

Review Article

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Review on Biochemical Changes Associated with Storage of Fruit Juice

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ABSTRACT

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Intake of fresh vegetables and fruits has an inverse association risk for developing non-communicable diseases primarily the coronary heart disease and nutritional disorders. It also reduces risk of obesity and to maintain a healthy body weight. High perishable nature of fruits and vegetables is detrimental in availability of these products for longer time so single strength or blended fruit juices can be alternative for nutritional security to human health. A wide range of biochemical changes occur during storage of juices at different temperature which reduces the nutritional value. However, proper understanding of these changes will help us to maintain the nutritional quality during processing and storage of fruit juice. The common biochemical changes studied are TSS (Total Soluble Solids), Ascorbic acid, titratable acidity, pH, sugar, non-enzymatic browning and microbial growth.

Introduction

Intake of fruit and vegetables in human diet has significant role in reducing disease burden mortality in both developing and developed world. Low intake of these commodities had accounted for more than 2.6 million death throughout the world (Lock *et al.*, 2005). Fruits and vegetables are good sources of vitamins, minerals, dietary fibre, phytochemicals (bioactive compounds) and antioxidants. High intake of these products provides protection against many non-communicable diseases like cardiovascular disease (Lock *et al.*, 2005), stroke (He *et al.*, 2006), certain cancers (Gandini *et al.*, 2000; WCRF, 2007), type-2 diabetes mellitus (Carter *et al.*, 2010), the metabolic syndrome (Ford *et al.*, 2003) and coronary diseases

(Abd-Ghafar *et al.*, 2010). However, consumption of fresh fruits and vegetables is matter of availability in particular season as most of them are seasonal and are having very small shelf life. Thus, processed products of fruits and vegetables are of great significance which can be preserved for long duration with minimum loss in consumptive value. Among different processed products, fruit juices are gaining wider acceptability in dietary pattern throughout the world.

The fruit juice is very refreshing, delicious and soothing (Roger *et al.*, 2002). Abeyasinghe *et al.*, (2007) had reported the presence of antioxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activity

and many bioactive compounds like ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidine, vitamin-C, carotenoid and naringin in citrus fruit juice (Xu *et al.*, 2008). However, processed juice suffers a wide range of biochemical changes during storage so required to be processed properly, preserved under appropriate conditions with suitable additives. Pasteurization, low temperature storage and concentrates making are promising methods of fruit preservation. Use of chemical preservatives, however, plays a very important role in ensuring safety and quality of stored foods. Preservatives are chemical agents intentionally added to food products to prevent or inhibit spoilage caused by moulds, yeasts and bacteria. The most common preservatives which are being used in fruit processing industries are salts releasing sulphur dioxide and salts of benzoic acid. Thus, biochemical changes taking place during preservation of fruit juice and their regulation to improve keeping quality has been reviewed in this manuscript (Figure 1).

TSS variation in fruit juices during storage

A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids. Bhardwaj (2013) proposed about the gradual passage of storage time as a function of increase in TSS which may be due to greater hydrolysis of polysaccharides. However, this rise in TSS is functional to storage temperature and a direct relation has been reported between increase in TSS and storage temperature. This might be correlated with lower rate of hydrolysis of sugars, poly-saccharides and organic acids at lower temperature following the La Chatelier Principles of chemical reactions. Prasad and Mali (2000) had advocated about least increase in TSS under refrigerator storage of Kinnow juice due to reduced hydrolysis of

polysaccharides and acids. Bahardwaj and Nandal (2014) had also proposed similar finding to show that TSS of blended Kinnow juice is directly correlated with storage duration and increase in TSS is higher at ambient condition due to high rate of solubilization or hydrolysis of acid into sugars. Similar, finding have been confirmed by Deka and Sethi (2001) in mixed fruit juice spiced beverage, Singh and Mathur (1983) in cashew apple juice and Bhardwaj and Mukherjee (2011) in Kinnow juice.

