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MICROBIAL COUNT AND SHELF LIFE OF PHALSA (*GREWIA ASIATICA*) JUICE

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ABSTRACT:- The study investigated the shelf life of laboratory developed phalsa juice at room temperature. Phalsa was purchased from local market, juice was prepared and kept in sterilized bottles at room temperature. Physicochemical and microbial and oragnoleptic quality of the juice was examined till two weeks. Microbial activity in phalsa juice increased while organoleptic attributes such as texture (mouth feel), flavour, taste, colour and overall acceptability of phalsa juice were decreased during the study period. The pH and TSS value were decreased from 3.99 to 3.54 and from 11.22 to 9.55, respectively after 2 weeks storage. Total plate count also showed decline from 6.2×10^{-1} to 3.2×10^{-1} cfuml^{-1} whereas yeast and mould counts increased simultaneously from 2.6×10^{-1} and nil to 5.5×10^{-1} and 2.4×10^{-1} cfuml^{-1}, respectively during the storage. To increase shelf life of phalsa juice storage at refrigerated temperature with/without preservatives is recommended.

Key Words: Grewia asiatica; Juice; Shelf Life; Total Plate Count; Yeast; Mould; Pakistan.

INTRODUCTION

Phalsa fruit (*Grewia asiatica*), bark and leaves have high medicinal values, therefore, are widely used for treatment of various common diseases (Sinha et al., 2015). Phalsa fruits are low in calories and fat, and high in vitamins, minerals, and fiber. Phosphoserine, serine, and taurine are found dominant amino acids in juice (Hasnain and Ali, 1992). Phytochemical screening of fruits indicated the presence of carbohydrate, tannins, phenolic compounds, flavonoids, vitamin-C and steroids (Gupta et al., 2006). Amino acids such as proline, glutaric acid, lysine and phenylalanine, and carbohydrates, like glucose, xylose, and arabinose were identified by paper chromatography in ethanolic extract of fruit (Sharma and Sisodia, 2010).

The fruits are excellent commodity for making juice and squash, which are regarded as very nutritious beverages by indigenous people. A refreshing summer drink prepared from fruits, called *phalsay ka sharbat* (Phalsa drink) is available in food-stores. The phalsa drink is also regarded as a cardiac tonic among people. The fruit juices may be among with other nutrients to further enhance its nutritional contribution to the diet. The phalsa juice has a low glycemic index and can be taken to diabetics on account of having carbohydrates which break down more slowly. Such low glycaemic index

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foods are also believed to lessen the risk of coronary heart disease, and obesity. Nutritionally essential amino acids are present in pulp whereas phosphoserine, serine, and taurine are the dominant amino acids in juice. Pigments and total soluble solids have been obtained from pomace (Hasnain and Ali, 1992). Six micronutrients (Co, Cr, Cu, Ni, Zn and Fe) have been reported in phalsa juice. Iron was presented in the highest concentration while cobalt in the lowest amounts. Considering the nutritional benefits of phalsa, a study was planned to prepare phalsa juice and evaluate its shelf life at ambient temperature following the changes in chemical, microbiological and organoleptic properties.

MATERIALS AND METHOD

Phalsa fruits were procured from local market of Islamabad and brought to the laboratory of Food Science and Product Development Institute (FSPDI), National Agricultural Research Centre (NARC), Islamabad for processing. The fruit was washed thoroughly with tap water before maceration using electric blender and grinder. The pulp containing water was filtered through double layered muslin cloth to remove pulp and seed particles (Balaswamy et al., 2011). The juice was taken in sterilized bottles and stored at room temperature for two weeks. The samples were taken out from storage weekly and analyzed for total plate count, yeast, mould, total soluble solid and pH. The samples were also evaluated organoleptically during storage period. The experiment was conducted in triplicate and mean value was recorded.

Physico-chemical Analysis

Fruit juice was analysed for total soluble solid (°brix) using hand refractometer and pH measured potentiometrically using Orino 420 A+ pH meter according to AOAC (2012).

Microbial Analysis

Total plate, mould and yeast counts were determined according to the described method (FAO, 1992). A 50ml sample was homogenized with butter fields' phosphate buffer (pH 7.2) Serial dilution of samples was prepared transferring 1ml of blend sample to 9ml of sterile phosphate buffer in test tube. After each dilution, the contents were mixed in vortex-mixer for 10 sec. One ml from each dilution was transferred to petri dish with plate count agar and mixed with the medium in triplicate. After solidification the petri dishes were incubated at 35°C for 48h for total plate count and colonies formed on the surface and in the media were counted. The total count was calculated from mean count of the triplicate petri dishes. Similarly mould and yeast counts were determined using spread plate method. Yeast growth was checked on plate count agar amended with 40 ppm Chloramphenicol (added as antibacterial agent) while Potato dextrose agar was used for moulds detection. The media was incubated at 25°C for 96 h.

Sensory Analysis

Phalsa juice was evaluated for organoleptic attributes in terms of colour, flavour, texture (mouth feel) and overall acceptability (Larmond, 1997).

Statistical Analysis

The data generated was subjected to statistical analysis using Statistica

software version 8.1. Duncan's Multiple Range Test (DMRT) was applied to compare difference in the means at 5% level of significance.

