



Effect of crude stevia extract and chemical preservatives on the overall quality of sweet orange juice during storage

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Abstract

Peoples are more attracted towards juices than carbonated drinks as they supply a variety of vitamins, minerals with some of the phytonutrients that are playing crucial role in the human health. Commonly three types of juices are available in the market. Fresh squeezed juice, NFC means not form concentrate obtained by freezing after squeezing and RFC, reconstituted from concentrate. Sweet orange is one of the famous citrus fruit due to its good-looking cheerful color, flavor and tempting taste. In the present study, the juice was prepared by the selected ratio as per panel of judges and treatments were named SOS₀ to SOS₇ with different level of preservative. The combination of sweet orange juice and stevia extract was made at a ratio 7: 93. The treatments were analyzed physio-chemically and sensory for a total period of 120 days. The statistical results revealed that treatments have a significant ($P < 0.05$) effect on the physiochemical and sensory attributes of the juice. Among different treatments studied, the sample SOS₁ has shown the best results in terms of physiochemical and organoleptic characteristics.

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Introduction

Pakistan is the 10th largest producer of citrus. Among all citruses, Pakistan mainly exports mandarin and sweet oranges to other countries of the world. Pakistan share 1.46 % of the total mandarin market. Pakistan is exporting only 10 % of its production (TDAP, 2010) while the rest is locally utilized. In Khyber Pakhtunkhwa province of Pakistan, it is cultivated on 3,840 hectares with a production of 30871 tonnes (Agriculture Marketing Information Service, 2015). Sweet orange is (*Citrus sinensis* L. Osbeck) a hesperidium belongs to family *Rutaceae* is rich source of vitamin C, which plays a vital role in building one's immune system. It contains important phytochemicals like limonoids, synephrine, folic acid and so on (Etebu and Nwauzoma, 2014). It is composed of an external layer (orange peel) and internal layer. Outer layer is made-up of Flavedo (epicarp) and Albedo (mesocarp). Internal portion known as endocarp that contains vesicles or juice sac containing juice. (Izquierdo and Sendra, 2003). Orange juice is important according to nutritional point of view as 100 g edible portion of orange juice contains 47 kcal energy, 11.53 g carbohydrates, 9.15 g sugar, 2.38 g dietary fibre, 0.22 g fat, 0.71 g protein, 0.1 mg thiamine vitamin B₁, 0.04 mg riboflavin vitamin B₂, 0.4 mg niacin vitamin B₃, 0.255 mg pantothenic acid B₅, 0.052 mg of B₆ vitamin, 0.09 mg folate vitamin. B₉, 10 mg magnesium, 12 mg phosphorous, 169 mg potassium and 0.08 mg zinc (USDA, 2011).

Increased intake of sugar has shown adverse effects on human health and vice versa. As an indispensable part of our food system, its little bit difficult to get rid of sugar from our diet. Below such precedence sugar might be replaced with a surrogate. Stevia (*Stevia rebaudiana*) is less or low caloric crop sweetener. Many countries of the world used stevia instead of sugar. Stevia (*Stevia rebaudiana* Bertoni) belongs to the family (Asteraceae), comprises of 230 different physiologically versatile species, from perennial and annual herbs to shrubs and sub shrubs which are grown in hilly areas and stream lodgers. The two important species took great importance known as, *S.*

rebaudiana and *S. phlebophylla*, acquire comparably highest quantity of steviol glycosides (SGs) which gives sweet perception (Ijaz and Malik, 2015). In comparison with sucrose, the pure steviol glycosides are 100-300 times sweeter than sucrose, while crude stevia leaf is 10-15 times sweeter than sucrose (Savita *et al.*, 2004). Recommended ADI (acceptable daily intake) of steviol glycoside is 4.1 mg/kg body wt. per day, according to joint consultation of FAO and WHO (Efsa, 2010). It was in 2003 that stevia was introduced for the first time in Pakistan by tissue culture technique (Talha, 2012).

Keeping in view the due importance of sweet orange juice and stevia as low caloric sweetener, the present study was designed to investigate the effect of crude stevia extract in combination with chemical preservatives on the physio-chemical, organoleptic and storage quality of sweet orange juice.

Materials and methods

Procurement of sweet orange

The fully mature sweet orange fruits were procured from the orchards of Kaka Sahib, Nowshera and were processed in the Laboratory of Food Science and Technology section, Agriculture Research Institute Tarnab, Peshawar for the extraction of juice.

