Research Paper

Influence of Combine Treatments on Enzymatic Browning, Microbial Growth, and Sensory Attributes of TomEJC Mango Pulp

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ARTICLE INFO

Article history:
Received: 04 June 2023
Revised version received: 08 July 2023
Accepted: 28 July 2023

Citation:
Fernando, H.R.P., Rukshana, M.H.F., Kodithuwakkuarachchi, C.D., Jayawardane, N.W.I., Dharmathilake, T., Manage, U.L. (2023). Influence of combine treatments on enzymatic browning, microbial growth, and sensory attributes of TomEJC mango pulp, *Tropical Agriculturist,* 171 (3), 69-80.

Abstract

Enzymatic browning is one of the main issues with TomEJC mango pulp preservation. Generally, different physical and chemical treatments are used to overcome the browning occurred by enzymes. Direct heating creates a color change and caramel taste in TomEJC mango pulp due to its higher sugar concentration. The current research aimed to study the effects of steam blanching and chemical treatments on browning, microbial growth, and sensory attributes of TomEJC mango pulp at -12±3 °C. Steam blanching with 0.1% sodium metabisulphite (SMS) treated samples exhibited significant improvements in Browning Index (BI), polyphenol oxidase (PPO) activity, pulp color, microbial growth, and sensory properties. Steam blanching with citric acid (CA) treatments showed low BI, PPO activity, color changes, and microbial growth. However, satisfied sensory properties were obtained after the steam blanching with SMS treatments. Unblanched SMS treated samples also exhibited higher performances than both steam blanched and unblanched controls. Furthermore, ready-to-serve (RTS) drinks prepared by steam blanching with SMS treated samples showed significantly higher scores for sensory properties such as color, odor, taste, and overall acceptability. Thus, RTS drinks prepared by steam blanching with SMS treated samples received higher acceptability from the panelists. The lowest microbial growth was also recorded in the same sample. Therefore, it can be concluded that steam blanching with SMS (0.1%) was the best treatment for the preservation of TomEJC mango pulp at -12±3 °C with minimum browning. Moreover, steam blanching is a more effective method for heating TomEJC mango pulp than direct heating to overcome sugar caramelization.

Keywords: **Enzymatic browning, Mango, Physicochemical and sensory properties, Pulp preservation, Steam blanching**

Introduction

Mango (*Mangifera indica* L.) is known as the king of fruits due to its' wonderful taste, higher nutritional value, and additional health benefits (Ntsoane *et al.*, 2019). TomEJC is one of the remarkable mango varieties in Sri Lanka, with elevating demand in the world fruit market. TomEJC has great consumer demand and higher prices in the local and export markets due to its excellent taste, special textural quality, golden color, and large fruit size (Bandara *et al.*, 2020). However, post-harvest losses of TomEJC mango are higher due to its perishable juicy nature. Large scale growers directly approach the market, and they control the price. Therefore, growers do not sell second, and third grade fruits in low prices, and destroy large quantities. Some producers and collectors produce dehydrated mango chips and fresh mango juice in very small quantities, but this is not sufficient to reduce post-harvest losses.

Preservation of mango pulp is one of the acceptable solutions to minimize postharvest losses for TomEJC mango. Mango pulp is used for the production of mango juices, nectars, RTS drinks, jam, fruit cheese, and various other kinds of beverages. It can also be used in puddings, bakery fillings, fruit meals for children and flavors for industry, and also to make the most delicious ice-creams, yoghurt, and confectionary (APEDA, 2022). However, microbial spoilage and enzymatic browning after pulp extraction are major problems in TomEJC mango pulp production (Jayathunge, 2020). Therefore, extracted mango pulp is required to be subjected to an extensive preservation process for sterilization of microbes and inactivation of spoilage enzymes naturally present in mango before storing (Siddiq *et al.*, 2017). Natural enzymes such as polyphenol oxidase (PPO) and peroxidase (PO), cause browning of the mango pulp, and spoil the color and flavor. Pectin methyl esterase degrades the natural pectin of polysaccharides present in the cell walls of fruits, and changes the texture of fruit pulp (Kaushik *et al.*, 2017).

