

PHYSICO-CHEMICAL, MICROBIOLOGICAL AND SENSORY STABILITY OF CHEMICALLY PRESERVED MANGO PULP

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Abstract

The effect of sodium benzoate (SB) and potassium metabisulphite (PMS) at various concentrations on chemical, microbiological and sensory quality of mango pulp during storage was assessed. Inhibitory activity of the chemical preservatives and their effect on chemical and sensory attributes was tested periodically by simulating the industrial mango pulp storage in the lab (30-42°C in the dark), for a period of 90 days. Protein, fats, decreased while ash content and total soluble solid (TSS) increased during the storage period. A slight progressive decline in pH was observed with a proportional increase ($p < 0.05$) in the acidity of the stored pulp samples. Significant inhibition of the total bacterial count (TBC) was observed on applying the specified concentrations, however PMS was shown to be more inhibitory. Storage time significantly ($p < 0.05$) increased the CFU/g of the pulp samples as the maximum growth was observed after 90 days of storage. Sensory characteristics of the juice prepared from treated mango pulp samples were affected negatively on addition of preservatives however, the samples were accepted by the judges even after three months of storage.

Introduction

Mango (*Mangifera indica* L.), as an emerging tropical export crop is produced in about 90 countries in the world with a production of over 25.1 million tones. Asia is the main producer with 76.9% of the total world production, followed by America with 13.38%, Africa with 9% and less than 1% each for Europe and Oceania. Pakistan is the 5th largest mango producer with production of 938000 tones per year contribution with a share of 7.6% in the world market (Sauco, 2002). Mango is a short seasoned fruit and being highly perishable does not withstand even in cold storage. Therefore, most of the fruit processing industry in Pakistan preserves mango pulp for the manufacture of mango products around the year. Mango pulp is not generally consumed directly rather used as fillings for pastries, jams, sauces, fruit juices and drinks (Hussain *et al.*, 2003).

Being the cheapest among several methods of preservation, chemical preservation of mango pulp is the most common and widely used in Pakistan. The chemical preservatives are used to prevent the food spoilage due to microbial attack and thus are effectively used in combinations for better preservation. No single preservative is completely effective against all microorganisms (Chipley, 1983). Sodium benzoate (SB) and potassium metabisulphite (PMS) are commonly used as preservatives for long term storage of fruit pulp because of their better antimicrobial activity (Sofos & Busta, 1981; Manganelli & Casolari, 1983; LuÈcK, 1990). The effect of SB on the growth and survival of some yeast strains, food poisoning and spoilage organisms has been widely reported (Warth, 1985; Sofos *et al.*, 1986).

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Concentration of SB has a direct impact on the inhibition of microorganism (Ogiehor & Ikenebomehclearly, 2004) as higher concentration of SB manifested the greater antimicrobial effect, exercised on various species of *Aspergillus* (Gould, 1989; Ogunrinola *et al.*, 1996). Among the chemical preservative tested in single doses of 1000-ppm PMS and 500-ppm SB showed no sign of micro flora up to 90 days. Besides having an inhibitory effect on microflora in the stored pulp, addition of these chemicals may adversely influence its physico-chemical and sensory profile. It has been reported earlier that preservatives have influence on physico-chemical characteristics of mango pulp as storage caused an increase in acidity, brix, reducing sugars and decrease in sucrose (Hussain *et al.*, 2003).

The maximum levels for the use of these chemicals in fruit preparations, including pulp, purees and fruit as described in the Codex Standards adopted in 2001 and 2006 respectively are 1000 mg/kg SB as benzoic acid and 500 mg/kg PMS as residual SO₂ (Codex Standard, 1995). Indiscriminate and non judicious use of these preservatives is a great threat to the health and well being of the consumers and has been the cause of the appearance of resistant microorganisms over and over, leading to the occurrence of emerging food borne diseases (Gibbons, 1992; Kaur & Arora, 1999; Akinpelu, 2001). This situation calls for a complete investigation into setting optimum dose levels of these preservatives in mango pulp and their individual and synergistic effect on its various quality attributes under the local conditions.