Pre-treatment of sweet orange

Sweet oranges were sorted, graded and washed thoroughly with continuous stream of water to remove adherent dirt particles and other foreign materials. The pulp was then extracted through citrus extractor machine.

Procurement of stevia leaves

The fresh Stevia leaves were brought from Nuclear Institute for Food and Agriculture (NIFA), Peshawar Pakistan.

Post-treatment of stevia leaves at different temperatures and dilution

Fresh stevia leaves were dried in a dehydrator at 58±10°C. Leaves were crushed and ground in a grinder. The powdered leaves were packed in

polyethylene bags and then sealed airtight. The powdered stevia leaves were diluted in water at a ratio of 1:50 (Thakur and Joshi, 2010.; Ghasemzadeh *et al.*, 2012) at different temperatures (40°C, 50°C, 60°C and 70°C) (Bimkr *et al.*, 2011) for one hour. The resulted treatment was replaced against sucrose with increasing trend. Then, the material was filtered and stevia extracts obtained from different treatments were poured into glass bottles.

Best combination of stevia extract with juice

Different concentrations of diluted stevia extract with sweet orange juice were sensory analysed according to 9 point hedonic scale for colour, taste, flavour and overall acceptability. Stevia extract and sweet orange juice was taken in different ratios (1ml extract+99ml juice, 3ml extract+97ml juice, 5ml extract+95ml juice, 7ml extract+93ml juice, 9ml extract+91ml juice, 12ml extract+88ml juice).

Packing and storage of the juice

After, the best one through sensory evaluation was finally preserved with sodium benzoate (0.1%), potassium meta bisulphite (0.1%) and potassium sorbate (0.1%) at different ratios as shown in Table 01. All the treatments were packed in 250 ml plastic bottles and stored at room temperature (15°C-20°C).

Physiochemical analysis

All the samples were analysed physio-chemically for ascorbic acid, reducing sugars, non-reducing sugar, pH, acidity and total soluble solids by method of AOAC, (2012).

Sensory evaluation

All samples were sensory analysed for color, taste, flavour and overall acceptability by 10 trained judge's panel. Sensory evaluation was carried out at each 20 days interval during 4 months storage.

The evaluation was conceded out by using 9 points hedonic scale of Larmond (1997) by Yousaf *et al.*, (2016). The results are of scoring rate 1-9 awarded by panel of judges.

Statistical analysis

All the data concerning treatments and storage were analysed using Completely Randomized Design with two factors (storage, treatment). Using statistical software Statistix 8.1.

In case the data was found significant then least significant test was applied at 0.5% level of significance for mean comparison (Shah *et al.*, 2015).

Results and discussion

Ascorbic acid content

The result revealed that treatment have least significant effect on the ascorbic acid (AA) content of orange juice (37.55 to 33.80) as compared to storage showed a highly significant effect on the AA content of orange juice (48.53 to 16.08) preserved with different chemical preservatives. Both storage and treatments significantly ($P < 0.05$) affected ascorbic acid of sweet orange juice with crude stevia extracts as shown in Fig. 1. On account of its easy oxidation nature, the ascorbic acid is a powerful reducing agent.

Table 1. Proposed plan of study for research.

Treatments	Stevia extract (ml)	Juice (ml)	Preservatives
SOS ₀	7	93	-
SOS ₁	7	93	0.1% KMS
SOS ₂	7	93	0.1% SB
SOS ₃	7	93	0.1% PS
SOS ₄	7	93	0.05% KMS+0.05% SB
SOS ₅	7	93	0.05% KMS+0.05% PS
SOS ₆	7	93	0.05%PS+0.05%SB
SOS ₇	7	93	0.033% KMS+0.033% SB+0.033% PS

SOS= sweet orange+stevia extract, KMS= Potassium meta bisulfite, SB= Sodium benzoate, PS= Potassium sorbate.

The decrease in ascorbic acid content with storage might be due to light exposure to transparent plastic bottle of treatment. Vitamin C contents decrease (38.2-12.6mg/100gm) with increase in storage and temperature (Burdurlaet *al.*, 2006). Decreased in

ascorbic acid during storage from (54.9-14.7mg/100gm) was observed during the preparation of RTS with low calories fruit beverages using stevia as a sugar substitute (Jothi *et al.*, 2014).

