

## MICROBIAL COUNT AND SHELF LIFE OF STRAWBERRY JUICE

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**ABSTRACT:-** A study was conducted to investigate the influence of microbiological activity on different quality factors of strawberry juice during storage. Strawberry was purchased from local market and its juice was kept for two weeks at room temperature to check its shelf life. Total plate count (TPC), pH, flavor, color, yeast and moulds were checked weekly. Quality changes of strawberry juice during storage can be caused both by microbiological and physiological processes. At day 0; pH 3.2, color bright red, excellent flavor, TPC  $2.7 \times 10^2$  cfu g<sup>-1</sup> were recorded, while no yeast or mould were found. After one week, the pH 3.6, dull red color, TPC  $2.25 \times 10^2$  cfu g<sup>-1</sup>, yeast  $1.7 \times 10^3$  cfu g<sup>-1</sup> and mould  $1.6 \times 10^5$  cfu g<sup>-1</sup> were observed. While after two weeks (day 14) color was brown while pH 4.0, TPC  $1.9 \times 10^2$  cfu g<sup>-1</sup>, yeast  $2.47 \times 10^6$  cfu g<sup>-1</sup> and mould  $1.6 \times 10^5$  cfu g<sup>-1</sup> were noticed. Yeast, mould and pH were increased with the passage of time. Spoilage can be controlled by keeping the juice in refrigerator and using preservatives. Thus with good storage conditions, the shelf life of the strawberry juice can be increased satisfactorily.

*Key Words: Strawberry; Juice; Shelf Life; Total Plate Count; Yeast; Mould; Pakistan.*

### INTRODUCTION

The garden strawberry, *Fragaria × ananassa*, is a hybrid species that is cultivated worldwide for its fruit. The fruit is widely appreciated for its characteristic aroma, bright red color, juicy texture, and sweet-ness.

The garden strawberry was first bred in Brittany, France, in the 1750s via a cross of *Fragaria virginiana* from eastern North America and *Fragaria chiloensis*, which was brought from Chile by Amédée-François Frézier in 1714. The first garden strawberry was grown in France during the late 18th century. Prior to this, wild strawberries and cultivated selections from wild strawberry spe-cies

were the common sources for the fruit.

In worldwide 366,889t strawberry is produced. Strawberry is one of the high value crop that has been cultivated in different parts of Pakistan for a long time but its commercial plantation in Sindh started about ten years ago in Khairpur. Based on rough estimate strawberry plantation is done over 250 acres, in different areas of Khairpur i.e., Ripri, Garihimori, Pano Akil. The varieties grown in Pakistan are Chandelier, Corona and Stuff. These are mostly sour and small in size. Pakistan is producing a limited quantity of strawberries. These are a number of reasons for restricted production, like the

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climate, size and taste. By overcoming problems related to quality, quantity and perish-ability of the fruit, Pakistan can also export it to Europe, the United State and Middle Eastern countries.

Strawberries are well known for their extensive flavor and are widely used to prepare juices (Bood and Zabetakis, 2002). Factors such as maturity at the moment of processing or straw-berry variety, could substantially affect the content in aroma compound (Forney et al., 2000). There is no one variety of strawberry which possesses all the desirable characteristics. Some are superior to others only because of certain characteristics. Some of its superior varieties enjoy color that is very attractive.

Nutritionally, strawberry contains low calorie carbohydrate, fibers and provides more Vitamin C than oranges. The main constituents of fruits per 100g are, Vitamin C (64.0 mg), water (91.75 g), protein (0.61 g), fat ( 0.37 g), carbohydrate (7.02 g), fiber (2.3 g), calcium (14 mg) and potassium (160 mg).

The pH of strawberry ranges between 3.27 and 3.80, which helps in stabilizing the color. Acidity ranges between 0.58% and 1.35 %, citric and malic acid are primary organic acid contributing excellent flavor. The main characteristics related to the quality of ripe strawberry are texture, flavor (soluble sugar and organic acid) and color (anthocyanin content). During ripening starch is converted into sugar, principally sucrose, glucose and fructose; the total concentration of sugar is 4% in the juice of strawberry.