The present work was carried out in order to investigate the inhibitory effect of SB and PMS when used at various concentrations (0.00, 0.05, and 0.1%) in controlling the growth and survival of microflora of mango pulp stored under ambient conditions. The study further explored the effect of incorporating these preservatives in the mango pulp on physico chemical characteristics and its sensory attributes.

Materials and Methods

Procurement of the materials: A local mango variety (*chaunsa*) was selected and ripened fruit, uniform in size, colour and weight was obtained from the local market of Multan. The fruit was thoroughly washed to remove dirt, dust, pesticide residues and microflora on the surface of the fruit. Sodium benzoate (NaC₆H₅CO₂) (Merck 6290) and Potassium metabisulphite (K₂S₂O₅) (Merck 106357) were purchased from the local supplier.

Pulp extraction, packaging and storage: Mangoes were passed through a mango pulper to separate pulp from the stones and skin and the pulp obtained was mixed with chemical preservatives as per treatment combination presented in Table 1. The treated pulp samples (2kg each) were transferred to UV-sterilized zip lock polyethylene bags (7x12 inches) and stored under ambient conditions (30-40°C) in the dark for a period of 90 days and analyses were carried out after every 30 days (Hussain *et al.*, 2003; Hashmi *et al.*, 2007).

Physico-chemical analyses of the pulp: The following methods (Anon., 2000) were used to determine protein, fat and ash content in stored mango pulp samples: micro Kjeldahl for protein (N x 5.7) (method 960.52) (Glass Model Pyrex-1); incineration at 550°C for ash (method 923.03)(PCSIR-Lhr); defatting in a Soxhlet apparatus (J.P.Selecta–Spain) with 2:1 (v/v) chloroform/methanol for lipids (method 920.39C). Total soluble solids (TSS) were determined directly in each sample by using refractometer (Atago 3810-Japan) and expressed as Brix. Acidity and pH determination (Jenway 3510-UK) was carried out by the same methods (Anon., 2000). The experiment was repeated twice and the values are presented as means (SD±).

Table 1. Treatment combinations (mg/kg) of various chemical preservatives used in mango pulp.

| Treatment | Sodium benzoate (as benzoic acid) | Potassium meta bisulfite (as SO ₂) |
|-----------|--------------------------------------|---|
| T0 | -- | -- |
| T1 | 1000 | -- |
| T2 | -- | 500 |
| T3 | 500 | 500 |
| T4 | 500 | -- |
| T5 | -- | 1000 |

Microbiological assay: The determination of the total microbial contamination of the pulp samples was performed after 30 days until three months by the method (with some modification) outlined in compendium of methods for the microbiological examination of foods (Anon., 1992). Nutrient agar, (The media used in the present investigation were obtained from Difco, Detroit, MI, unless specified otherwise) was used for periodical determination of total bacterial count (TBC) in the stored mango pulp sample. Nutrient medium was suspended/litre of distilled water, mixed thoroughly, pH adjusted at 7.2 (25°C) (Jenway 3510-UK), heated with frequent agitation and boiled for 1 minute to completely dissolve the ingredients and autoclaved at 121°C for 15 minutes. One-gram sample was taken from each treated pulp sample using aseptic techniques, placed in labeled sterile dilution bottles and made into a volume of 100 ml by distilled water to achieve 10⁻¹ suspension under sterile conditions. The contents were mixed thoroughly and aliquots were serially diluted and enumerated onto Nutrient agar. Plates were subsequently incubated (Memmert 100-Germany) for 48h at 37°C and TBC was calculated using colony counter. The experiment was repeated twice and reported data represent mean values (CFU/g) of these measurements.

