste. Banana slices treated with CA and CC had more overall preference than the others. However, after vacuum-frying, vacuum-fried banana slices treated with mixed agent solution that contained ascorbic acid caused formation of off-colours as shown in higher redness value (a*). Sour taste was found in samples treated with CA and CC and less sour taste than control was found in TA samples. In overall preference evaluation, CC sample was perceived to have significantly highest R-more values while the others were less than control (α = 0.05).

Key words: Banana, Musa spp. AAA group, enzymatic browning, antibrowning agents, inhibitory activity on browning, vacuum-fried banana slices, synergistic effect, bipolar R-index, degree of colour change, change in lightness.

Introduction

As bananas are one of the world’s most traded fruit in both fresh and processed forms, the market quality and consumer acceptability of fresh banana and processed banana are significantly influenced by the colour of the fruit. For vacuum-fried banana slice products which are popular in Thailand, bananas are prepared by peeling and slicing before vacuum-frying. In this preparation step, as a result in slicing and waiting for processing, there is accumulation of cell fluids, especially the phenolic compounds, on the cut surface and their exposition to oxygen, leading to browning 1. Phenolic compounds undergo oxidation to brown compounds that discoulour fruits, reducing their quality 2. Discolouration is known as enzymatic browning which results from the action of a group of enzymes called polyphenol oxidase (PPO). PPO has been reported to occur in all plants and exists in particularly high amounts in mushroom, banana, apple, pear, potato, avocado and peach 3. PPO catalyzes, in the presence of oxygen, the oxidation of mono- and di-phenols to o-quinones; these products are highly reactive and can either polymerize spontaneously to form high-molecular-weight compounds or brown pigments, or react with amino acids and proteins to enhance the brownish colour produced 3, 4. Inhibition of enzymatic browning can be achieved by a number of strategies that can be divided into three classes, depending on whether they affect the enzymes, substrates or reaction products, although in some cases, two or three targets can be affected at the same time. In addition, enzymatic inhibition can be reversible or irreversible; the latter case often achieved by physical treatment (heat), while chemicals may act in one or another way 4. The control of enzymatic browning has always been a challenge to the food industry. For using chemical treatments, several types of chemicals are used in the control of browning; some act directly as inhibitors of PPO, others by rendering the medium inadequate for the development of the browning reaction, still others act by reacting with the products of the PPO reaction before these can lead to the formation of dark pigments 5.

Therefore, the main purpose of this study was to find the most effective antibrowning agents for banana slices by using individual and combination treatments. The selected antibrowning agents were applied in vacuum-fried banana slices and sensory discrimination of this product was evaluated.
Material and Methods

Material: Banana fruits (Musa spp. AAA group) with outside diameter of 12 to 16 cm were purchased from Huay Kwang Local Market, Bangkok, Thailand. Samples were prepared for experiment when the fruit attained a peel colour index of 6 according to the CSIRO banana ripening guide. For chemical composition, banana flesh has 4.17±0.48% of starch, 5.62±0.07% of protein, 2.65±0.39% of fat, 0.90±0.11% of crude fibre, 1.73±0.42% of ash and 71.77±1.52% of moisture content. The pH and total soluble solid were 4.94±0.37 and 26.9±0.6°Brix. The titratable acidity (as % citric acid) was 0.43±0.01%. The mean values of banana peel colour coordinates were: L* = 47.57±1.75, a* = 2.24±0.38 and b* = 31.21±2.72. For surface colour measurement of banana slices, the mean values were: L* = 46.88±1.09, a* = 0.90±0.55 and b* = 16.93±1.52.

Antibrowning agent treatments: Individual treatment. Antibrowning agents used in the experiment can be classified into four groups that are carboxylic acids (acetic, formic, lactic, oxalic, succinic and tartaric acids), ascorbic acid, sulfur-containing amino acid (cysteine) and others (calcium chloride and sodium chloride). For determination of each antibrowning agent effect, concentration of each dipping solution was 1% (w/v). After evaluation, three antibrowning agents were selected for study on synergistic effect of mixed antibrowning agents.

Combination treatment. Selected agents were used in combination with ascorbic acid, citric acid and lactic acid that are widely used commercially. Each dipping solution for synergistic effect study was prepared from 0.5% (w/v) selected antibrowning agent and 1% (w/v) mixed agent.