The enzymatic browning is predominantly initiated by the phenolic compounds' oxidation into quinones. Besides, quinones polymerization into melanoides (red brown and red pigments) by oxidative enzymes, mainly PPO leads to undesirable browning (Ranveer *et al.*, 2010). This problem is considerable importance to fruit pulp preservation as it affects the color, appearance and nutritional quality (Queiroz *et al.*, 2008). PPO exhibits optimum activity at pH between 5 and 7. However, PPO activity may be inhibited by heat or removal of one of its necessary components, namely, O_2, enzyme, Cu^{2+} or substrate (Lambrecht, 1995). Therefore, factors influencing PPO activity, including the type and amount of endogenous phenol compounds, presence of oxygen, and pH can be targeted to prevent enzymatic browning (Moon *et al.*, 2020). There are a large number of previous findings to inhibit PPO activity of mango pulp, but there are several limitations in TomEJC mango pulp with its' high sugar concentration, low pH, and bright golden pulp color. Therefore, this study aimed at studying TomEJC mango pulp preservation, aiming to prevent browning and to maintain its color, texture, and sensory properties in cold storage conditions.

Materials and Methods

Plant materials:

Mango fruits of variety TomEJC (*Mangifera indica* L.) were obtained from DSI Farms (Private) Limited, Girandurukotte, Sri Lanka. The maturity of mango was considered as 90–100 days after the fruit set. The fruits had a golden yellow peel color, light yellow cream pulp and soluble solids concentration of 9.5 ^{0}Brix, approximately. Only well-developed fruits were carefully selected based on uniformity in shape, color, size and free from pests and diseases. Mangoes were treated with artificial ethylene fumigation at room temperature for 24 hours using an airtight ripening chamber to obtain equal ripening before pulp extraction.

Mango sample preparation and pulp extraction:

Selected mango samples were cleaned with running tap water to remove impurities and immersed in 0.1% (v/v) sodium hypochlorite solution for 5 min to reduce the microbial load. The samples were air dried for 2 hours before pulp extraction. The peels of mango samples were removed, sliced and de-stoned by a stainless-steel sharp knife.

Treatments:

The mango lot was divided into two groups for pulp extraction. The first group of mango slices was steam blanched for 1 min and then pulped using a pulping machine (Comminutor MODEL D, USA). The second group was pulped directly without steam blanching. Both pulp groups contained a control (no treatment), 0.1% sodium metabisulphite (SMS) treated and 0.3% citric acid (CA) treated samples in triplicates. The SMS and CA levels of the treatments were selected by preliminary studies done at the laboratory. (Table 1)

Table 1. Anti-browning treatments

Symbols	Treatments
T_1	Steam blanched + Control
T_2	Steam blanched + 0.1% SMS
T_3	Steam blanched + 0.3% CA
T_4	Unblanched + Control
T_5	Unblanched + 0.1% SMS
T_6	Unblanched + 0.3% CA

SMS= sodium metabisulphite and CA= citric acid

Packaging and storage:

Treated mango pulp samples were transferred into sterilized polythene zip bags (7×12 inches) and stored under freezing condition (-12±3°C) for 35 days and samples were evaluated every 7 day intervals. Sensory evaluation and microbial growth were tested at end of the storage.

Evaluation of pulp samples:

Browning Index:

The Browning Index was determined by the method described by Arora *et al.* (2018). A five gram of each mango pulp sample was homogenized with 25 ml of 60% ethanol and kept overnight in the covered condition in the dark. After 24 hours, each sample was filtered to obtain a clear solution. Absorption of clear solution was taken at 440 nm using 60% ethanol as blank with an UV spectrophotometer (Genway 3600, Japan). Calculation was done by the following equation:

$$\text{Browning Index (\%)} = \frac{\beta - \beta^{\circ}}{\beta^{\circ}} \times 100$$

Where: - Browning of sample was evaluated as absorbance at 440 nm at 0 day

β° - Browning of sample was evaluated as absorbance at 440 nm at × day

Polyphenol oxidase activity:

The enzyme activity of polyphenoloxidase was measured according to the method reported by Ueda *et al*. (2000) with minor modifications. The mango pulp (10 g) was homogenized with 12 times (120 mL) volumes of cold (-20 °C) acetone. The collected residue was washed with excess volumes of cold acetone and a little ethyl ether, and dried. The dried residue was mixed with a 5 g of acetone powder, and it was suspended in 1 5 mL of 0.1 M phosphate buffer (pH 7.2). Then, stirred gently overnight at 4 °C. The aqueous extract was centrifuged at 15,000 rpm for 15 min, and the supernatant was used as the enzyme solution. The enzyme solution (1 mL) was incubated with 2.5 mL of 0.1 M phosphate buffer (pH 5.5), which contained 0.01 M 4-methylcatechol as the substrate for 10 min at 25 °C. The enzyme activity was determined by the increase in absorbance at 400 nm. One unit of the enzyme activity was defined as a 0.001 absorbance increase per min under the employed assay conditions. All operations were carried out at 4 °C as a triplicate.

