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Effect of Different Spices on Enzymatic Browning of Banana Pseudostem Juice

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Authors' contributions

This work was carried out in collaboration between all authors. Author LS designed the study, performed the statistical analysis and wrote the final draft of the manuscript. Authors PG and LS wrote the protocol and managed the analyses of the study. Author RC managed the literature searches and the analyses of the study. Authors RC and PG wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aim and Background: Banana pseudostem is an agricultural waste with potential health benefits but highly susceptible to browning caused by polyphenol oxidase enzyme (PPO). The aim of present study is to investigate the effect of different spices on browning of pseudostem juice.
Methods: In present study, extracts of spices namely Clove, Cardamom, Cinnamon and Ginger were added to banana juice at different concentrations (4,8,12%) and their effect on browning and color was determined.
Results: Significant effect of spice type ($p < 0.05$) was observed on PPO while no significant effect of spice was observed on color. The ginger (4% and 8%) and clove (12%) exhibited higher inhibition of PPO activity while highest L value (lightness) was found for juice sample treated with cardamom (12%).
Conclusion: Spices exhibited promising effect in inhibiting browning of pseudostems' juice but color parameter did not act as an efficient marker for evaluating the same.

Keywords: Banana; pseudostem; browning; polyphenol oxidase; spices.

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1. INTRODUCTION

Banana (*Musa Paradisiaca* L.) which belongs to the family Musaceae is an herbaceous plant widely grown in tropical and subtropical countries of the world. It holds an important place in food (unripe) and fruit (ripe) basket owing to its rich nutritious value [1]. The waste generated after harvesting is used for preparation of biodegradable ropes, plates and other daily use materials. Fruit and other parts of the plant such as flower, pesudostem, rhizome and leaf sheath are also used as traditional medicine for human diseases [2,3].

Banana Pseudostem, owing to its richness in nutrients and dietary fiber, is used as vegetable, soup preparation, pickle, natural coagulant in the treatment of spent coolant wastewater, paperboard manufacturing, fiber products, pulping in paper industry, bio-fertilizer, etc. [4,5]. The juice of pseudostem also possesses anilithiatic activity i.e. they have capability to dissolve the pre-formed stones and prevent formation of further stones in urinary bladder [6]. The medicines and decoctions prepared from this trunk part prove very effective against alcohol intoxication, diahorrea, cystitis, gonorrhoea, cholera, otalgia, haemoptysis and tonic for blood and venal diseases [7,8]. Recently, this part of plant has been targeted for development of sports drink and functional beverages due its high mineral content such as 874 mg/L potassium, 88 mg/L sodium, 357.8 mg/L chloride, 130 mg/L calcium and 116 mg/L magnesium [9].

It has been observed that the pseudostem juice rapidly turns brownish on extraction due to presence of tannins (1.32 mg/L) which are highly susceptible to enzymatic browning reaction catalyzed by enzyme polyphenol oxidase [9]. This undesirable phenomenon not only reduces economic value but also reduces its acceptance and preference by consumers. Sundry anti-browning agents like sodium bisulfite, kojic acid, L-cysteine, 4-hexylresorcinol, and glutathione have been extensively studied for prevention of enzymatic browning [10]. However, their use is often discouraged due to their associated side-effects or toxicity [11]. The focus in present time thus shifted towards incorporation of natural additives and compounds to achieve desired food quality and consumer's safety. The different natural food extracts extensively used to prevent the browning includes honey dip for mango fruit [12] green tea extract for apple juice [13], spice extracts (cinnamon, basil, lemongrass, sage, clove)for apple slices and juice [14] etc. However, no study was found where spice extract was utilized to prevent the browning of banana pseudostem and its by-products. Hence, the present study was aimed to evaluate the effect of different spice extracts (Clove, Cardamom, Cinnamon and Ginger) and their concentrations on browning of pseudostem juice.

2. MATERIALS AND METHODS

2.1 Material

The pseudostems of banana tree were purchased from the Azadpur mandi, Delhi and stored at 4°C until used. Spices namely Cinnamon, Ginger, Cardamom and Clove were obtained from local market at Kundli (Sonipat, Haryana).

2.2 Chemicals and Reagents

Sodium Potassium Tartarate, Copper Sulphate, Triton X-100 and Catechol were purchased from Sisco Research Laboratory Pvt Ltd. (India). Sodium Carbonate was supplied by Thermo Fischer Scientific India Pvt Ltd. (India). Disodium hydrogen phosphate and Sodium dihydrogen phosphate were provided by RFCL Limited (India). Standard Bovine serum albumin was obtained from Qualikems Fine Chemicals Pvt Ltd. (India). Folin-Phenol reagent was purchased from HiMedia. Ethanol was obtained from HiTech Chemicals (India). All the chemicals and reagents used were of analytical grade.