Table 2. Mean score of judges for the Colour, Taste, flavour and overall acceptability of sweet orange juice with added crude stevia.

Treat	Storage Interval						% Dec	Mean	
	0	20	40	60	80	100			120
Colour Score									
SOS0	6.5	6.1	5.3	4.7	4.1	3.8	3.3	48.69	4.84
SOS1	7.9	7.7	7.4	7.2	7.0	6.7	6.6	16.43	7.20
SOS2	7.6	7.1	6.9	6.7	6.4	6.2	6.1	19.02	6.71
SOS3	6.5	6.2	5.7	5.5	5.3	5.1	5.0	24.04	5.62
SOS4	7.6	7.4	7.1	6.9	6.7	6.5	6.2	18.48	6.92
SOS5	6.7	6.5	6.3	5.8	5.5	5.2	5.1	23.66	5.88
SOS6	6.9	6.7	6.5	6.2	5.8	5.4	5.4	22.33	6.13
SOS7	7.4	7.1	6.7	6.5	6.3	6.0	5.8	21.79	6.54
Taste Score									
SOS0	6.5	6.2	5.3	4.7	4.2	3.8	3.3	49.46	4.86
SOS1	7.9	7.7	7.4	7.2	7.0	6.8	6.6	16.39	7.22
SOS2	7.6	7.1	6.9	6.7	6.4	6.2	6.2	18.97	6.73
SOS3	6.6	6.2	5.8	5.5	5.3	5.1	4.9	24.89	5.63
SOS4	7.7	7.4	7.1	7.0	6.7	6.5	6.2	18.43	6.94
SOS5	6.7	6.6	6.3	5.8	5.5	5.3	5.2	23.59	5.90
SOS6	7.0	6.7	6.5	6.2	5.9	5.4	5.4	22.56	6.15
SOS7	7.4	7.3	6.7	6.5	6.3	6.0	5.8	22.27	6.58
Flavour Score									
SOS0	6.5	6.2	5.3	4.7	4.2	3.9	3.4	48.47	4.87
SOS1	7.9	7.7	7.5	7.2	7.0	6.8	6.6	16.37	7.23
SOS2	7.6	7.1	6.9	6.7	6.4	6.3	6.2	18.95	6.74
SOS3	6.6	6.3	5.8	5.5	5.3	5.1	5.0	24.09	5.65
SOS4	7.7	7.4	7.1	7.0	6.7	6.5	6.3	18.41	6.95
SOS5	6.8	6.6	6.3	5.8	5.5	5.3	5.2	23.56	5.91
SOS6	7.0	6.7	6.5	6.2	5.9	5.5	5.4	22.24	6.16
SOS7	7.4	7.3	6.8	6.5	6.4	6.0	5.8	21.70	6.60
Overall Acceptability Score									
SOS0	6.5	6.1	5.3	4.7	4.1	3.8	3.3	49.23	4.83
SOS1	7.9	7.7	7.4	7.2	7.0	6.7	6.6	16.46	7.21
SOS2	7.6	7.1	6.9	6.7	6.4	6.2	6.1	19.74	6.71
SOS3	6.5	6.2	5.7	5.5	5.3	5.1	4.9	24.62	5.60
SOS4	7.6	7.4	7.1	6.9	6.7	6.5	6.2	18.42	6.91
SOS5	6.7	6.5	6.3	5.8	5.5	5.2	5.1	24.22	5.88
SOS6	6.9	6.7	6.5	6.2	5.8	5.4	5.4	21.74	6.13
SOS7	7.4	7.2	6.7	6.5	6.3	6.0	5.8	21.62	6.56

Reducing and non-reducing sugar content

The stevia extract fortified sweet orange juice were analysed for reducing sugar during storage. Both storage and treatments significantly ($P < 0.05$) affected reducing sugar of sweet orange juice with crude stevia

extracts. The results showed that increasing storage duration significantly increased juice reducing sugar value (2.48 to 3.38) (Fig. 2). Whereas, the non-reducing sugar decreased with storage from 6.55 to 3.11 (Fig. 3).