Slicing and dipping: Banana fruits were washed with water, peeled and sliced into pieces with 5 mm thickness. Ten banana slices were treated in 500 ml of dipping solution prepared from antibrowning agents for 3 min and drained. The excess liquid was removed on cheese cloth. Samples were kept on the laboratory bench at room temperature for 3 h.

Vacuum-frying of banana slices: Banana slices were prepared, treated with dipping solution and drained before vacuum-frying. Laboratory vacuum-fryer (2 kg capacity, Kasetsart Kampangsaen University, Nakornprathom, Thailand) was used with adjusted temperature at 90±2°C and pressure at 70 mmHg vacuum for 1.5 h.

Colour measurement: The changes in surface colour of the treated banana slices and control (without treatment) were measured by a Hunter colourimeter (HunterLab DP-9000, Reston, Virginia, USA) at 10 min intervals for 3 h. The colour was recorded using the CIE-L*, a*, b* scale, where L* represents lightness, a* represents chromaticity on a green (-) to red (+) axis and b* represents chromaticity on a blue (-) to yellow (+) axis. Ten replications were done for measurement of L*, a* and b*. Numerical values of a* and b* were converted into Hue angle (Hue = tan⁻¹(b*/a*)) and Chroma (Chroma = (a²+b²)1/2). The degree of colour change was expressed by DL*, Da* and Db* values.

Sensory discrimination: The 20 trained panelists from Department of Food Science and Technology, School of Science, University of the Thai Chamber of Commerce, were recruited for sensory discrimination. The panelists were asked to rate the colour intensity, appearance, firmness, sour taste and overall preference of treated banana and vacuum-fried banana slices when compared to control without treatment. The panelists specified if they thought these attributes were more than (sure, unsure), the same (sure, unsure) or less than (sure, unsure) the labeled control sample. The R-index approach was used in order to determine if panelists would be able to distinguish differences in attributes of banana and vacuum-fried banana slices when compared to the control.

Data analysis: Data were analyzed using the analysis of variance (ANOVA) and significant differences were separated using Duncan’s multiple-range tests when the F values of ANOVA showed significance at P≤0.05 (SPSS v 10.0 SPSS Inc. Chicago, IL, USA).

For sensory discrimination, the bipolar R-index approach requires that the panelists rate the attributes as more (sure, unsure), same (sure, unsure) or less (sure, unsure) than the labelled control (Table 1). This value provides both magnitude and the direction of the difference that was perceived by the panelists. The bipolar R-index value is calculated as follows:

\[ R_{\text{More}} = \frac{([a_1(f_1+g_1+h_1)+b_1(g_1+h_1)+c_1h_1] + [1/2(a_1e_1+b_1f_1+c_1g_1+d_1h_1)])}{(a_1+b_1+c_1+d_1)} \]

\[ R_{\text{Less}} = \frac{([a_2(f_2+g_2+h_2)+b_2(g_2+h_2)+c_2h_2] + [1/2(a_2e_2+b_2f_2+c_2g_2+d_2h_2)])}{(a_2+b_2+c_2+d_2)} \]

To determine if the attribute in question is either more or less in intensity, a frequency table was prepared with the number of discriminators compared to the proportion of more or less values recorded. Of the two measures, either more or less, one direction was chosen based on which had the higher number of cumulative responses. Therefore, it was possible to determine whether a significant difference existed between the samples using a one-tailed test at α= 0.05.

Table 1. Bipolar R-index.

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Results and Discussion

Effect of each antibrowning agents on banana slices: In this research, effect of each antibrowning agents on banana slice browning was investigated using tristimulus colourimetry that can be applied directly to cut surfaces.

The data obtained was given in L* (lightness), a* (greenness or redness) and b* (blueness or yellowness). From previous research, the tristimulus colourimeter was an effective means of measuring banana pulp colour, as measurements were closely correlated with those obtained by visual matching with colour.
Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph. Duncan’s multiple range tests (P = 0.05) were performed. From DL* values (Fig. 1), based on Duncan’s multiple range tests (P = 0.05), the effectiveness of antibrowning agents may be divided into three groups. Cysteine and calcium chloride were the best inhibitor group that showed least change in lightness. Tartaric acid, formic acid, oxalic acid, ascorbic acid and citric acid belonged to a medium inhibitor group followed by the weak inhibitor group of lactic acid, acetic acid, succinic acid and sodium chloride. The latter group was the most ineffective, showing severe browning on banana slices during the 3 h period and their DL* values were not significantly different from the control (P > 0.05). Cysteine, tartaric acid and calcium chloride gave lowest Db*. This result shows that treated samples with these antibrowning agents had more yellowness while Db* values of treated samples with ascorbic acid, oxalic acid, citric acid and formic acid were not different from control (Fig. 3). Acetic acid, lactic acid, succinic acid and sodium chloride gave higher Db* values than control.