Total phenolic content:

The total phenolic content was determined using a method previously described by Zhang *et al*. (2006). A volume of 12.5 mL of 80% methanol was added to 1.5 grams of mango pulp sample and centrifuged for 25 min at 11000 rpm. A volume of 0.1 mL was taken from a clear solution and mixed with 2.5 mL water + 0.15 mL Folin-Ciocalteu and incubated for 30 min at room temperature. Thereafter, 300 µL of Na2CO3 added and kept for 2 hour in dark condition. Absorbance of clear solution was measured at 765 nm using 150 mL Folin-Ciocalteu + 2.5 mL distilled water as a blank using an UV spectrophotometer (Genway 3600, Japan). Gallic acid was used as the standard to calculate the total phenolics equalents. All samples were measured in a triplicate.

Total soluble solids content:

Total soluble solids (TSS) content was measured using a digital refractometer (ATAGO N-1, Japan) as a Brix value.

pH value:

pH value was determined using a bench top pH meter at 25±3 °C.

Color change:

Color of the pulp samples were measured by a Royal Horticulture Society (RHS) color chart.

Microbial analysis:

Microbial analysis was done before sensory evaluation to ensure the safety of the pulp for consumption. A total plate count (TPC) was determined using solidified plate count agar plates in triplicates (SLS 516:1, 1991).

Sensory evaluation:

RTS drinks were prepared from each mango samples, and presented to a sensory evaluation using a hedonic scale in accordance with the method described by Larmond (1977). The panel members were selected on the basis of their knowledge and working experience of the mango and mango products (researchers, research staff and university students of the Food Research Unit). The sample was assessed for colour, odour, taste, mouth feel and overall acceptability by 25 members. The hedonic scale was prepared on a 9 point scale, where 9= Like extremely, 8=Like very much, 7=Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extreme.

Statistical design and data analysis:

Completely randomized design (CRD) was used as the experimental design for the study. All the tests described above were performed in triplicate unless otherwise specified. Statistical analysis, two-way ANOVA will be performed using the Minitab version 17 to compare the mean differences between groups. Turkey post hoc tests will be used to find the least significant difference between the compared groups ($P < 0.05$). ANOVA was used to analyze the differences between samples based on specific treatments, while Spider Web was used to describe the sensory properties of the different treatments.

Results and Discussions

The browning rate of preserved TomEJC mango pulp samples was varied on different treatments at -12±3 °C during 35 days storage. The BI of all pulp samples increased with the progress of the storage. Maximum BI values were reported in both controls compared to other treatments. Unblanched control sample exhibited the highest BI compared to the blanched control (Figure 1). The treatment, steam blanched + SMS (0.1%) showed the maximum reduction of BI after unblanched + SMS treatment. Both blanched and unblanched treatments with SMS were performed well compared to all other treatments. As well, steam blanched + CA (0.3%) treatment also showed a lower BI value compared to samples treated with SMS. However, steam blanched + SMS treatment exhibited a significantly lower ($P < 0.05$) BI when compared to all treatments. Previous studies reported the effect of chemical preservatives including SMS on browning, microbial growth, and overall acceptability of mango pulp for long term storage. Shakhale *et al*. (2012) found that 0.2% SMS controlled the microbial growth and PPO activity while maintaining the sensory properties of Kesar mango pulp kept in glass containers for 90 days. Besides, the steam blanching used in the current study might also be responsible for reduction of BI of TomEJC mango pulp. In agreement, Robinson *et al*. (1993) reported that the inhibition of 50% PPO activity of Kensington mango pulp by the heat treatment (80 °C for 15 min).