2.3 Spice Addition to Pseudostem Juice

The juice was extracted from inner soft core material of banana pseudostem. Spices were ground to powder and 5g was mixed with ethanol-water (1:1; v/v, 50 ml). The solutions were kept in a water bath at 37°C for 1 hour. The material was then filtered using Whatman No. 1 filter paper to obtain the spice extract. The freshly collected juice was distributed (100 mL) in different beakers and 4%, 8% and 12% of spice extracts were added. The samples were stored at 25°C for 30 min.

2.4 Polyphenol Oxidase (PPO) Extraction

The enzyme was extracted by mixing 5 mL juice with 8.5 mL sodium phosphate buffer (pH 6.5,

0.1 M) and 1.5 mL Triton-X (5%). After mixing, the sample was centrifuged at 4800×g for 5 min at 25°C (Sigma, 3-18KS, Germany). The supernatant was collected and filtered, and stored at - 20°C until used. The protein content of enzyme solution was determined by the [15] using Bovine serum albumin (BSA) as standard.

2.4.1 Determination of PPO activity

PPO activity of control and treated samples were analyzed at 25°C by measuring the absorbance at 420 nm for 3 min at 30 second interval using UV/VIS Spectrophotometer (Elico, SL159, India) [16]. The reaction assay for the sample contained 0.6 mL of 0.1 M catechol solution, 0.3 mL of 0.2 M freshly prepared standard sodium phosphate buffer (pH 5.5) and 0.1 mL of enzyme extract. The reference cuvette contained 0.6 mL of catechol solution and 0.4 mL of 0.2 M standard sodium phosphate buffer (pH 5.5). The Molar extinction coefficient of catechol was taken as 3450 M^{-1} $cm^{-1}$and the enzyme activity was expressed in Units/mg protein/min.

2.5 Color

The color parameters such as color difference (ΔE, Eq. 1), yellowness index (YI, Eq. 2) and browning index (BI, Eq. 3) for banana pseudostem juice samples were calculated by measuring the intensity of lightness (L), redness (a), yellowness (b), using hand hold chroma-meter (KONICA MINOLTRA, CR-400, JAPAN) calibrated with a standard white board [17,18].

$$(\Delta E) = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$YI = \frac{142.86 \times b}{L} \quad (2)$$

$$BI = \frac{[100(x-0.31)]}{0.17} \quad (3)$$

Where, $x = \frac{(a+1.75L)}{(5.645L+a-3.012b)}$

2.6 Statistical Analysis

All the experiments were in triplicates. The means and standard deviation of the triplicates were calculated and are represented in figure and table. Since there were two variables explored at a time i.e. a) spice concentration and b) spice type, we analyzed the mean values by applying two-way ANOVA on them in order to determine the change in a particular property (such as color, PPO etc.) due to effect of these two variables. Analysis of data was thus carried out using two-way ANOVA (on mean values, not individually at each replicated value i.e. without replication) at a probability level of P = 0.05 on Microsoft excel.

3. RESULTS AND DISCUSSION

3.1 Effect of Spices on PPO Enzyme Activity

3.1.1 Spice type

Different spices exhibited variable and significant (P=0.05) effect on the browning reaction of banana pseudostem juice. Samples treated with 12% clove exhibited lowest enzyme activity (3.008×10^{-4} Units/mg protein/min) followed by 4% ginger (3.34703×10^{-4} Units/mg protein/min), 8% ginger (3.78374×10^{-4} Units/mg protein/min) and 8% clove (4.21003×10^{-4} Units/mg protein/min) while the PPO enzyme activity in control samples was found to be 5.33×10^{-4} Units/mg/min protein (Fig. 1). Thus the most effective spice additive for the reduction of browning in banana pseudostem juice was found to be clove (12%) which inhibited the PPO activity by 43.52% compared to the control sample. The results are in accordance with study by Essa et al. [19] who evaluated the anti-browning effect of different spices on apple juice and showed that at room temperature, the better inhibition of browning occurs on treatment with clove, ginger and cardamom in comparison to untreated, nutmeg and cinnamon treated samples. Inhibitory effect of clove oil was also reported by Chen et al. [20] on fresh cut lettuce.

