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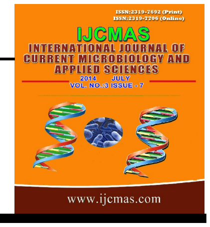

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Original Research Article

Effect of storage and Preservatives on Antioxidant Status of some Refrigerated Fruit Juices

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Keywords

Fruit juices, antioxidant, flavonoids, phenolics, ascorbic acid

A B S T R A C T

Limiting or preventing the growth of undesirable microbial flora in food products is one of the main goals of food preservation. A number of preservation methods have been designed to extend the shelf-life of the food products, not only by reducing the microbial growth, but also to maintain the antioxidant potential to serve the consumers' needs. The aim of the present study was to compare the changes in the contents of antioxidant biomolecules like total flavonoids, total polyphenols and ascorbic acid of seven fruit juices available in local markets of Kolkata, before and after storage in refrigerated conditions. Effect of sodium benzoate as preservative was also adjudicated during storage. It was observed that the flavonoids content, total phenol contents and ascorbic acid contents of the fruit juices have been deteriorated after 10 days of refrigeration if no preservative was added. However, the juices retained some of their bioactives on addition of the preservative, even after 10 days refrigeration. Retention of flavonoids and phenolics indicated that sodium benzoate might not only inhibiting microbial growth, but also scavenged free radicals generated due to oxidation over time. The study indicated a plausible way of preserving commercial fruit drinks during refrigerated storage.

Introduction

Many nutritional factors are widely considered to be critical for human health. Among them, free radicals have been of concern as one of the factors contributing to chronic degenerative disease (Parkin et al, 2001). Due to the increased prevalence of chronic degenerative diseases, people are more aware of their food consumption. In the body, free radicals are derived from two sources – endogenous sources, e.g. nutrient metabolism and the ageing process, and exogenous sources, e.g. air pollution (Patthamakanokporn et al, 2008). Once formed, these highly reactive radicals can start a chain reaction and can damage important cellular components such as

DNA, proteins or the cell membrane. To prevent free radical damage, the body has a defense system of antioxidants, which include several enzyme systems (e.g. SOD, CAT) and small molecules like vitamin E, beta-carotene, and ascorbic acid. Some phytochemicals (e.g. polyphenols, flavonoids and lignans) obtained from foods are also important for these purposes (Boyer and Liu, 2004).

It was reported that regular consumption of fruit juices, rich in polyphenols, can enhance the protective effects against numerous degenerative diseases. Moreover, regular fruit juice consumption was reported to reduce the risk of several chronic diseases. Nowadays, there are many types of fruit juices available commercially in the market. The consumption of the fruit juices is popularizing rapidly as they are convenient, nutritious and ready-to-drink (Zabidah et al, 2011).

Fruits like orange, apple, black grapes, sweet lime, litchi, pomegranate and mango serve as good sources of many antioxidative phytochemicals. Moreover, juices of these fruits can serve as a judicial consortium of antioxidants for human health benefits. Of these antioxidative phytochemicals, flavonoids and other polyphenolics are the most important. As for example, Pomegrante juice contains tannins, ellagic tannis, anthocyanins, catechins, gallic and ellagic acid as antioxidant chemicals (Manukumar and Thribhuvan, 2014). Citrus fruits contain ascorbic acid as the major antioxidative constituent. Apples contain flavonoids like quercetin, catechin, phloridzin and phenolic acids like chlorogenic acid and coumaric acid (Boyer and Liu, 2004). Again, these fruits are replete with ascorbic acid, which is one of the major small molecule antioxidant of human physiological system.

Commercial preparation of these fruit juices, available in the local markets, are usually devoid of preservatives and can lose their effectiveness with respect to their antioxidant capacities on preservation. This is a typical problem as fruit juices are consumed usually after keeping in refrigerator for some times. This leads to a possibility of deterioration of the quality and the antioxidative capacities of the juices. The present study was designed to investigate the changes of antioxidant activity during storage at a low temperature (in a refrigerator at 4-6^{o}C) and using a common food preservative – sodium benzoate, which usually inhibits the growth of mold, yeast and some bacteria (Stanojevic et al, 2009).

Materials and Methods

Chemicals

All chemicals used in this study were purchased from Merck or SRL, India and were of AR grade. Double distilled water was used throughout the study.

Procurement of samples

The fruit juice samples were selected through non-probable, non-random purposive sampling method. The fruits selected were – orange, apple, black grapes. sweet lime, litchi, pomegranate and mango. All samples were purchased from local markets at Barasat, Kolkata. The juices were designated, respectively, as – OJ, AJ, BJ, LJ, LTJ, PJ and MJ for further studies.