Sensory evaluation of mango pulp samples: Ready to serve drinks (pulp 8%, acid 0.2% and sugar 16 Brix) were prepared from each sample of mango pulp and were presented to a panel of judges for sensory evaluation for colour, taste, flavour and overall acceptability using an hedonic scale in accordance with the method described by Larmond (1977). The panel members were selected on the basis of their ability to discriminate and scale a broad range of different attributes of mango and mango products. An orientation program was organized for the panel members to brief them the objective of the study. The drink samples were brought to the sensory analysis lab and were served to the panelists. The judges were provided with prescribed questionnaires to record their observation. The information contained on the performa was 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely. The panelists expectorated the drinks and rinsed mouth using distilled water between samples. Sensory testing was made in the panel room completely free of food/chemical odour, unnecessary sound and mixing of daylight. The experiment was repeated twice and the values are presented as means (SD±).

Statistical analysis: Data were statistically analyzed, using analysis of variance (Steel *et al.*, 1997). Duncan's Multiple Range Test was applied to assess the difference between means (Duncan, 1955). Significance was defined at $p \leq 0.05$. Values are means of two experiments (SD±).

Table 2. Treatments effect on physico-chemical characteristics of preserved mango pulp.

| Treatment | Protein (%) | Fat (%) | Ash (%) | TSS (Brix) | Acidity | pH |
|-----------|--------------------------|--------------------------|--------------------------|------------|--------------------------|------------|
| T0 | 0.57±0.014 ^a | 0.63±0.014 ^a | 0.36±0.007 ^b | 18.60±0.40 | 0.69±0.032 ^a | 3.81±0.087 |
| T1 | 0.51±0.024 ^c | 0.60±0.015 ^{ab} | 0.40±0.010 ^a | 18.20±0.48 | 0.64±0.025 ^{ab} | 3.84±0.063 |
| T2 | 0.53±0.021 ^{bc} | 0.57±0.021 ^b | 0.38±0.008 ^{ab} | 18.03±0.41 | 0.62±0.022 ^{bc} | 3.85±0.081 |
| T3 | 0.51±0.025 ^c | 0.59±0.018 ^b | 0.36±0.007 ^b | 18.14±0.40 | 0.62±0.023 ^{bc} | 3.85±0.071 |
| T4 | 0.56±0.016 ^{ab} | 0.60±0.017 ^{ab} | 0.38±0.007 ^{ab} | 17.89±0.37 | 0.59±0.019 ^c | 3.90±0.058 |
| T5 | 0.51±0.025 ^c | 0.59±0.019 ^b | 0.38±0.009 ^b | 18.24±0.35 | 0.61±0.022 ^{bc} | 3.84±0.092 |

Means (± SD) sharing similar superscripts in a column are statistically non-significant (p<0.05)

Table 3. Storage effect on physico-chemical characteristics of preserved mango pulp.

| (Days) | Protein (%) | Fat (%) | Ash (%) | TSS (Brix) | Acidity | pH |
|--------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|------------|
| 0 | 0.62±0.010 ^a | 0.68±0.008 ^a | 0.36±0.005 ^b | 17.39±0.31 ^b | 0.52±0.010 ^c | 3.94±0.065 |
| 30 | 0.55±0.009 ^b | 0.58±0.010 ^b | 0.38±0.007 ^a | 18.02±0.31 ^{ab} | 0.63±0.013 ^b | 3.86±0.055 |
| 60 | 0.50±0.009 ^c | 0.57±0.010 ^b | 0.38±0.006 ^a | 18.41±0.24 ^{ab} | 0.67±0.014 ^a | 3.82±0.059 |
| 90 | 0.46±0.015 ^d | 0.56±0.009 ^b | 0.39±0.009 ^a | 18.91±0.33 ^a | 0.69±0.017 ^a | 3.78±0.060 |

Means (± SD) sharing similar superscripts in a column are statistically non-significant (p<0.05)

Results and Discussion

Physico-chemical analyses of the mango pulp: Incorporation of chemical preservatives exhibited a significant (p<0.05) effect on physico chemical profile of mango pulp (Table 2). Addition of 500 mg/kg of SB alone did not show any effect on protein content of the samples while rest of the combinations (Table 1) decreased protein content. Benzoic acid at the concentration applied exerted no effect on lipid content; however SO₂ was able to decrease the fat content in mango pulp samples. SB at the concentration of 1000 mg/kg increased ash content while TSS content of all the treated samples remained unchanged. The results revealed that benzoic acid only increased the acidity of mango pulp with a corresponding decrease in pH value of the samples (Table 2).