Ascorbic acid variation in fruit juices during storage

Ascorbic acid degradation is common in all consumable items during storage and can occur aerobically as well as anaerobically. However rate of aerobic degradation is 100 to 1000 times faster than anaerobic degradation. Vitamin C is light and heat sensitive, the concentration of Vitamin C follows first order kinetics and thus storage time affects Vitamin C content (Heldman and Singh, 1981). Blasco *et al.*, (2004) had also reported two different degradation pathways of vitamin C during storage. Aerobic degradation was observed in the beginning due to presence of oxygen in the bottle but after prolonged heating the bottle becomes saturated with vapor and becomes oxygen deficient which induced anaerobic degradation. Carvalho *et al.*, (2007) reported a decreasing trend in vitamin C content in cashew apple juice blended with coconut water during storage. Similar trend was also reported in cashew apple juice by Maia *et al.*, (2001) and Costa *et al.*, (2003). Kabasakalis *et al.*, (2000) had found 29-41% of ascorbic acid loss after 4 months when fruit juices were stored at room temperature while Burdulu *et al.*, (2006) found 27.3 -45.3 % loss in ascorbic acid when orange juice was stored at 28⁰C for 2 months and was in line to findings of Burdulu *et al.*, (2006). Majumdar *et al.*, (2009) reported 74% loss in ascorbic

acid in cucumber + litchi + lemon blended juice stored for 6 months.

The stability of ascorbic acid in consumable items is also affected by temperature, pH, sunlight and the presence of metals like copper and iron (Bhargawa *et al.*, 2014). Thus, storage condition has greater influence over ascorbic acid retention in food components. In presence of light and temperature ascorbic acid is oxidized to dehydroascorbic acid so causing significant loss in beverages and nectars (Ahmed *et al.*, 2008; Kalra and Tondon, 1984). Ahmed *et al.*, (2008) studied storage quality of citrus juice and reported that ascorbic acid contents decreased significantly at all storage intervals which depends on the processing methods, storage duration and light exposure.

Storage temperature is one of the measure contributing factors for ascorbic acid degradation during storage as it is highly thermal sensitive. Pasteurization of fruit juice produces p-vinylguaiacol (PVG) and induces ascorbic acid degradation (Naim *et al.*, 1997). Lotha *et al.*, (1994) reported that Kinnow mandarin fruit stored at ambient temperature (9⁰C-24⁰C; RH 65-90%) have life of 22 days and the amount of ascorbic acid was reported to decrease where the fruit was stored at refrigerated temperature (3⁰C; RH 70-80%) with a shelf life of 60 days. Cvetković and Jokanović (2009) reported that after 30 days of storage at 4-8⁰ C overall loss in ascorbic acid was from 81.01% to 90.27% in thermally pasteurized samples. According to Vikram *et al.*, (2005), the degradation was rapid at higher temperatures. The ascorbic acid degradation rate in the orange–carrot juice stored at 2⁰C was less than in the juice stored at 10⁰C, and in the pasteurized juice it was greater (Torregrosa *et al.*, 2006).

Application of materials with anti-oxidant properties or antimicrobial properties has

potential to improve retention of ascorbic acid during storage of fruit juice. Sulphur dioxide acts as good antioxidant for ascorbic acid so SO₂ fumigation or using sulfites, metabisulphites of sodium or potassium reduces oxidation and microbial degradation of ascorbic acid. El-Ashwah *et al.*, (1981) stated that sulfites and meta-bi-sulphite of sodium or potassium can be added to fruit juices as potential sources of sulfur dioxide to stabilize ascorbic acid. The application of KMS (potassium meta-bi-sulphite) to reduce the loss of ascorbic acid during the storage of leafy vegetables has also been reported by Negi and Roy (2000). Mathooko and Kinyi (2002) proved that sodium metabisulfite had significant effect on stabilizing the ascorbic acid and its concentration had directly influenced the ascorbic acid retention. Zheng and Wang (2001) had also reported upto eighty percent loss in ascorbic acid content in Kinnow nectar however, significant retention in ascorbic acid was reported in aloe juice blended Kinnow nectar confirming positive impact of phenolic components present in aloe juice. Jain and Khurdiya (2009) in aonla juice study confirmed that pasteurization action at low temperature, use of sulphites and low temperature storage improved ascorbic acid retention.