Storage experiment

Sodium benzoate was added in a proportion of 0.05% (w/v) of the juices (Yadav et al, 2014). The samples were analyzed for respective bioactives at 0 day (after immediate addition of sodium benzoate) and after 10 days storage at a refrigerator (4-6°C). Samples without the preservative and kept for 10 days in refrigerator were also analyzed for comparative purposes.

Estimation of total flavonoids content

Colorimetric aluminum chloride method was used for flavonoid determination following a published procedure (Gupta et al, 2012). Briefly, 0.5 ml solution of each sample in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1ml of 1 M potassium acetate solution, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV-Vis spectrophotometer (model – Systronics 2202). Total flavonoids content were calculated using a calibration curve of quercetin as standard and the results were expressed as μg quercetin equivalent/ml juice.

Estimation of total phenolics content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Gupta et al, 2012).The samples (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) were then added. The absorbance of the reaction mixture was then measured at 765 nm with a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of μg gallic acid equivalent/ml juice.

Estimation of ascorbic acid content

Stock iodine solution was standardized by titrimetric method using standard (N/100) sodium thiosulphate solution followed by addition of 1% starch solution, when the solution turned blue and continue the titration until the blue colour just discharged. The juice samples were then titrated with the standard iodine solution following a standard procedure (Das, 2010). The results were expressed as mg ascorbic acid/100 ml juice.

Statistical analyses

All samples were analyzed in quadruplicate and the results were expressed as mean±SD.

Results and Discussion

Estimation of total flavonoids content

It is observed that the flavonoids content of the fruit juices have been deteriorated after 10 days of refrigeration if no preservative were added (Fig 1). However, it was observed that the juices retained some of their flavonoids on addition of the preservative, even after 10 days refrigeration (Fig 1). Fruit juices with preservative apparently do not show any significant difference than without preservative content fruit juices in 0 day (Fig 1). This indicated that sodium benzoate probably does not react with the phytochemicals of the fruit juices.

Phenolic compounds such as flavonoids are the most active and common antioxidants present in fruit and vegetables. These compounds have an antioxidant function which result from a combination of chelating properties and scavenging of free radicals as well as inhibition of oxidases and other enzymes (Bramorski et al, 2010). The results indicated that the flavonoids can be preserved by the use of sodium benzoate and the juices can be consumed with same effectivity as of fresh ones, even after refrigerated storage.

Estimation of total phenolics content

Here results shows that black grape and pomegranate contain high amount of total phenol than the others. The reason of it is that they contain more anthocyanins than the other juices. It was observed that the total phenol contents of the fruit juices have been deteriorated after 10 days of refrigeration (Fig 2). However, it was observed that the juices retained some of their total phenol on addition of the preservative, even after 10 days refrigeration (Fig 2). Fruit juices with preservative apparently do not show the significant difference than the fruit juices of day 0. This suggested that sodium benzoate did not react with the polyphenolics of the juices. Probably with the use of preservative, sodium benzoate, the reduction of the total phenol content in commercial fruit juices is prevented to some extent. A previous study reported that preservation of Noni (*Morinda citrifolia*) juice in refrigerator reduced total phenolics content of the juice (Yang, 2007). However, addition of sodium benzoate would improve the total phenolics content, probably by inhibiting the growth of unwanted microorganisms, which could oxidize the bioactive phytochemicals.

Estimation of Ascorbic acid content

It is observed that the ascorbic acid contents of the fruit juices have been deteriorated after 10 days of refrigeration (Fig 3). However, it was observed that there was no significant change in ascorbic acid content of all juices except mango, in addition of the preservative, even after 10 days refrigeration (Fig 3). So preservative has no further effect on retaining the ascorbic acid content in commercial fruit juices. Fruit juices with preservative apparently did not show any significant difference than the fruit juices without preservative of day 10. The reason is that they might react with the oxidants slowly. But probably with the use of preservative, sodium benzoate, the reduction of the ascorbic acid content in commercial fruit juices is decreased to some extent (Fig 3).

The consumption of fruit juices is beneficial and the health effects of fruits are attributed, in part, to ascorbic acid, a natural antioxidant which may inhibit the development of major clinical conditions including cardiovascular diseases and cancer (Rekha et al, 2012). Ascorbic acid is highly bioavailable and is consequently the most important water soluble antioxidant in cells, effectively scavenging reactive oxygen species (ROS). When relating the antioxidant activities of fruit juices to health and disease risk, it is important to consider the contribution of ascorbic acid in addition to that of phenolic compounds with antioxidant activity (Gardner et al, 2000). So addition of preservatives to commercial fruit juices might help in maintaining the quality of the juices even after keeping in refrigeration for a longer period.