Storage time had shown a pronounced effect on physico-chemical attributes of chemically preserved mango pulp. Progressive decrease in protein and fat content of pulp sample was observed over the entire storage period of 90 days (Table 3). Similarly, a significant (p<0.05) decreasing pattern in ash content was noticed when the samples were tested after every 30 days. TSS was not affected up to a period of 60 days storage, however, the increase in TSS was apparent in the last month of the storage period. About half of the soluble sugars of mango pulps are mainly composed of fructose, with about 30% sucrose and 20% glucose. The high sugar content of pulps from ripe fruits might be attributed to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during ripening (Germain & Linden, 1981; Favier *et al.*, 1993).

Amin *et al.*, (2008) reported the effect of the time of fruit harvest on most of the fruit quality attributes. The authors showed a significantly higher TSS value, measured in the fruit harvested at noon as compared to other times of the day.

Addition of preservatives increased the acidity of the mango pulp samples until 60 days and no further change in acidity could be observed in the last month. Interestingly, the pH of the stored pulp samples decreased concomitantly for 90 days but the differences remained non significant (Table 3). Germain *et al.*, (2003), however correlated the changes in lipid profile of mango pulp samples with the state of ripening of the mangoes. Abbassi *et al.*, (2009) attributed the increase in pH and the decrease in titratable acidity with increased storage time of the mangoes.

The results pertaining to the increase in acidity and decrease in pH during storage of mango pulp are in complete agreement with other researchers (Fulya *et al.*, 1999; Doreyyapa *et al.*, 2001). The increase in acidity may be ascribed to rise in the concentration of weakly ionized acid and their salts during storage. This increase in acidity might also be due to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and uronic acid (Hummel & Okay 1950; Iqbal *et al.*, 2001; Hussain *et al.*, 2008).

pH plays dual role in the fruit juices by acting as a flavour promotion and preservative factor. Decrease in pH of the fruit pulp samples proportional to increase in acidity has been confirmed by several researchers and may be attributed to the presence of SB in the pulp samples (Bajwa *et al.*, 2002; Hussain *et al.*, 2008).

Microbiological evaluation of the mango pulp: Fig. 1 revealed inhibitory effects of PMS and SB on the microbial growth of the mango pulp at concentrations commonly used in the food industry. The highest inhibitory effects on bacterial growth in mango pulp samples were exerted by PMS alone at a concentration of 1000 mg/kg followed by combination of PMS and SB at 500 mg/kg each. Increasing SB concentration from 500 mg/kg to 1000 mg/kg reduced the growth velocity to two times and 500 mg/kg of PMS was shown to be equally effective as compared to 1000 mg/kg of the SB (Fig. 1). The results of the present study also demonstrated an inhibitory effect of PMS and SB in mango pulp stored for a period of three months.

Fig. 2 indicates the bacterial growth pattern at various time intervals and the systematic increase in CFU/g of the mango samples, corresponding to the storage time. The growth was found to be highest at the termination of the storage period which may be attributed to the variability in the temperature and the chemical changes, specifically alteration in pH of the system that would take place resulting from the presence of the chemical preservatives in the samples.