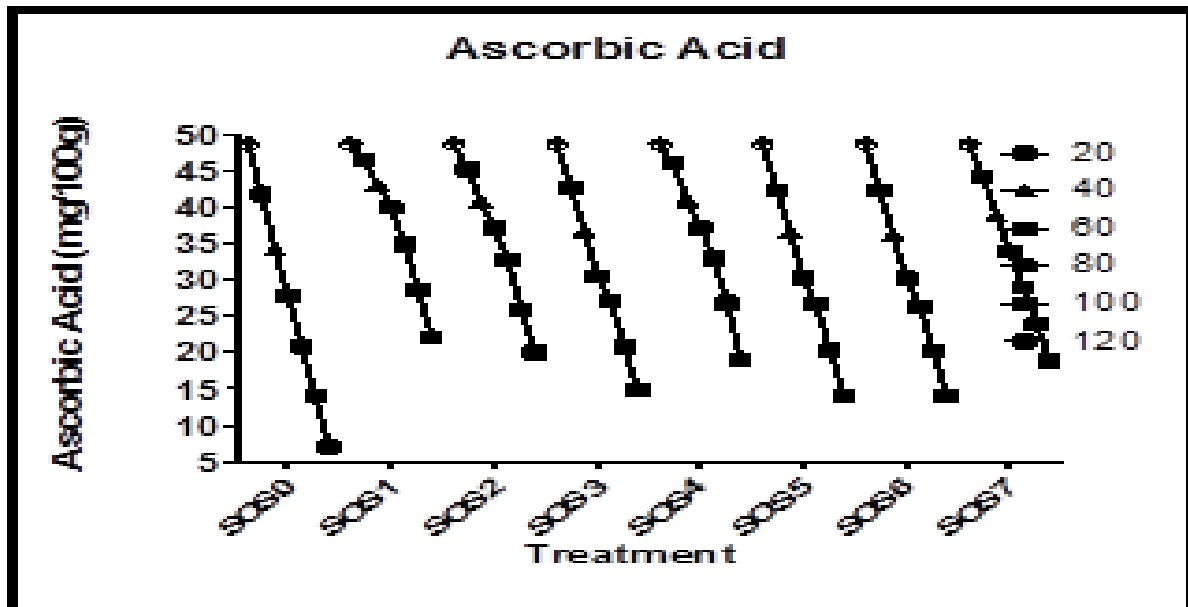


Fig. 1. Effect of crude stevia extracts and chemical preservatives on the ascorbic acid of sweet orange juice.

The increase in reducing sugars is due to reduction of sucrose into glucose and fructose. There is even a possibility of starch hydrolysis due to the presence of citric acid, therefore reducing sugar content may increase during prolonged storage. The increase in reducing sugar could be due to the conversion of non-reducing sugar through the process of glucogenesis to

reducing sugar and also organic acid hydrolysis in the stored sample. Kumar Singh and Sharma, (2017) reported that total sugar increasing with storage due to conversion/hydrolysis of polysaccharides like pectin, cellulose and starch in simple sugar and non-reducing sugar to reducing sugar.