Acidity variation in fruit juices during storage

The titratable acidity of fruits or fruit juice includes the organic acids predominantly present in fruits (Table 1). These organic acids are of high nutritional values and are useful in extending shelf life of fruit juice during storage. However, these are highly sensitive to temperature, storage condition and duration. The organic acids undergo degradation during storage which might be due to conversion of acids into sugar and salt by invertase enzymes as suggested by Jain *et al.*, (1986). Singh *et al.*, (2005) advocated that

titrable acid content in bael beverage was significantly decreased during 6 months storage, which is in conformity with Singh *et al.*, (2009) for 74 days storage of Kinnow juice. Bhardwaj and Nandal (2014) had reported decrease in acidity of stored Kinnow juice due to oxidation as the period of storage advanced but the decrease was lower under refrigerated storage condition due to enzymatic inactivation. These findings are in conformity with Sethi *et al.*, (1980) in long duration Kinnow juice storage at ambient and refrigerated condition. Pareek *et al.*, (2011, 2015) had also reported declining trend of acid loss in mandarin juice during 180 days of storage which was found to be declined at low temperature and high humidity due to reduced conversion of acids into sugar and salt by invertase enzymes. They had further advocated the minimum loss of acidity during storage when treated with KMS and the loss was found to be reduced as the concentration of KMS increased.

pH variation in fruit juices during storage

The pH of fruit juice is negative function of natural acidity in the juice, thus increase in pH is accompanied with decrease in acidity of fruit juice during storage (Rehman *et al.*, 2014). Rehman *et al.*, (2014) described that the possible reason for increase in pH with prolonged storage of Kinnow juice may be the acid hydrolysis of the poly-saccharides into mono-saccharides and di-saccharides which are responsible for increase in sweetness and decrease in sourness (Dhaka *et al.*, 2016). The results of present investigation are in line with the findings of Alaka *et al.* (2003). The acidity of juice is also influenced by preservatives added and storage condition. Nwachukwu and Ezeigbo (2013) revealed a synergism between pasteurization; acidification and sodium benzoate treatment and reported an increase in pH of soursop juice and decrease in acidity which was more

in pasteurized juice in comparison to low value in sodium benzoate treated juice.

Sugar variation in fruit juices during storage

The fruit juice contains various reducing and non-reducing sugars which tend to change during storage due to various interconversion processes. The increase in total sugars could be result of hydrolysis of polysaccharides like pectin, cellulose and starch into simple sugars as reported by Singh and Mathur (1983) in cashew apple juice. They had proposed that an increase in reducing sugar during storage may be due to gradual conversion of non-reducing sugar and acids into reducing sugars. Ahmed *et al.*, (2008) reported that a significant increase in reducing sugar during storage of citrus juice which may be due to acid hydrolysis of sucrose (non-reducing sugar) to glucose and fructose. Similar observations were also reported by Babsky *et al.*, (1986), and Pruthi *et al.*, (1984). However, these decline in non-reducing sugar was slow and non-significant at initial phase of storage while significant at later stage. A similar decline in non-reducing sugar was observed by Sandi *et al.*, (2004), during storage of fruit drinks.

The accumulation of reducing sugars has been reported to be more in sulphur dioxide or KMS preserved fruit juice which could be attributed to acid induced hydrolysis of polysaccharides and disaccharides into monosaccharides (reducing sugar) due to formation of sulphurous acid (Pareek *et al.*, 2015). Similarly, Garg *et al.*, (2008) had also reported increase in reducing and total sugar content during storage in blended Indian gooseberry juice treated with 0.05% KMS. Amin *et al.*, (2008) had also observed pronounced similar effect of chemical preservatives like potassium meta-bi-sulphite (KMS), citric acid and sodium benzoate (SB)

on sugar content of mango pulp. Shahnawaz *et al.*, (2015) reported a non-significant but higher total sugar and reducing sugar in orange juice treated with sodium-benzoate (1.0g/1000 ml). The rising temperature during storage also accelerates hydrolysis of acids and poly-saccharides into simple sugars (Bhardwaj and Nandal, 2014). Sarmah *et al.*, (1981) observed considerable increase in reducing sugar content in single strength Kinnow mandarin juice in the samples at room temperature as compared to those kept at low temperature.