Inhibitory effect of PMS and SB in specified combinations and concentration levels in relation to storage periods have been presented in Table 6. The highest level of contamination in mango pulp samples was observed in control (no preservative added) after 90 days of storage while the minimum growth was shown in the presence of 1000 mg/kg PMS. Periodical analysis of the mango pulp samples for the TBC showed a progressive increase in the growth though the rate of growth varied with different treatment combinations with exception of 1000 mg/kg of PMS where the growth remained stagnant for 60 days suggesting the PMS to be relatively inhibitorier in mango pulp. The results of the present study substantiated that none of the two preservatives used was able to completely inhibit the bacterial growth in the specified concentrations for a period of 90 days storage; however, the preservative had been synergistically active in inhibition of the microbial growth in the pulp samples.

A previous study on inhibitory effects of 1000-ppm PMS (Hussain *et al.*, 2003) confirmed the results of the present study suggesting that PMS is more effective against the microflora of the stored pulp samples at 1000 ppm.

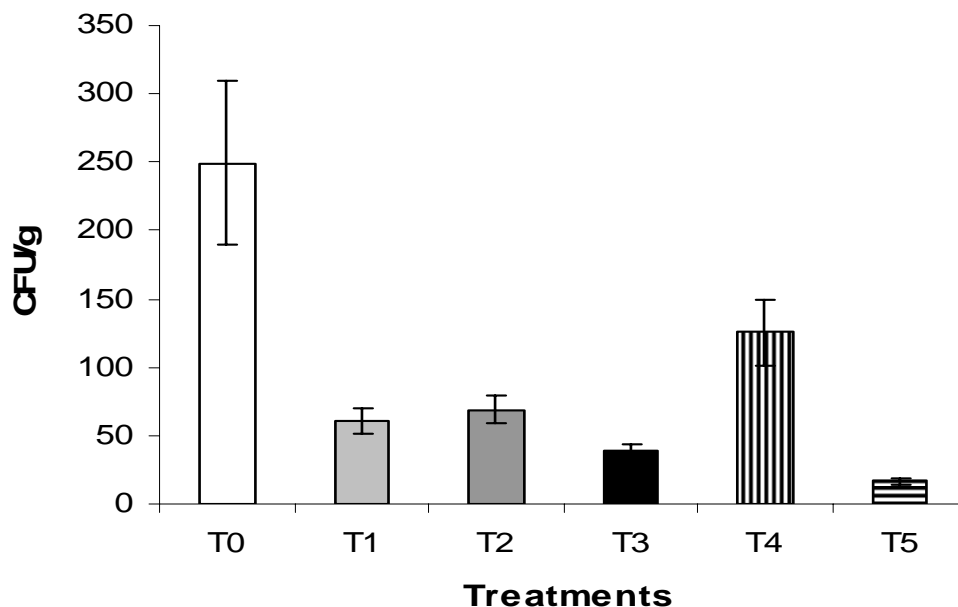


Fig. 1. Effect of SB and PMS on microbial growth of stored mango pulp T0=control, T1=1000 ppm SB, T2=500 ppm PMS, T3=500 ppm SB + 500 ppm PMS, T4=500 ppm PMS, T5=1000 ppm PMS. The values are means of at least two experiments (SD \pm) ($\alpha=0.05$).