Several types of chemicals are used in the control of browning: some act directly as inhibitors of PPO, others by rendering the medium inadequate for the development of the browning reaction, still others act by reacting with the product of the PPO reaction before these can lead to browning; some act directly as inhibitors of PPO, others by rendering the medium inadequate for the development of the browning reaction, still others act by reacting with the product of the PPO reaction before these can lead to the formation of dark pigments. Antibrowning agents can be divided into many groups based on their mechanism on inhibition of enzymatic browning. In this research, there were four groups of the agents used for browning inhibition. Tartaric acid, formic acid, oxalic acid, citric acid, lactic acid, acetic acid and succinic acid are acidulants which are carboxylic acids. Ascorbic acid is one types of reducing agents. Cysteine can be classified as sulfur-containing amino acid and other antibrowning agents, as other halides, are calcium chloride and sodium chloride.

The optimum pH for PPO has been reported as ranging from acid to neutral, in most fruits and vegetables, optimum PPO activity is observed at pH 6.0-6.5, while little activity is detected below pH 4.5. Richardson et al. selected “SL” value (L value at initial-L value at given time) to express degree of browning. Therefore, to compare the effective of each antibrowning agent on browning of banana slices, the difference between L*, a* and b* values before and after browning were computed and expressed as DL*, Da* and Db* values (Figs 1, 2 and 3, respectively). DL*, Da* and Db* were used as an index for selection of effective antibrowning agents.

The samples with higher amount of DL*, Da* and Db* values represented more browning, more greenness (less redness) and more blueness (less yellowness), respectively. From DL* values (Fig. 1), based on Duncan’s multiple range tests (P = 0.05), the effectiveness of antibrowning agents may be divided into three groups. Cysteine, tartaric acid and calcium chloride gave lowest Db*. This result shows that treated samples with these antibrowning agents had more yellowness while Db* values of treated samples with ascorbic acid, oxalic acid, citric acid and formic acid were not different from control (Fig. 3). Acetic acid, lactic acid, succinic acid and sodium chloride gave higher Db* values than control.

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and Hyslop \(^\text{12}\) reported that irreversible inactivation of PPO can be achieved below pH 3.0. However, these acids may leave off-flavors and promote tissue softening and therefore must be used with care \(^\text{10}\). Son \(\text{et al.}\) \(\text{9}\) reported that some carboxylic acids that were oxalic and tartaric acid showed only a slight change in lightness of apple slices. Citric acid and lactic acid were medium inhibitors, whereas acetic acid, succinic acid and formic acid were weak inhibitors. This report gave some consistent results as in banana slices that some of acidulants, as tartaric acid and oxalic acid, were medium inhibitors, while lactic acid, acetic acid and succinic acid cannot inhibit browning.

Cysteine is thiol-containing compound that is also reducing agent that inhibits enzymatic browning. Cysteine was reported to have a good effectiveness at high concentration (5 mM) \(^\text{13}\). However, the threshold of acceptance should be carefully checked by means of sensory analysis. Cysteine directly reacts with the PPO and forms stable complexes with copper \(^\text{14}\). However, it is also known that cysteine reacts with quinine products and forms colourless conjugated compounds \(^\text{9}\). As reported by Son \(\text{et al.}\) \(\text{9}\), there was no change in the L value of apple slices dipped in a 1% solution of cysteine. The same result was obtained in this experiment that cysteine gave lowest DL* value. Guerrero-Beltrán \(\text{et al.}\) \(\text{15, 16}\) also reported that peach purees containing ascorbic acid and cysteine maintained their colours individually for 9 days.