3.1.2 Spice concentration

The concentration of spice had no significant effect on PPO activity (P=0.05). However, increase in concentration of cardamom, ginger and cinnamon from 4% to 12% resulted in an increase in PPO activity (in Units/mg protein/min) from 11.1 x 10^{-4} to 17.7 x 10^{-4}, 3.34 x 10^{-4} to 5.82 x 10^{-4} and 7.16 x 10^{-4} to 8.60 x 10^{-4} respectively. The increase observed in PPO activity may be attributed to the enzyme-substrate contact, release of bound PPO or generation of more PPO during cutting and juice extraction from pseudostems [20]. On the other hand, change in clove concentration from 4% to 12% reduced the activity from 9.09 x 10^{-4} to 3.01 x 10^{-4}. The PPO enzyme activity is directly

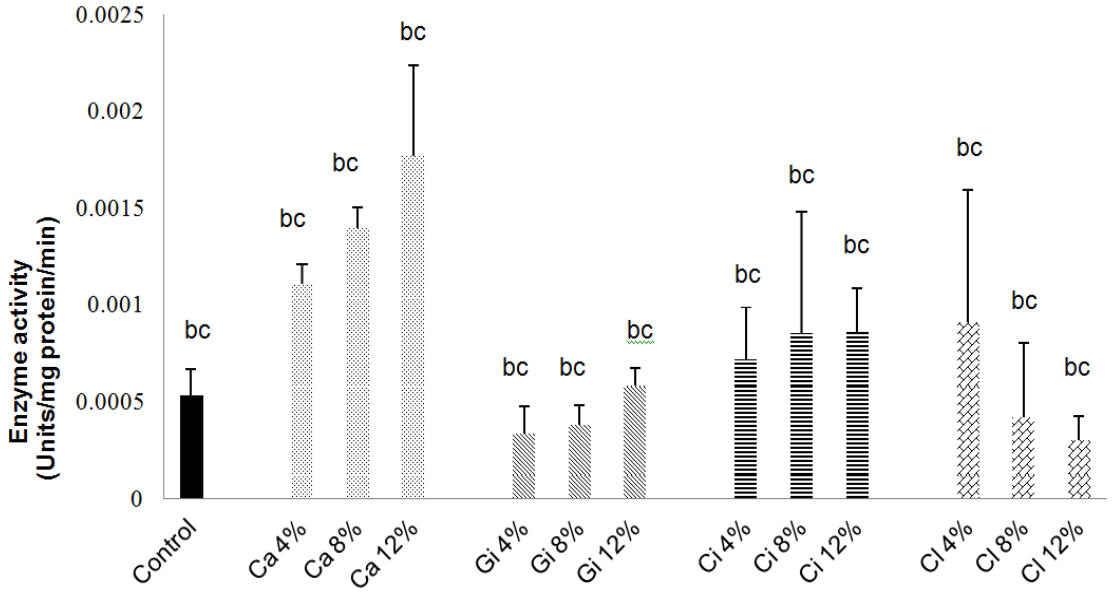

Fig. 1. Effect of spice concentration on PPO enzyme activity

The superscripts 'a' and 'b' denotes significant and non-significant effect of concentration respectively and superscripts 'c' and 'd' denotes significant and non-significant effect of spice type respectively on PPO enzyme activity at P=0.05. (Where Ca=Cardamon, Gi=Ginger, Ci=Cinnamon, Cl=Clove)

proportional to browning reaction taking place in different fruits and vegetables. This means that reduction in PPO activity found in present study upon clove treatment is due to the anti-browning effect of clove. This anti-browning effect of clove has also been studied by Eissa et al. [14] where 0.05% clove extract inhibited PPO activity upto 90% in apple juice.

3.2 The Effect of Spices on Color of Pseudostem Juice

The juice prepared from banana pseudostem and treated by variable spice concentrations were evaluated by CIE color system. Variation in 'L', 'a' and 'b' values was observed with the change in spice concentration and type (Table 1). It was observed that for all spices except clove, the lightness 'L' value was found to decrease initially at lower spice concentrations followed by an increase at higher concentrations. Samples treated with ginger (8% and 12%) and cardamom (8% and 12%) had more lightness compared to control samples. The highest L value (42.89) was observed at 12% concentration of cardamom and lowest L value (38.17) was observed at 8% concentration of clove. At constant spice concentrations (8% and 12%), the L value followed the order: clove<cinnamon<ginger< cardamom while at 4% concentrations the trend of L value changed to cinnamon<clove<ginger<cardamom. However, their effect was found non-significant at *P*=0.05.

Increase in concentration, reduced the 'a' (redness) value of ginger (from 0.91 to 0.66) and cardamom (from 0.66 to 0.34) treated samples, while for cinnamon, increasing trend was observed (from 0.91 to 2.00). Similarly, 'b' value (yellowness) also increased for cinnamon (from 0.38 to 2.34) and cardamom (from 0.42 to 1.08) treated samples. The highest value of 'a' and 'b' (2.00 and 2.84), and Browning Index (BI) was observed for 12% concentration of Cinnamon (59.142), which indicates more browning [20] whereas lowest BI was found in 12% ginger (56.415) treated samples. After ginger, the lowest BI was observed for 8% and 4% clove (56.435 and 56.537 respectively).