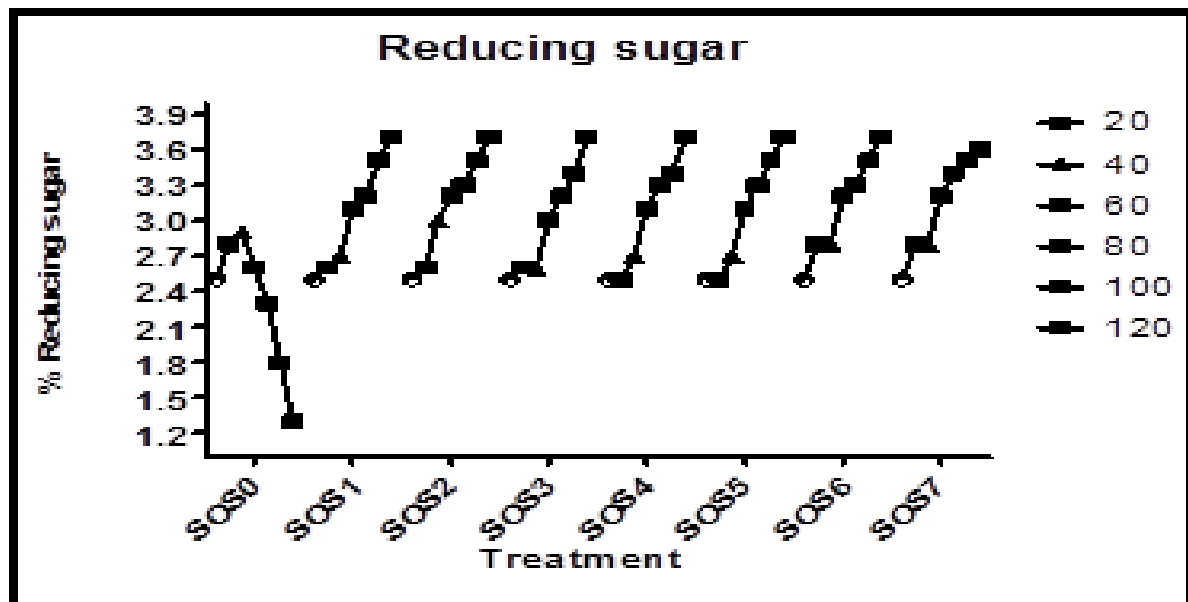


Fig. 2. Effect of crude stevia extracts and chemical preservatives on the reducing sugar of sweet orange juice.

It was stated previously that increase in reducing sugar of citrus juice is the result of acid hydrolysis of sucrose to glucose and fructose. The current results

are in line with the findings of Sandi *et al.* (2004). During their study on pasteurized yellow passion fruit juice, the non-reducing sugar contents were found in

decreased ratio from (5.6-2.9%). The acid content in beverage usually considered to the anhydrous citric acid content. The consumer acceptability relegate to the sugar acid ratio attribute of the final product. The present study revealed the acid content of sweet orange juice fortified with crude stevia extract analysed through NaOH neutralization increase

(0.86- 1.27) with storage. Both storage and treatments significantly ($P < 0.05$) affected the acidity of sweet orange juice with crude stevia extracts as shown in Fig. 4. The increase in acidity was attributed to the degradation of free sugars into carboxyl acids. The increase in acidity was also reported by Pareek *et al.*,(2015) in mandarin juice extraction and storage.

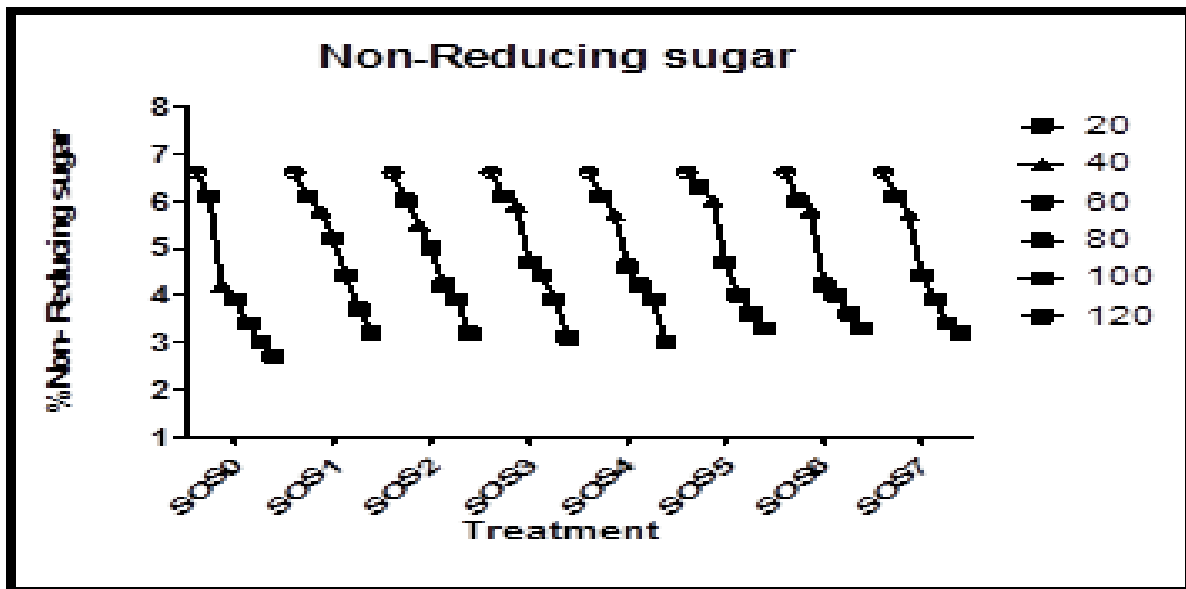


Fig. 3. Effect of crude stevia extracts and chemical preventives on the non-reducing sugar of sweet orange juice.