Ascorbic acid is reducing agent that causes chemical reduction of colourless o-quinones resulting from the PPO reaction back to o-diphenols \(^\text{17}\). Reductants are irreversibly oxidized during the reaction, which means that the protection they confer is only temporary since they are consumed in the reaction. When all the reducing agent added is oxidized, the o-quinones from the PPO reaction may undergo further oxidation reactions (not involving PPO), and finally rapid polymerization leading to the formation of brown pigments. Due to the oxidative nature of enzymatic browning, reducing agents can also be applied in the prevention of discoloration \(^\text{1}\). Ascorbic acid is probably the most widely used antibrowning agent, and in addition to its reducing properties, it also slightly lowers pH. Ascorbic acid reduces the o-benzoquinones back to o-diphenols, and it also has a direct effect on PPO \(^\text{11}\). Son \(\text{et al.}\) \(\text{9}\) found that ascorbic acid was effective antibrowning agent, however, its reducing power was depleted within 20 min. In this research, ascorbic acid was found to be an intermediate inhibitor as seen in its DL* value that was lower than the control.

For other antibrowning agents, sodium chloride was known to inhibit PPO according to its ability in decreasing pH. However, chloride is a weak inhibitor and it required higher concentration to elevated inhibition level that may cause the change in product taste \(^\text{1}\). This seemed to be same with this result which shows that 1% sodium chloride cannot be used to inhibit browning in banana slices. The DL* value was not significantly different with the control. Calcium chloride has also been reported to reduce browning \(^\text{1}\). Rosen and Kader \(^\text{18}\) reported that 1% calcium chloride gave a lighter colour than water-treated control slices of pear. This was most likely due to the PPO inhibition by chloride ion \(^\text{1}\) and the same result was found in this experiment. Ponting \(\text{et al.}\) \(\text{19}\) also found that calcium chloride can cause almost as much inhibition on ‘Newton Pippin’ apples.

### Synergistic effect of mixed antibrowning agents

More effective preservation of fresh-cut products can frequently be achieved using a combination of treatments \(^\text{1}\). In many cases, the enhanced activity of the combined ingredients is additive, although synergism is often claimed for experimental blends of antibrowning agents \(^\text{2}\). Three antibrowning agents with smallest DL* and Db*; calcium chloride, cysteine and tartaric acid; were selected to use in studying on synergistic effect of mixed antibrowning agents. The synergistic effect on selected antibrowning agents was examined by mixing 0.5% of calcium chloride, cysteine and tartaric acid with 1% of citric acid, lactic acid and ascorbic acid. Samples treated with 0.5% tartaric acid and 1% ascorbic acid (TA), 0.5% calcium chloride and 1% ascorbic acid (CA) and 0.5% cysteine and 1% citric acid (CC) gave the best browning inhibition (Figs 4-6). The results show that lightness was found to be constant in treated samples with TA and CA, while samples treated with CC gave an increase in lightness. All of these treatments also gave more yellow banana slices.

These results show that a strong synergistic effect occurred by using combination treatments of antibrowning agents. Ponting \(\text{et al.}\) \(\text{19}\) also obtained that combination of 0.1% calcium chloride...
and 1% ascorbic acid resulted in the lowest loss of reflectance or browning readings in the case of ‘Newton Pippin’ and ‘Golden Delicious’ apples. Based on the research of Son et al. 9, apple browning can be effectively prevented by combining 1% ascorbic acid or citric acid with less than 0.02% oxalic acid as a dipping solution. Pizzocaro et al. 20 reported that a 0.2% solution of citric acid resulted in little or no inhibition of PPO in ‘Golden Delicious’ apple cubes, and that dipping the apple cubes in a solution of 1% ascorbic acid and 0.2% citric acid resulted in 90-100% inhibition of PPO. However, Janovitz-Klapp et al. 21 reported that combining 50-70 mM citric acid with ascorbic acid exhibited little effect on the PPO activity of ‘Red Delicious’ apples. Rocha and Morals 9 also found the contrary results in using combination of ascorbic acid, citric acid and calcium chloride in minimally processed ‘Jonagored’ apples. An ascorbic acid was the most efficient chemical treatment in reducing the PPO activity of ‘Jonagored’ apple cubes.

As seen, each compound has its own strength and weakness in terms of variable potency, availability, safety and cost. However, its appears that TA, CA and CC have strong potential for browning inhibition. Therefore, these three treatments were selected to apply in banana slices prepared for vacuum-frying.

**Effect of mixed antibrowning agents on vacuum-fried banana slices:** Banana slices were treated with TA, CA and CC before vacuum-frying. After vacuum-frying, the colour measurements were done and compared with the control without treatment and no delay time before frying. All treatments were not significantly different in L* and b* values (Fig. 7). However, the a* value of samples treated with TA and CA was increased significantly (P≤0.05), while samples treated with CC gave less redness and its yellowness was not different with the control (P>0.05). Ascorbic acid can cause formation of off-colours and/or off-flavor. Therefore, samples treated with solution containing ascorbic acid gave off-colour as shown in higher redness value (a*) 1. All of these treatments were used to determine sensory discrimination of banana and vacuum-fried banana slices using bipolar R-index.