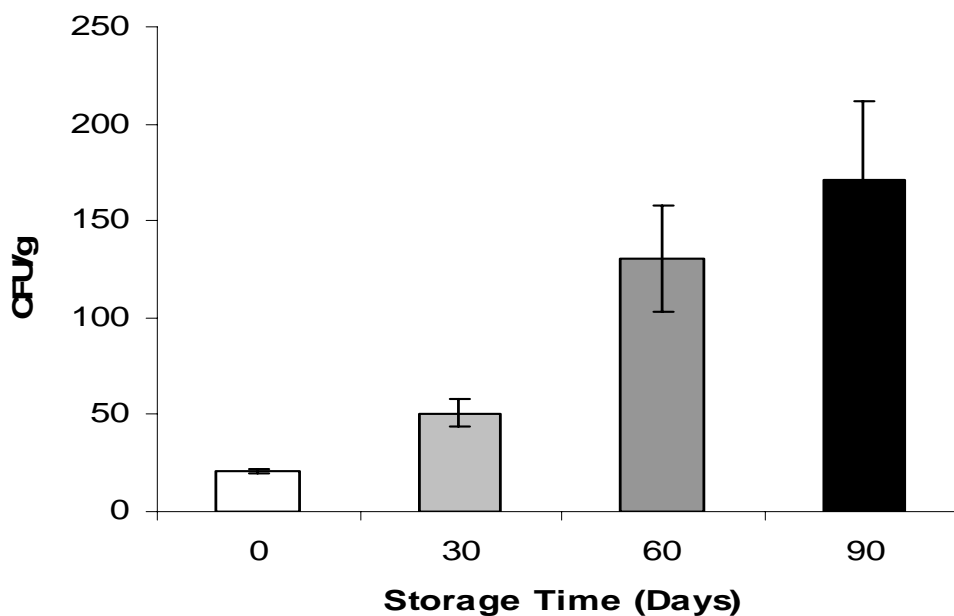


Fig. 2. Effect of storage on microbial growth of mango pulp preserved with SB and PMS at various concentrations. The values are means of at least two experiments (SD \pm) ($\alpha=0.05$).

Sensory evaluation of mango pulp: Organoleptic evaluation of the ready-to-serve mango drinks prepared from the treated pulp samples was carried out for colour, flavour, taste and overall acceptability. It is evident that addition of chemical preservatives greatly influences these attributes with a little loss in pulp quality (Table 4). The results pertaining to the effect of addition of chemical preservatives to mango pulp are presented in Table 4. Concentration and synergistic addition of PMS and SB seem to have no effect on their ability to act differently for deteriorating the colour, flavour and overall acceptability of stored mango pulp. However, the exception was noticed in taste scores showing greater variability in relation to treatment combination and the concentration of these chemical compounds (Table 4).

Table 4. Treatments effect on sensory characteristics of preserved mango pulp.

| Treatment | Colour | Flavour | Taste | Acceptability |
|-----------|-------------------------|-------------------------|--------------------------|-------------------------|
| T0 | 7.25±0.175 ^a | 7.25±0.142 ^a | 7.00±0.171 ^a | 6.75±0.122 ^a |
| T1 | 6.75±0.183 ^b | 6.25±0.151 ^b | 5.75±0.175 ^d | 6.00±0.171 ^b |
| T2 | 6.25±0.168 ^b | 6.25±0.142 ^b | 6.25±0.142 ^{bc} | 6.00±0.154 ^b |
| T3 | 6.75±0.203 ^b | 6.25±0.280 ^b | 6.54±0.221 ^b | 6.25±0.260 ^b |
| T4 | 7.25±0.175 ^a | 6.25±0.151 ^b | 6.00±0.178 ^{cd} | 6.00±0.136 ^b |
| T5 | 6.50±0.202 ^b | 6.50±0.221 ^b | 5.75±0.168 ^d | 6.00±0.171 ^b |

Means (± SD) sharing similar superscripts in a column are statistically non-significant (p<0.05)

Table 5. Storage effect on sensory characteristics of preserved mango pulp.

| (Days) | Colour | Flavour | Taste | Acceptability |
|--------|-------------------------|-------------------------|-------------------------|-------------------------|
| 0 | 7.67±0.139 ^a | 7.50±0.129 ^a | 7.33±0.116 ^a | 7.17±0.096 ^a |
| 30 | 6.67±0.143 ^b | 6.33±0.121 ^b | 6.33±0.100 ^b | 6.33±0.106 ^b |
| 60 | 6.50±0.146 ^b | 6.00±0.136 ^c | 5.83±0.136 ^c | 6.00±0.118 ^c |
| 90 | 6.33±0.126 ^b | 6.00±0.141 ^c | 5.36±0.089 ^d | 5.17±0.058 ^d |

Means (± SD) sharing similar superscripts in a column are statistically non-significant (p<0.05)