RESULTS AND DISCUSSION

The pH decreased significantly during two weeks of storage from 3.99 at zero day to 3.54 after 14 days of storage (Table 1). The decrease in pH may be due to conversion of pectin into pectic acid, which increases acidity and decreases pH of the juice. Similar results were observed by Mehmood et al. (2008) who studied the effect of pasteurization and chemical preservatives on the quality and shelf stability of apple juice stored at ambient temperature for three months. The decrease in pH was also observed by Hussain et al. (2011) who worked on apple and apricot blended juices stored with sodium benzoate at refrigeration temperature for three months. Total soluble solids (TSS) of phalsa juice also decreased significantly from 11.12 on zero day to 9.55 after two weeks of storage at room temperature. The decrease in total soluble solids might be due to break down of polysaccharides into oligo and monosaccharides and to further fermented products of lower molecular weights. These results are in agreement with those reported by Zeb et al. (2009) on preservation of grape juice containing sodium benzoate and potassium sorbate and stored at room temperature for one month.

Table 1. Variation in pH and TSS of phalsa juice on storage

Storage period (days)	pH	TSS (°Brix)
0	3.99^{a}	11.12^{a}
7	3.70^{b}	10.98^{ab}
14	3.54^{c}	9.55^{b}

Means followed by same letters do not differ significantly at 5 % level.

Changes in the Microbial Activity of Juice during Storage

Changes in the number of total plate count (TPC), mould and yeast were also observed in phalsa juice on storage (Table 2). On 0 day of juice storage TPC and yeast counts were 6.2×10^{-1} cfuml^{-1} and 2.6×10^{-1} cfuml^{-1}, respectively and mould count were recorded as nil. The TPC reduced to almost half of the original counts, after two weeks storage at ambient temperature, whereas yeast and mould counts increased significantly to 5.5 x 10^{-1} and 2.4 x 10^{-1} cfuml^{-1}, respectively on storage under same conditions (Table 2).

Table 2. Variations in total plate count, yeast and mould of phalsa juice on storage

(cfu ml^{-1})

Storage period (days)	Total plate count	Yeast	Mould
0	$6.2^{a}\times10^{-1}$	$2.6^{c}\times10^{-1}$	Nil
7	$4.2^{b}\times10^{-1}$	$4.5^{b}\times10^{-1}$	$1.2^{b}\times10^{-1}$
14	$3.2^{c}\times10^{-1}$	$5.5^{a}\times10^{-1}$	$2.4^{a}\times10^{-1}$

Means followed by same letters do not differ significantly at 5% level.

TPC value decreased with the passage of time because of sugar fermentation resulting in significant increase in the acidity (decrease in pH) of juice which did not favour most of the bacterial growth. When

Table 3. Gulf standards of total plate count, yeasts and moulds in juices

Microbial count	Maximum count anticipated	Maximum count permitted
Total plate count	5.0×10^{3}	1×10^{4}
Yeast	1×10^{2}	1×10^{3}
Mould	1×10^{2}	1×10^{3}

Source: Gulf standards, (2000)

Table 4. Effect of storage on the sensory attributes of phalsa juice

Storage period (days)	Colour	Texture	Taste	Flavour	Overall acceptability
0	9.0[a]	8.8[a]	8.7[a]	9.0[a]	8.9[a]
7	7.6[b]	7.0[b]	8.2[ab]	8[ab]	7.5[b]
14	4.0[c]	5.0[c]	4.0[b]	4.2[b]	4.5[c]

Means followed by same letters do not differ significantly at 5% level.

fermentation process speeded up the yeast growth also increased rapidly because of favourable conditions (Morgan et al., 2012). Phalsa juice remained microbiologically acceptable during the storage period as per Gulf standards for TPC, yeast and moulds in juices (Table 3).

Effect of Storage on Sensory Qualities

The colour of the fresh juice was bright clear red and scored 9.0. With the passage of time the juice changed its colour and became dark and after 14 days of storage it turned into brown and scored 4.0 (Table 4). Decline in colour may be due to degrading of anthocyanin as reported by Zeb et al. (2009) in grape juice stored at ambient temperature.

The change in colour in the phalsa juice on storage is also expected on account of modification in the ionic structure of anthocyanin molecules by the acidity value produced on storage. Colour change has also been observed by Shakoor et al. (2013) while studying strawberry juice.

The score for texture/mouth feel of the phalsa juice also showed a decline during 2 weeks storage period. The score of 8.8 of the attribute reduced to 7.0 after one week storage which further reduced to 5.0 till storage ending. These results are in agreement with the earlier finding of Shakoor et al. (2013), Hussain et al. (2011) and Zeb et al. (2009).

The taste and flavour score of phalsa juice also showed gradual but significant decline during storage. The score for both the attributes reduced to almost half of the original values after 14 days of storage. Decreases may be due to change in pH and acidity of juice. These results are in agreement with findings of several research workers (Hussain et al. 2011; Zeb et al. 2009 and Shakoor et al. 2009).

Since all the quality attributes were impaired during storage this leads to diminution of the overall quality and hence did with overall acceptability score.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Ms. Ambreen Akhtar Saddozai	Research planning and microbial analysis, Data collection
2.	Mr. Amer Mumtaz	Technical input at every step, Results and discussion
3.	Dr. Saeeda Raza	Conceived the idea, References
4.	Dr. Shahzada Arshad Saleem	Literature review and writing introduction

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