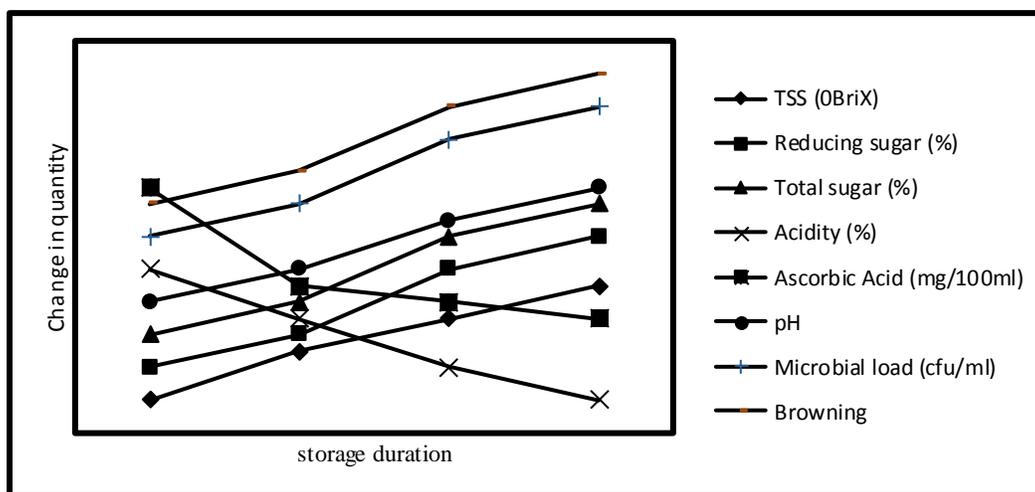
Browning of fruit juice during storage

Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as Maillard reaction. HMF (hydroxymethylfurfural), formed in the Maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food (Arribas-Lorenzo and Morales, 2010).

Table.1 Sources and importance of various organic acids

S.No.	Organic Acid	Fruits	Importance
1	Citric Acid	Citrus fruits, tomato, berries, pineapple, pear, cherries and lettuce	Inhibits formation of stones in kidney and stimulates the breakdown of smaller kidney stones.
2	Malic Acid	Apple, pear, water melon, apricots, bananas, blackberries, cherries, grapes, kiwi, lychees, mango, nectarines, oranges, peaches, pears and strawberries, tomato, broccoli and rhubarb	It is used in fibromyalgia and chronic fatigue syndrome. It is also used to reduce muscle soreness and increase energy levels.
3	Oxalic Acid	Leafy green vegetables, rhubarb and beets	Excess use of oxalic acid has potential risk of stone formation, however limited use can prevent further development of kidney stones.
4	Tartaric Acid	Grapes, banana, tamarind, apricots, apples and avocados	It is used as an additive and flavoring agent in food industries.

Fig.1 Common trend of change in biochemical parameters of fruit juice during storage



The formation of HMF is accelerated by storage time and temperature (Serra-Cayuela *et al.*, 2014). However, Nie *et al.*, (2013) had proposed temperature dependent three sugar-amino acid (glycine) models for Maillard reaction and reported that the mechanism is completely temperature dependent and attributed to selective sugar types, pH, temperature, and heating time. Saini *et al.*, (2000) observed that the browning in mango pulp upto 83.33% can be reduced by the application of potassium meta-bi-sulphite. Bhardwaj (2013) had reported a liner increase in non-enzymatic browning during storage of blended juice of Kinnow for 6 month. Bhardwaj and Nandal (2014) reported that low temperature lead to a decline in the enzymatic and non-enzymatic browning reaction in blended Kinnow juice in presence of KMS.

Microbial contamination of fruit juice during storage

Microbial contamination is an important aspect of food safety and standards and is measured in colonies forming units per unit of volume. Rahman *et al.*, (2011) had reported that the desirable fungal count in all products should be in order of 10^3 - 10^5 (cfu/ml). The microbial contamination can occur at any step from production to consumption but the microbial growth during storage depends on quality of packaging, storage temperature and preservatives added. Hashmi *et al.*, (2007) reported that the mango pulp stored at ambient temperature (30^0 C - 36^0 C) with 0.2% KMS showed negligible microbial growth. Chauhan *et al.*, (2002) reported that microbiological population (total plate counts, and yeast and mold counts) increased during storage of sugarcane juice at room temperature in comparison to storage at refrigeration temperature. Nwachukwu and Ezeigbo (2013) had also reported lower microbial load in sodium benzoate treated and

pasteurized soursop juice, while Ogiehor *et al.*, (2004) and Akhtar *et al.*, (2009) reported lower microbial growth due to KMS treatment. Amin *et al.*, (2008) showed reduced microbial growth in during storage of fruit juice by using Potasium sorbate, Sodium Benzate and KMS, while Deka and Sethi (2001) reported inhibitory effect of spices (ginger juice) towards microbial growth. Yigeremu *et al.*, (2001) and Mutaku *et al.*, (2005) concluded that preservatives significantly reduced the microbial growth in comparison to untreated papaya juice.

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