Changes in pH

The pH changes were substantial throughout the storage of each treatment. Overall decreasing trend of pH ranged from 3.9 to 3.06 was obvious from results

that acidity increasing during storage of 120 days. Both storage and treatments significantly ($P < 0.05$) affected the pH of sweet orange juice with crude stevia extracts as shown in Fig. 5.

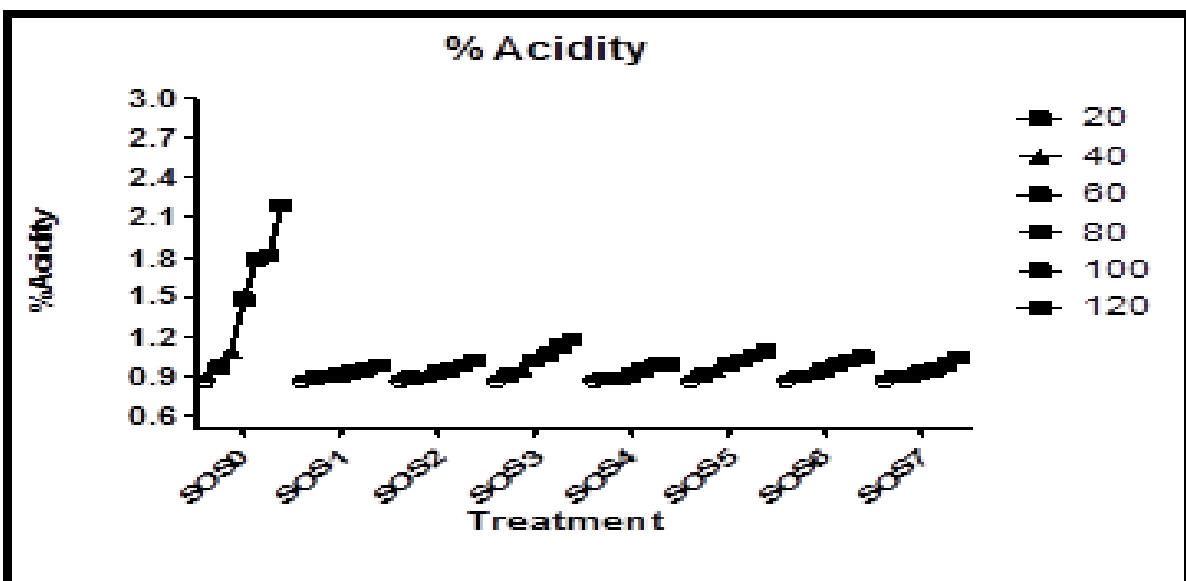


Fig. 4. Effect of crude stevia extracts and chemical preventives on the acidity of sweet orange juice.

The reason behind the decrease in pH is the acid production in juice with the increase in the time of storage. (Jothi *et al.*, 2014) also recorded decreased in pH during storage from (3.96-3.76) during the preparation of RTS with low calories fruit beverages using stevia as a sugar substitute.

Effect on total soluble solids

Storage significantly ($P < 0.05$) affected the TSS value of sweet orange juice with crude stevia extracts as shown in Fig. 6. While the treatment had no considerable effect on the TSS value of juice. The results showed a substantial decreased in TSS range

from 12.5 to 10.10 °Brix. The decreased in TSS might be due to the conversion of non-reducing sugar to reducing sugar and other organic acid. The fortified extract, stevia had no effect of the TSS level of juice.

Highest juice TSS (12.5 °brix) was observed in fresh fruit juice of sweet orange. While lowest fruit juice TSS (10.4 °brix) was observed in juice kept for 120 days in storage. During storage maximum decrease was observed in SOS₀ (58.94%), whereas minimum decrease was observed SOS₁ (8.76%). Stevia is low caloric or no calories and its adding in drink did not cause any increase in TSS (Balaswamy *et al.*, 2014).