During storage, these antioxidants react with free radicals, produced by aerial oxygen, and depleted. So, the concentration of phenol, flavonoids and ascorbic acid decrease during storage, even though stored at refrigerator. For these reason, fruit juices contained less amount of antioxidant than fresh one after 10 days storage. The present study indicated that sodium benzoate containing fruit juices retained higher amount of antioxidants than control juices during storage. One reason might be the fact that sodium benzoate has potent free radical scavenging activity (Marak et al, 1987), especially potent hydroxyl radicals, and can scavenge noxious radicals generated during storage.

Figure.1 Variation in total flavonoids content of the seven fruit juices for 10 days of refrigeration at 4-6°C with or without preservative

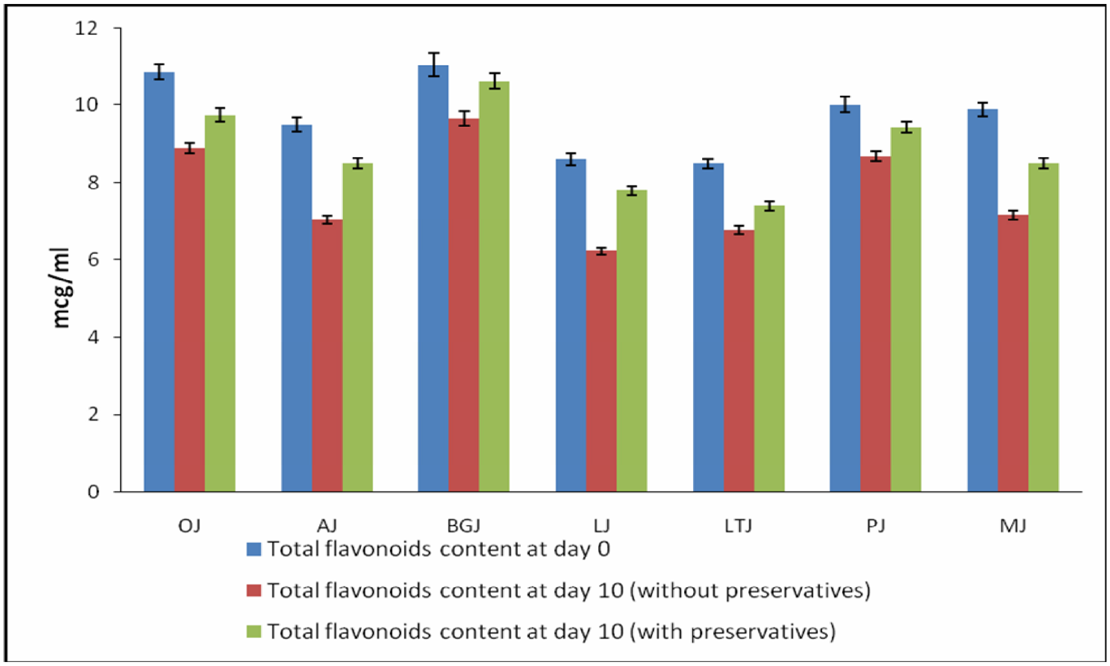


Figure.2 Variation in total phenolics content of the seven fruit juices for 10 days of refrigeration at 4-6°C with or without preservative