**Figure 6.** DL*, Da* and Db* values of banana slices after 3 h treatment with 0.5% cysteine in combination with 1% citric acid (cys0.5ci1), lactic acid (cys0.5lac1) and ascorbic acid (cys0.5as1), respectively.

**Figure 7.** L*, a*, b* Chroma and Hue angle values of vacuum-fried banana slices after treatment with TA, CA and CC in comparison with no treatment (control), respectively.

**Sensory discrimination:** The R-index measures the degree of difference between the control and treated samples in terms of the probability of discriminating between the two in paired comparison. The bipolar R-index method generated 2 R-indices (R-more and R-less) for banana and vacuum-fried banana slices after 1 h treatment with TA, CA and CC in comparison with samples without treatment (control). Depending on the panelists’ responses, the value of R-more and R-less could have larger or smaller sample sizes. The sum of each bi-polar R-index (R-more plus R-less) is not equal to 100% (Table 2 and 3) because they were calculated directly from the responses and some values were shared in calculation.

**Banana slices:** For all treated samples, more panelists indicated that treated samples had lower colour intensity than control, whereas their appearances were better and they had significantly (α = 0.05) more sour or tart taste. As known, tartaric acid is found in grapes and wines and gives them a tart taste, whereas citric acid found in citrus fruits gives them a sour taste. All samples had no significant difference in firmness. However, samples treated with CA and CC had more overall preference than control and samples treated with TA.

**Vacuum-fried banana slices:** Banana slices treated with TA, CA and CC were fried in vacuum-frying at 90±2°C and 70 mmHg vacuum for 1.5 h. Vacuum-fried banana slices were determined for sensory discrimination using bipolar R-index. More panelists indicated that treated samples had significant difference in colour intensity than control. TA and CA samples gave more colour intensity while CC samples gave less (Table 3). The CC was estimated to be effective antibrowning agent that did not gave off-colour after frying. However, better appearance of all treated samples was found significantly. For firmness discrimination, CA samples gave more firmness while TA and CC samples less than the control. Firmness of vacuum-fried banana slices treated with CA may be caused from calcium treatments that help maintain...
Conclusions
From DL* values, the effectiveness of antibrowning agents may be divided into three groups. The best inhibitor group contained calcium chloride and cysteine. Tartaric acid, formic acid, oxalic acid, ascorbic acid and citric acid belonged to a medium inhibitor group. Lactic acid, acetic acid, succinic acid and sodium chloride can be classified in the weak inhibitor group. According to the lowest change of L* and b* (DL* and Db* values) that represented less browning and blueness, three compounds, tartaric acid, cysteine and calcium chloride, were selected to used in combination treatment. The results show that lightness was found to be constant in treated samples with 0.5% tartaric acid and 1% ascorbic acid (TA) and 0.5% calcium chloride and 1% ascorbic acid (CA) and 0.5% cysteine and 1% citric acid (CC) with banana slices without treatment (control).

**Table 2.** R-Index (% discrimination) comparing banana slices after 1 h treatment with 0.5% tartaric acid and 1% ascorbic acid (TA), 0.5% calcium chloride and 1% ascorbic acid (CA) and 0.5% cysteine and 1% citric acid (CC) with banana slices without treatment (control).

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**Table 3.** R-Index (% discrimination) comparing vacuum-fried banana slices after treatment with 0.5% tartaric acid and 1% ascorbic acid (TA), 0.5% calcium chloride and 1% ascorbic acid (CA) and 0.5% cysteine and 1% citric acid (CC) with vacuum-fried banana slices without treatment (control).

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**Sour taste**

- Critical R-index value using a one-tailed test at α = 0.05.
- Bold italized values indicate that more responses were selected from R-index more or R-index less by the panelists.

**Overall preference**

- Critical R-index value using a one-tailed test at α = 0.05.
- Bold italized values indicate that more responses were selected from R-index more or R-index less by the panelists.

**Results**

- Sour taste was found in samples treated with CA and CC and less sour taste than control was found in TA samples. In overall preference evaluation, CC samples were perceived to have significantly higher R-more values.
References