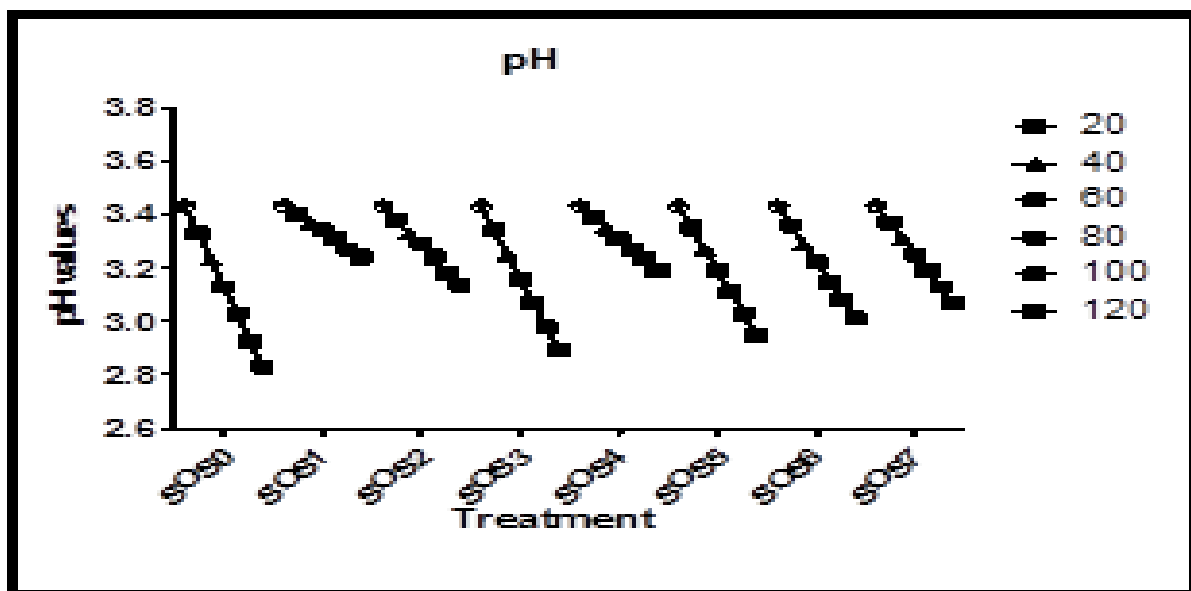


Fig. 5. Effect of crude stevia extracts and chemical preventives on the pH of sweet orange juice.

Changes in Colour

Both storage and treatments significantly ($P < 0.05$) affected colour of sweet orange juice fortified with crude stevia extracts as shown in Table 02. The mean values for treatment showed that sweet orange juice treated with SOS₁ significantly retained colour value (7.22). Increasing storage duration significantly reduced juice colour value. During storage maximum decrease was recorded in SOS₀ (83.08%), whereas minimum decrease was observed in SOS₁ (16.43%). Colour of organic compounds is due to carotenoids (chromophores). Chromophores depend on oxygen, chlorine or other reducing agents. During storage double bonds convert to single bond. This conversion leads to degradation in colour e.g., phenolphthalein is

colourless in acid while it gives pink colour in base due to change in its structure. The decrease in colour is also because of synergistic effect of steviosides and polyphenols existing in the juice (Kaushik *et al.*, 2010).

Effect on taste

Both storage and treatments significantly ($P < 0.05$) affected the taste of sweet orange juice with crude stevia extracts as shown Table 02. The mean values for treatment showed that sweet orange juice treated with SOS₁ significantly retained taste value (7.22). Increasing storage duration significantly reduced juice taste value. Highest juice taste value (7.16) was observed in fresh fruit juice of sweet orange. While

lowest fruit juice taste value (5.16) was observed in juice kept for 120 days in storage. Inversion of the sugars during storage change the taste of the product, such type of defect occurs due to the presence of free

water molecules. The decreased in taste value (7.4-5.2) was also reported by Jothi *et al.*, (2014) during the preparation of RTS with low calories fruit beverages using stevia as a sugar substitute.