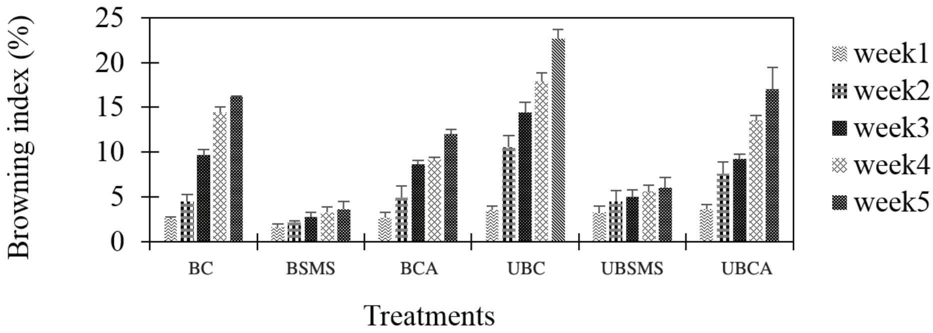

Figure 1. Browning Index of control and treated TomEJC mango pulp samples at -12±3 °C for 35 days.

Vertical bars indicate standard error of the mean for three replicates. BC=blanched control, BSMS=blanched + sodium metabisalphite, BCA= blanched + citric acid, UBC= unblanched control UBSMS=unblanched + sodium metabisalphite, and UBCA= unblanched + citric acid.

PPO activity of the control and treated samples were measured after 35 days. The highest PPO activity was observed in unblanched control (77±2.3) followed by steam blanched control (52±2.1) samples. Steam blanched + SMS treated sample exhibited the lowest PPO activity (Table 2), and it was significantly lower compared to all the other treated samples and controls. Thereafter, steam blanched + CA and unblanched SMS treatments showed second and third lowest PPO activity values respectively with significance to both controls. Shakhale *et al*. (2012) reported that 0.2% SMS treatment reduce the PPO activity in Kesar mango pulp. Furthermore, Robinson *et al.* (1993) and Guerrero-Beltrén & Barbosa-Cénovas (2006) reported that different thermal treatments reduce the PPO activity of Kensington and Alphonso mango pulp in different levels.

In the current study, SMS (0.1%) and steam blanching were applied together as a combined treatment. The combine treatment was effective on the PPO activity, significantly. The results of PPO activity were coincided well with the results of Browning Index. Therefore, it can be confirmed that the SMS, CA, and steam blanching reduced the enzymatic browning in various levels through inactivation of the PPO activity.

Table 2. PPO activity of control and treated TomEJC mango pulp samples at -12±3 °C after 35 days.

Treatments	PPO activity (Units/ gram)
Steam blanched+ control	52±2.1[b]
Steam blanched+ SMS (0.1%)	07±1.4[d]
Steam blanched+ CA (0.3%)	21±1.6[c]
Unblanched+ control	77±2.3[a]
Unblanched+ SMS (0.1%)	22±4.2[c]
Unblanched+ CA (0.3%)	26±3.1[c]

PPO activity values presented in mean ± SD of three replicates. Means followed by the same letter are not significantly different at P ≤ 0.05.

According to the results (Figure 2) there was no significant difference ($P > 0.05$) in the total phenolic content in TomEJC mango pulp samples stored at -12±3 °C for 35 days. The total phenolic content of control and treated samples did not show a strong correlation with Browning Index or PPO activity. However, the phenolics content of most of the samples reduced in progressing with the storage. However, the lowest total phenolics contents were observed in blanched and unblanched controls, as same as the samples recorded the highest browning. Robles *et al.*, (2013) reported that decline in phenolic compounds in fresh cut fruits and fruit pulp is of oxidative nature, resulting in tissue browning. The results of the current study also agree with previous findings. Besides, steam blanched + SMS sample exhibited slightly highest total phenolic content than unblanched SMS sample. This may be due to steam blanching (heat treatment) is an effective pre-treatment to control enzymatic browning (Liu *et al.*, 2019).

Color change of the pulp samples were measured by a RHS color chart. The initial color of all pulp samples was bright yellowish orange color. SMS treated samples in both blanched and unblanched groups retained the bright yellow color than other samples while CA treated samples turned yellowish brown in the progressing of the storage. Multi factors are responsible for the colour change during the processing and storage of fruit pulps such as, non-enzymatic and enzymatic browning, pH, oxygen, acidity, packaging material and the duration and temperature of storage (Ahmed, 2002). The main browning methods related to fruit pulps are enzymatic and non-enzymatic browning reactions. Enzymatic browning of mangoes is caused by oxidation of phenolic compounds by polyphenol- oxidases and peroxidases. This reaction triggers a succession of other reactions, leading to the formation of brown pigments known as melanin.