Strawberries are non-climatic fruit with limited shelf life and

complex surface structure due to its high sensitivity to chemical and microbial deterioration during postharvest storage and handling. It may be harvested at full maturity to achieve the maximum quality in relation to flavor and color. As a consequence, strawberries should be harvested ready for consumption and due to this; strawberries fruit has a very short shelf-life at its best quality (Larsen et al., 1992).

Prices of fresh strawberry at the beginning of season is between Rs.100 and Rs.120 per kg in big cities. The wholesale price of the fruit comes down to Rs 50 per kg during the second fortnight of March when the crop production is at its peak. At that time it is economical to prepare its juice and store to consume during off season.

Strawberries may be contaminated during harvesting, postharvest handling and processing, the most common pathogen is hepatitis A virus introduced via an application of contaminated irrigation water or by contact with infected food handlers (Ayala Zavala et al., 2004). Natural reservoirs of enteric bacteria such as Salmonella and Escherichia coli 157 are found with those of hepatitis A virus and are spread by animal faeces. Hence, these pathogenic bacteria may contaminate strawberries that grow in close association with soil and harvested by hand (Harris et al., 2003).

Strawberry pigment extract can be used as a natural acid/base indicator due to the different color of the conjugate acid and conjugate base of the pigment. Strawberries contain , an antioxidant that has been studied in relation to Alzheimer's disease and to kidney failure resulting from diabetes. In

addition to being consumed fresh, strawberries can be frozen, as well as dried and used in prepared foods, such as cereal bars. Strawberries are a popular addition to dairy products, as in strawberry-flavored ice cream, milkshakes, smoothies, and yogurts. Depending on area, strawberry pie, strawberry rhubarb pie, or strawberry shortcake are also popular. The aim of present studies was to investigate the shelf life of strawberry juice (microbial and physical changes) at room temperature.

### MATERIALS AND METHOD

Strawberries were purchased from local market of Islamabad, washed thoroughly, processed into juice and stored at room temperature to determine the shelf life of prepared juice at Food Science and Product Development Institute, National Agricultural Research Centre (NARC), Islamabad. The prepared juices were analyzed for sensory evaluation by a panel of 10 judges a (Larmond, 1977). The parameters included color, taste and flavor. The juice was also tested for microbiological analyses i.e., total plate count, yeast and moulds (FAO, 1992). According to this method 50ml sample was homogenized with Butter field's phosphate buffer (pH 7.2). Serial dilutions of juice were prepared by transferring 1ml of sample in 9ml of sterile phosphate buffer in test tubes. After each dilution, the contents were mixed in vortex-mixer for 10 sec. One milliliter volumes of each dilution were transferred to Petri dish with plate count agar and mixed with medium in triplicate. After solidification 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 the mean count of triplicate of Petri dishes, considering the dilutions.

For yeast and mould spread plate method was used. Yeast growth was checked on plate count agar amended with 40 ppm Chloramphenicol (added as antibacterial agent). Potato dextrose agar was used to detect moulds. About 0.1ml inoculum of each dilution was placed on the center of solidified potato dextrose agar and plate count agar in a Petri plate and used a sterile bent glass rod for spreading. The inoculum was spread on the surface of media and incubates at 25°C for 96h.

### RESULT AND DISCUSSION

The considerable changes in quality parameter were reported in the sample (strawberry juice) during storage at room temperature (Table 1). It is evident that the pH value of fresh juice was 3.2. Deirdre et al. (1999) had also reported the pH range of strawberry juice as 3.4-3.6 which shows it is acidic in nature. The pH of the juice increased with the passage of time. At the start of study, the pH of the sample was 3.2, while after 7 days the pH increased to 3.6 and further increase in pH i.e., 4.0 was recorded after two weeks of storage (Table 1). The increase in the pH is might be due to the increase in alcoholic contents in the juice during storage (Forney et al., 2000).