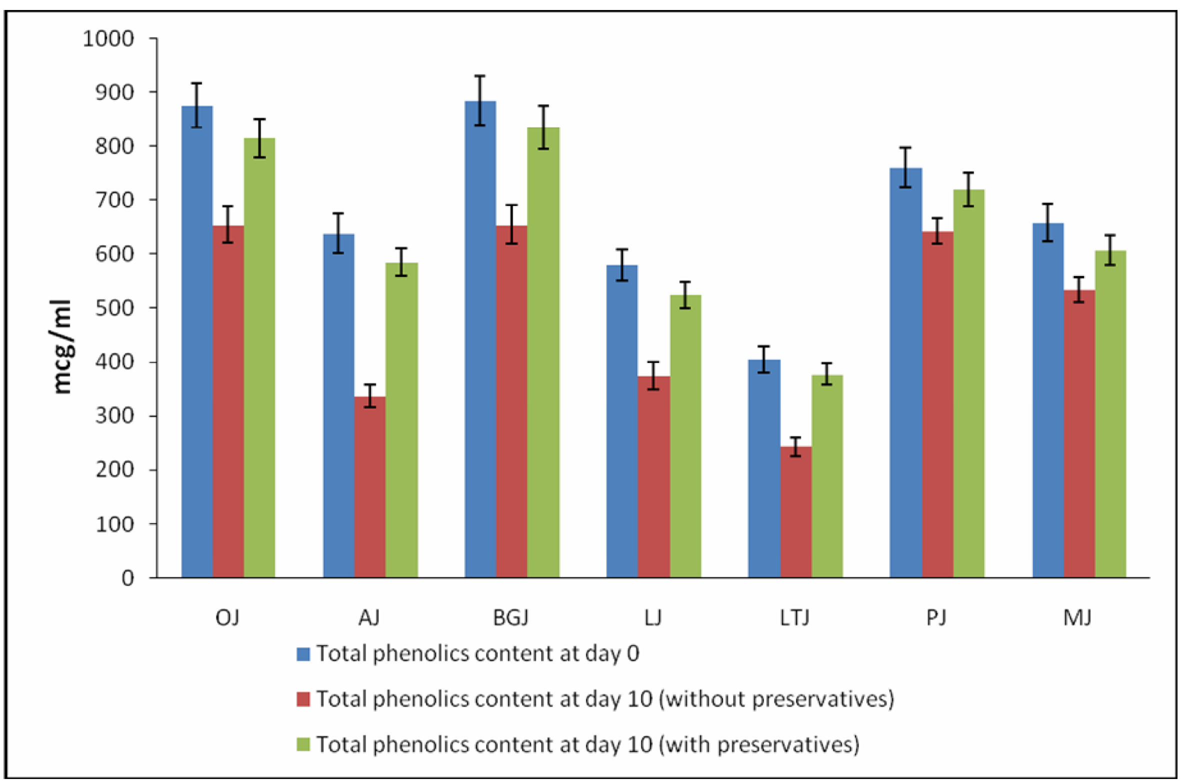
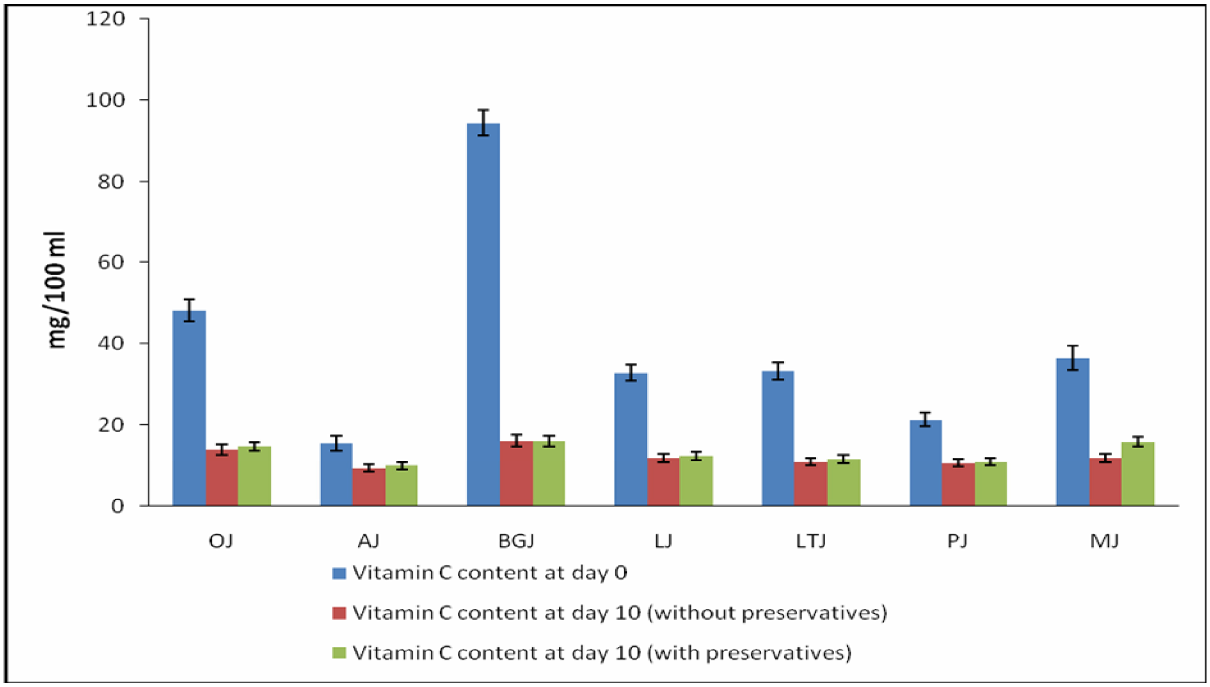


Figure.3 Variation in ascorbic acid content of the seven fruit juices for 10 days of refrigeration at 4-6°C with or without preservative



The major conclusion arising out of this study was that commercial fruit juices containing phenolics, flavonoids and ascorbic acid, might lose some of their antioxidant molecules during storage for 10 days in refrigerator. Addition of sodium benzoate, however, had protective ability of the antioxidant biomolecules. Commercial fruit juices, available in the local markets, can be preserved for at least 10 days, if 0.05% (w/v) sodium benzoate could be added. By this way, the antioxidant status of the juices could be maintained. The study would indicate a possible way to preserve fruit juices by normal consumers in the households.

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