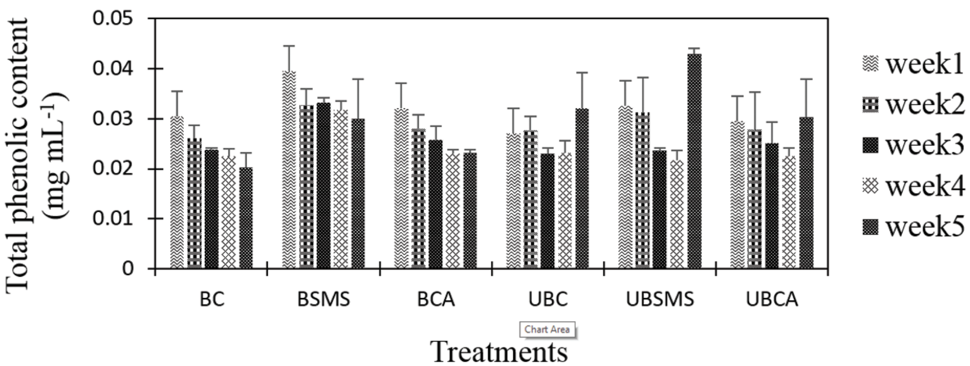

Figure 2. Total phenolic content of control and treated TomEJC mango pulp samples at 12±3 °C for 35 days.

Vertical bars indicate standard error of the mean for three replicates. BC=blanched control, BSMS=blanched + sodium metabisalphite, BCA= blanched + citric acid, UBC= unblanched control UBSMS=unblanched + sodium metabisalphite, and UBCA= unblanched + citric acid.

The Maillard reaction, one of the main non-enzymatic browning reactions, is a set of processes between reducing sugars and amino acids, leading to the formation of brown colors melanoides (Diop, 2021). However, previous findings explained that SMS and CA both treatments slow down the enzymatic browning through the inhibition of PPO and PO activity of fruit pulp. Therefore, the result of current study indicated a lower color change in SMS and CA treated samples than the others.

The total plate count (TPC) test was conducted after 35 days of storage before use for sensory evaluation (Table 3). Mango pulp was not exceeded the acceptable level (104 CFU/mL) of microbial count except unblanched control. The acceptable microbial count range of the fruit juice is from 1.4×10^4 CFU/mL to 2.6×10^5 CFU/mL for human consumption (Oranusi, 2012). All treated mango pulp samples exhibited an acceptable microbial growth range for human consumption.

Mango pulp subjected to treatments (blanched + sodium metabisalphite, blanched + citric acid, unblanched + sodium metabisalphite, and unblanched + citric acid) was evaluated for sensorial properties by a semi- trained panel. The effect of blanching, chemical treatment, and storage of pulp on sensory attributes such as color, odor, taste, mouth feel, and overall acceptability were determined. The average score for the color of RTS drinks prepared by different treated TomEJC mango pulp samples was observed in the range of 5.32 to 7.48 (Table 4). The highest score was obtained by steam blanched + SMS added samples, while the lowest score was obtained by unblanched CA treated samples. The average score for odor of RTS drinks prepared by different treated TomEJC mango pulp samples ranged from 6.04 - 7.2 a 9 point hedonic scale. Steam blanched + SMS added samples had the highest score of the others (Table 4). The average score for the taste of RTS drink samples ranged from 4.00 to 7.26. Unblanched CA treated samples received the lowest taste score, while steam blanched + SMS added samples received the highest. The highest average score for both mouthfeel and overall acceptability (6.96 and 7.96 respectively) was exhibited by the RTS drink prepared by a TomEJC mango pulp sample of steam blanched + SMS.

Table 3. Microbial analysis of control and treated mango pulp samples after 35 days storage

Sample	Total plate count (CFU/mL)
Blanched + control	$1.59 \times 10^4 \pm 0.03$
Blanched + SMS	$0.33 \times 10^4 \pm 0.11$
Blanched + CA	$0.42 \times 10^4 \pm 0.07$
Unblanched + control	$3.12 \times 10^4 \pm 0.70$
Unblanched + SMS	$0.56 \times 10^4 \pm 0.91$
Unblanched + CA	$0.77 \times 10^4 \pm 0.16$

Microbial count values presented in mean ± SD of three replicates. CFU= colony forming units, SMS=sodium metabisalphite, and CA= citric acid.