At day 0, the color of the sample was bright clear red. On day 7, a little change in the color was observed. However, a dark red color was recorded later. On day 14, brown

**Table 1. Shelf life of strawberry juice**

Day	pH	Color	Flavor	Total plate count (cfug <sup>-1</sup> )	Mould (cfug <sup>-1</sup> )	Yeast (cfug <sup>-1</sup> )
Day 0	3.2	Bright, clear red	Excellent	2.7×10 <sup>2</sup>	Nil	Nil
Day 7	3.6	Dull red	Satisfactory	2.25×10 <sup>2</sup>	1.4×10 <sup>3</sup>	1.7×10 <sup>3</sup>
Day 14	4.0	Brown	Unsatisfactory	1.9×10 <sup>2</sup>	1.6×10 <sup>5</sup>	2.47×10 <sup>6</sup>

color was recorded. Studies of Pino et al. (2001) and Silas et al. (2006) showed that a progressive change from a bright, clear red to dull red and finally to brown darkening in the color of strawberry occur during the storage at room temperature. They also noticed that color changes occurring in the juice could partially be overcome by storage at low temperature.

Strawberries are well known for their exquisite flavor and are widely used to prepare juices (Bood and Zabetakis, 2002). The flavor of fresh strawberry juice was excellent, while after 7 days storage, the flavor was satisfactory. The flavor became unsatisfactory after 14 days of storage. With the passage of time the flavor of the sample was degraded due to compositional changes in the sample. Strawberry juice flavor compounds have shown low stability during storage in various studies (Mosqueda-Melgar et al., 2008). Lundahl et al. (2006) observed pronounced losses of ascorbic acid in strawberry juice with marked change in flavor during storage. Alcohols have been identified as constituents of the aroma profile in strawberries. The major alcohol identified in strawberry juice was 1-butanol which was affected after a week of storage. After a long period of storage at room

temperature, an increase in 1-butanol concentrations can render an unpleasant flavor to the product, due to its strong, pungent smell and taste.

Factors such as maturity at the moment of processing or strawberry variety, could substantially affect the color, flavor, pH and microbiological characteristics of strawberry juice (Forney et al., 2000). The total plate count decreased from 2.7×10<sup>2</sup> to 2.25 ×10<sup>2</sup> cfug<sup>-1</sup> during one week storage at room temperature. After 2 weeks it decreased to 1.9×10<sup>2</sup> cfug<sup>-1</sup> due to low pH of the sample. Bacterial growth is eliminated to a very large extent by acidity, bacterial spores will not germinate in juice with the pH less than 4.5 and vegetative cells of pathogenic bacteria will not grow at pH less than 4.0 (Smelt et al., 1982).

Moreover, no mould and yeast growth was observed in the sample at 0 day. After 7 days 1.4×10<sup>3</sup> cfug<sup>-1</sup> yeast, 1.7×10<sup>3</sup> cfug<sup>-1</sup> mould were recorded. After 2 weeks 1.6×10<sup>5</sup> cfug<sup>-1</sup> yeast and 2.47×10<sup>6</sup> cfug<sup>-1</sup> mould were recorded. The reason of yeast growth is due to the presence of sugars (carbohydrates) in the sample.

According to the Gulf standards the maximum anticipated and permitted count of TPC for juices is 5.0 × 10<sup>3</sup> and 1 × 10<sup>4</sup>, respectively

(Table 2). While in the present study the TPC is lower than the anticipated value as well as permitted value. However the bacterial load is decreased with the passage of time. This is due to the lower pH than that of the pH needed for bacterial growth.

The Gulf standards show that the yeast and mould count should not be more than  $1 \times 10^3$  cfu ml<sup>-1</sup> in juices. Present study shows that at day 0, there was no yeast growth in the sample but with the passage of time the yeast growth was observed. The microbiological status of strawberry juice is not affected very much during storage at room temperature if it is carefully handled. However, some studies have shown a significant increase in the microbiological populations of strawberry juice during storage. The TPC increased during storage in some cases. Moreover, mould and yeast growth has also been observed by various researchers during their work on strawberry juice (Amanatidou et al., 2003).