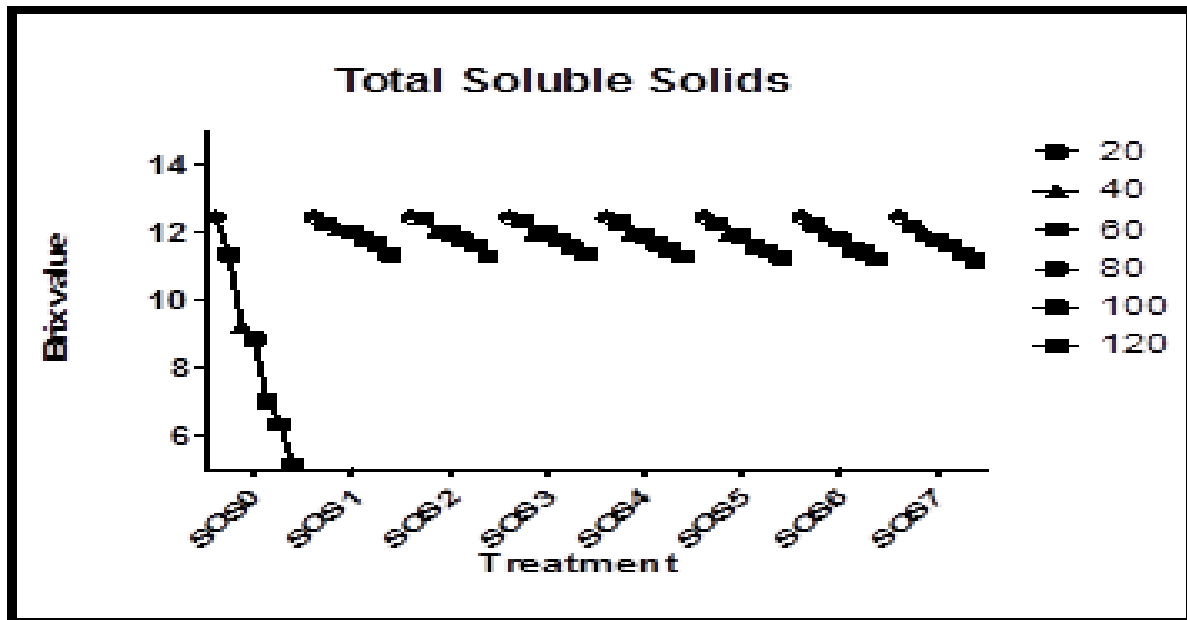


Fig. 6. Effect of crude stevia extracts and chemical preventives on the TSS of sweet orange juice.

Effect on flavour

Both storage and treatments significantly ($P < 0.05$) affected the flavours of sweet orange juice with crude stevia extracts as shown in the Table 2. The mean values for treatment showed that sweet orange juice treated with SOS₁ significantly retained flavour value (7.24).

Increasing storage duration significantly reduced juice flavour value. Highest juice flavour value (7.19) was observed in fresh fruit juice of sweet orange. While lowest fruit juice flavour value (5.17) was observed in juice kept for 120 days in storage. During storage maximum decrease value was recorded in SOS₀ (83.18%), whereas minimum decrease value was observed in SOS₁ (16.37%). The difference in flavor might be due to storage conditions and storage time. Similar observation during research on physiochemical and sensory properties of orange drink were also noticed by (Jain *et al.*, 2003). A decrease in flavor during storage study on 2-Methyl-3-furanthiol and methional in stored orange juice of beverage was also reported by Bezman (2001).

Effect on the overall acceptability of orange juice

Both storage and treatments significantly ($P < 0.05$) affected the overall acceptability of sweet orange juice with crude stevia extracts as shown in Table 02. The mean values for treatment showed that sweet orange juice treated with SOS₁ significantly retained overall acceptability value (7.23). Increasing storage duration significantly reduced juice overall acceptability value. Highest juice overall acceptability value (7.14) was observed in fresh fruit juice of sweet orange. While lowest fruit juice overall acceptability value (5.15) was observed in juice kept for 120 days in storage. During storage maximum decrease value was recorded in SOS₀ (83.08%), whereas minimum decrease value was observed in SOS₁ (16.46%). Decrease in overall acceptability was also observed by (Jothi *et al.*, 2014).

Conclusion

In the current study, sensibly sweet orange juice was preserved in different ratios of chemicals. It was observed that treatments and storage had significant effect on the quality of sweet orange juice with added crude stevia extract. Crude stevia extract (1:50)

extracted at 50 °C was selected by the panel of judges on the base of sweetness perception. Crude stevia extract was used in sweet orange juice up to 7% according to the high sensory score results. On the basis of different analysis and parameters, it was accomplished that sample SOS₁ (sweet orange juice+ crude stevia extract+ 0.1% KMS) remained the best one in case of physiochemical and organoleptic characteristics followed by treatment SOS₄ (sweet orange juice + crude stevia extract + 0.05% KMS+0.05% sodium benzoate).

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