The RTS drink samples prepared by the blanched and CA added samples exhibited the second highest scores (6.84 and 6.76) after the blanched + SMS samples. However, the unblanched samples, both added SMS and CA represented the poor sensory attributes compared to the steam blanched samples. Thus, it can be suggested that steam blanching is an important hydrothermal treatment to preserve quality characteristics of TJC mango pulp (Figure 3). Furthermore, steam blanching was more effective with the addition of SMS (0.1%) to maintain the sensory properties of TomEJC mango pulp. The combined treatment, steam blanching with SMS well maintained the pH level, total soluble solids, and titratable acidity (data not presented) of TomEJC mango pulp. Therefore, this may lead to protect sensory properties at acceptable levels.

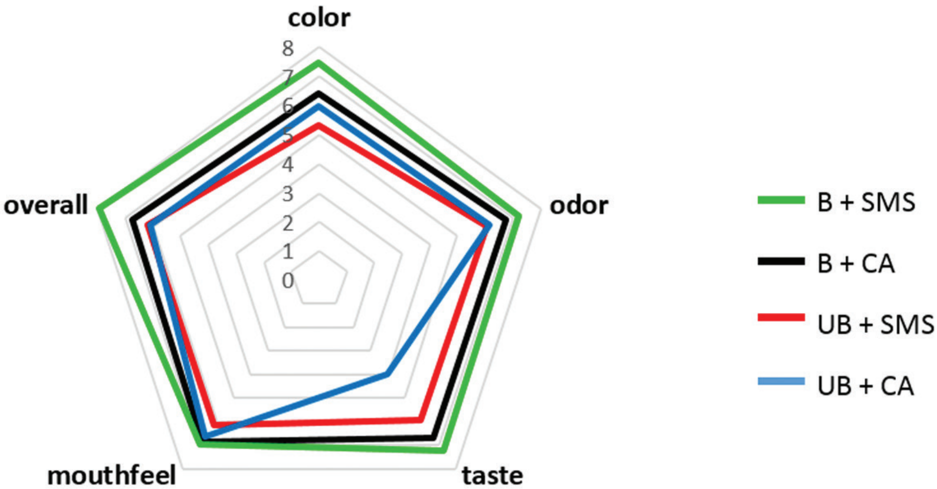

Figure 3. Sensory evaluation of ready-to-serve drinks produced by treated TomEJC mango pulp samples in terms of different sensory attributes

Table 4. The average rating of samples by 25 panelists during sensory evaluation

Sample	Color	Odor	Taste	Mouth feel	Overall acceptation
B + SMS	7.48	7.2	7.26	6.96	7.96
B + CA	6.44	6.76	6.68	6.84	6.76
UB + SMS	5.32	6.04	5.96	6.16	6.2
UB + CA	6	6.16	4	6.64	6.12

The values of sensory properties presented as the mean of three replicates. B= blanched, UB= unblanched, SMS=sodium metabisalphite, and CA= citric acid.

Besides the preliminary studies (FRU, 2022) done by our research group, we observed that the direct heating destroyed the color, texture, and other sensory properties of TomEJC mango pulp. Especially, direct heating produced a caramel color and taste due to the nature (high sugar and low fiber) of TomEJC mango pulp. Therefore, the authors suggest that wet heating (steam blanching) is the best hydrothermal treatment to preserve TomEJC mango pulp. Besides, combined treatments such as steam blanching and chemical addition (SMS and CA) at permitted levels (0.1 and 0.3%, respectively) were more effective for TomEJC mango pulp preservation.

Conclusion

The chemical treatments (sodium metabisalphite 0.1% and citric acid 0.3%) with or without steam blanching showed the results in varied levels of preservation of TomEJC mango pulp. The significant results in browning index, polyphenol oxidase activity, color, microbial growth, and sensory properties were exhibited in steam blanching with SMS treated samples. Steam blanching with CA treated samples showed low browning index, polyphenol oxidase activity, color change, microbial growth and good sensory properties after the steam blanching with SMS treatments. Unbalanced SMS treated samples also exhibited a higher performance than in both controls Thus, RTS drink prepared by steam blanching with SMS treated pulp samples received higher acceptability from panelists. In addition, the lowest microbial growth was also recorded in the same sample. Therefore, authors conclude that the treatment, steam blanching with SMS was the best for preservation of TomEJC mango pulp at -12±3 °C with minimum color change (browning). Moreover, steam blanching is an effective method for heating TomEJC mango pulp than direct heating to overcome sugar caramelization.

Acknowledgements

The authors would like to express special gratitude for all laboratory and staff members of the Food Research Unit, and the Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka.

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