The freshly prepared juice got the higher score and overall acceptability was 9 (Table 3). But after 7 days there was little change in colour, taste and flavor and overall acceptability was 5. After 14 days overall acceptability was 1.5. This shows the progressive deterioration

**Table 2. Gulf standard for microbiological criteria for juices**

Count	(cfu/ml <sup>1</sup> )	
	Maximum count anticipated	Maximum count permitted
Total plate count	$5.0 \times 10^3$	$1 \times 10^4$
Yeast	100	$1 \times 10^3$
Mould	100	$1 \times 10^3$

**Table 3. Sensory evaluation of strawberry juice**

Duration (day)	Color	Flavor	Taste	Overall acceptability
0	9	9	9	9
7	6	5	4	5
14	2	1	Not fit to taste	1.5

and quality degradation of juice stored at room temperature.

It is thus concluded that the strawberry juice quality is affected during storage by microbiological and physiological processes. However the spoilage can be controlled by processing and storage under aseptic/sanitation conditions. The shelf life of the strawberry juice can be increased in a satisfactory way by keeping in refrigeration and by using preservatives.

**LITERATURE CITED**

Amanatidou, A. Slump, R. A. and Smid, E. J. 2003. Microbial interactions on minimally processed carrots under elevated oxygen and carbon dioxide concentrations. *Acta Horti-culturae*, 600: 621-628.

Ayala-Zavala, J. F. Wang, S. Y. Wang, C. Y. and González-Aguilar, G. A. 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT-Food Sci. and Technol.* 37: 687-695.

Bood, K. G. and Zabetakis, I. 2002. The biosynthesis of strawberry flavor (II): Biosynthetic and molecular biology studies. *J. Food Science*, 67: 2-8.

- FAO, 1992. Manual of Food Quality Control, 4. Review, Microbiological Analysis FAO Food and Nutrition paper. Food and Agriculture Organization of the United Nation, Rome.
- Forney, C. F. Kalt, W. and Jordan, M. A. 2000. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. Hort. Science, 35: 1022-1026.
- Harris, L.J. Farber, J.N. Beuchat, L.R. Parish, M.E. Suslow, T.V. Garrett, E.H. and Busta, F.F. 2003. Outbreaks associated with fresh produce, incidence, growth and survival of pathogens in fresh and fresh-cut produce. Compr. Rev. Food Sci. Food Safe, 2:78-141.
- Larmond, E. 1977 Laboratory Methods for sensory evaluation of foods. Research branch, Canada. Dept. of Agriculture Pub No.1637.
- Larsen, M. Poll, L. and Olsen, C. E. 1992. Evaluation of the aroma composition of some strawberry (*Fragaria ananassa Duch*) cultivars by use of odour threshold values. Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 195: 536-539.
- Lundahl, D.S. McDaniel, M. R. and Wrolstad, R.E. 2006. Flavor, aroma, and compositional changes in strawberry juice concentrate stored at 20° C. J. Agric. and Food Chem. 16: 90-101.
- Mosqueda-Melgar, J. Raybaudi-Massilia, R. M. and Martín-Belloso, O. 2008. Nonthermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. Innov. Food Sci. and Emerging Tech. 9: 328-340.
- Pino, J. A. Marbot, R. and Vázquez, C. 2001. Characterization of volatiles in strawberry guava (*Psidium cattleianum Sabine*) fruit. J. Agric. and Food Chem. 49: 5883-5887.
- Silas, R. Ronald E. and Wrolstad, 2006. Effects of mold contamination and ultrafiltration on the color stability of strawberry juice and concentrate. J. Agric. and Food Chem. 60: 130-145.
- Smelt, J.P.P.M. Ratjes, J.G.M. Crowther, J.S. and Verriós, C.T. 1982. Growth and toxin formation by *colestridium* at low pH values. J. Appl. Bacteriol. 31: 525 - 529.