Table 6. Inhibitory effect of different preservatives (CFU/g) during three months storage at ambient temperature of mango pulp.

| Treatment | Storage time (days) | | | |
|-----------|--------------------------|---------------------------|--------------------------|---------------------------|
| | 0 | 30 | 60 | 90 |
| T0 | 25.00±0.51 ^{mn} | 102.00±5.33 ^{ef} | 355.00±8.20 ^b | 516.00±14.8 ^a |
| T1 | 21.00±0.80 ^{mn} | 42.00±0.53 ^{kl} | 84.00±2.88 ^{gh} | 97.00±6.05 ^{efg} |
| T2 | 24.00±0.54 ^{mn} | 51.00±2.40 ^{jk} | 92.00±5.31 ^{fg} | 109.00±2.21 ^e |
| T3 | 18.00±0.60 ^{mn} | 22.00±1.09 ^{mn} | 53.00±0.83 ^{jk} | 61.00±2.31 ^{ij} |
| T4 | 24.00±0.49 ^{mn} | 72.00±1.08 ^{hi} | 188.00±9.66 ^d | 217.00±4.91 ^c |
| T5 | 11.00±0.22 ⁿ | 16.00±0.23 ^{mn} | 11.00±0.30 ⁿ | 29.00±0.45 ^{lm} |

Means (± SD) sharing similar superscripts in a column are statistically non-significant (p<0.05)

Periodical analysis for colour scores manifested a drastic decline (p<0.05) in the first month of storage which did not alter in the last two months. The panelists clearly identified the changes in flavour profile of the samples rating the stored sample inferior as compared to the freshly prepared drinks. Storage time perpetually decrease flavour score until 90 days storage, nevertheless, the drink samples were still liked by the judges for colour and flavour (Table 5). The maximum deterioration was noticed in the pulp sample for taste and overall acceptability as a function of storage for three months. A uniform pattern of decline in these sensory attributes of the mango pulp samples is evident in relation to storage time albeit the samples were not rejected instantaneously for taste and overall acceptability at any time period (Table 5).

Numerous studies have confirmed the effect of adding chemical preservatives and subsequent storage at ambient temperature of the fruit pulp samples on the organoleptic characteristics. One study revealed that pulp could be preserved up to one year at ambient temperature by adding PMS alone (1200 mg/kg) or in combination with SB (600 mg/kg each) without a significant loss of sensory properties of the fruit pulp (Sonia *et al.*, 2003). Other studies (Kapse *et al.*, 1995 ; Mercadante & Rodriguez, 1998) corroborated the results of the current research reporting notable changes in sensory characteristics (appearance, smell and flavour) of the pulp fruit during ripening which altered its

physico-chemical profile by inter-conversion of the starch to soluble sugar, decrease in acidity, carotene content, modification in texture and conversion of protopectin to pectin.

In another study, pulp of *chaunsa* mango tested for various sensory attributes was rated highly acceptable to the judges (Akhtar *et al.*, 2009). A similar study (Hussain *et al.*, 2003) conducted on the sensory evaluation of chemically preserved mango pulp samples confirmed that all the samples tried for organoleptic evaluation were satisfactory up to 270 days of storage at ambient temperature.

Conclusions

This study demonstrated the inhibitory effects of SB and PMS on microbial growth in the mango pulp stored under ambient temperature and suggested that SB and PMS applied synergistically at concentrations of 500 ppm each had been equally effective as 1000 ppm PMS used individually. Further, these chemical preservatives had significantly affected the physico-chemical profile of the pulp samples with a pronounced increase in acidity and corresponding decrease in pH during storage for three months. Addition of SB and PMS adversely influenced the sensory attributes of the stored pulp however, the product remained acceptable after three months storage. The effect of these chemical preservatives on physico-chemical profile, microflora and organoleptic properties of stored mango pulp shown in this work constitutes a major contribution that can help the development of a safer and viable storage of mango pulp at industrial scale.

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