Apropos to color, browning is indicated by an increase in 'a' and 'b' value, decrease in 'L' value and increase in 'BI' [14,20]. Considering these parameters, 12% cardamom treatment was found most suitable as anti-browning agent, which had high 'L' value and low 'a' value. However, these results differ from the results of PPO activity in our study where best result was observed for clove treatment and

Table 1. Effect of spice concentration on color change of banana pseudostem juice

Spices	Concentration %	Change in color					
		L	A	b	Δ E	YI	BI
Control	0	40.62±0.09^{bd}	0.55±0.18^{bd}	1.24±0.38^{bd}	54.42±0.07^{bd}	-	-
Cl	4	39.20±1.17^{bd}	0.65±0.41^{bd}	1.01±0.23^{bd}	55.90±1.22^{bd}	1.409^{bd}	56.537^{bd}
	8	38.17±0.39^{bd}	0.62±0.12^{bd}	0.52±0.20^{bd}	56.98±0.40^{bd}	1.946^{bd}	56.435^{bd}
	12	39.46±0.49^{bd}	0.64±0.26^{bd}	1.28±0.73^{bd}	55.56±0.49^{bd}	4.646^{bd}	57.220^{bd}
Ci	4	38.94±0.09^{bd}	0.91±0.06^{bd}	0.38±0.23^{bd}	56.15±0.10^{bd}	1.394^{bd}	56.682^{bd}
	8	39.40±0.55^{bd}	0.92±0.12^{bd}	1.14±0.14^{bd}	55.66±0.55^{bd}	4.146^{bd}	57.269^{bd}
	12	40.54±0.87^{bd}	2.00±0.23^{bd}	2.84±0.62^{bd}	54.49±0.88^{bd}	9.997^{bd}	59.142^{bd}
Gi	4	39.58±0.72^{bd}	0.91±0.09^{bd}	0.87±0.35^{bd}	55.49±074^{bd}	3.128^{bd}	57.044^{bd}
	8	40.74±1.24^{bd}	0.83±0.07^{bd}	1.57±0.76^{bd}	54.30±1.27^{bd}	5.506^{bd}	57.501^{bd}
	12	40.85±0.58^{bd}	0.66±0.11^{bd}	1.17±0.55^{bd}	54.22±0.57^{bd}	4.092^{bd}	56.415^{bd}
Ca	4	39.64±0.33^{bd}	0.66±0.06^{bd}	0.42±0.32^{bd}	55.45±0.34^{bd}	1.526^{bd}	56.563^{bd}
	8	41.80±0.52^{bd}	0.39±0.21^{bd}	0.91±0.34^{bd}	53.26±0.52^{bd}	3.099^{bd}	56.736^{bd}
	12	42.89±0.52^{bd}	0.34±0.05^{bd}	1.08±0.31^{bd}	52.16±0.53^{bd}	3.597^{bd}	56.809^{bd}

The superscripts 'a' and 'b' denotes significant and non-significant effect of spice type and superscripts 'c' and 'd' denotes significant and non-significant effect of concentration, respectively on measured property at P=0.05. (Where Ca = Cardamon, Gi = Ginger, Ci = Cinnamon, Cl = Clove, ΔE = Total color difference, YI = Yellowness Index and BI = Browning Index)

poor result was observed for cardamom. This contradictory trend observed between color and PPO inhibition is due to the inherent properties of spices. The variation in juice color upon different spice treatment was brought by the inherent color of the individual spice. The color of clove and cinnamon extract was dark brown–reddish while ginger and cardamom extracts were light yellow in color. On the other hand, the variation in PPO activity owe to the antibrowning compounds present in the spices [20]. Chen et al. [20] got more browning in clove treated sample compared to eugenol treated sample. Eugenol is the phenyl propanoid component present in clove and is mainly responsible for the inhibition of PPO activity. Eissa et al. [14] also found in their study that the 'a' value of clove treated sample is higher than lemongrass treated samples which showed browning was more in clove treated sample.

Hence we conclude that compared to other spices used in this study, clove acted as a better antibrowning agent for psuedostem juice. Also, color solely may not be considered as an effective marker for determining the inhibition of browning process. The further research focusing on study of the effect of these spices on pseudostem juice for a specific storage time will give better understanding of browning and related aspects.

4. CONCLUSION

Enzymatic browning of banana pseudostem juice during processing and storage reduces its commercial value and consumer acceptance. Treatment of juice with different spices (natural additives) has variable effect on color and PPO activity. Significant effect of spice type occurred on PPO while non-significant effect was seen on color. Clove treated juice exhibited lowest PPO activity while cardamom was found best for color estimates. Conclusively, spices may prove beneficial as anti-browning agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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