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Impact of storage conditions on the health promoting attributes of lemon, *Citrus limon* juice

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ABSTRACT

Consumers are increasingly expecting the fruits to be tasty and attractive while being safe and healthful. The objective of the present study was to develop a lemon drink and to see the effects of different storage temperatures, presence of gasses and storage period over the bio-chemical quality of the drink. In the preparation of lemon drink, volume of lemon juice (*Citrus limon*) (10%), sugar (11%) and water (79%) were used. Lemon juice was filled in sterilized glass bottles, sealed and Pasteurized at 85oC for 15 minutes and stored at 37oC and 6oC with and without vacuum for 150 days. Biochemical changes in lemon drink were investigated after each 30 days for the period of 150 days. Following chemical parameters of fruit juice were determined : total soluble solid (TSS), acidity, pH, moisture, ash content, carbohydrate, protein, fat and ascorbic acid were quite similar for both varieties. The dark yellow color of lemon cultivar had eye appeal effect to the consumers. With the various nutritional benefits, the fruits could be recommended for commercial exploitation and preparation of different value added products. A gradual increase in acidity, total soluble solids, reducing sugars and total sugars was observed in all samples, while pH and ascorbic acid decreased gradually during storage studies. The declining trend in ascorbic acid contents of lemon drink was increased as a function of product storage. The overall results showed that samples without presence of gasses (sealed with vacuum) at 6°C gave best results for ascorbic acid retention.

KEY WORDS: LEMON DRINK, ASCORBIC ACID RETENTION, BIO-CHEMICAL PROPERTIES

INTRODUCTION

Fruits and vegetables are important sources of essential dietary nutrients such as vitamins, minerals and fibers. Since the moisture content of the fresh fruits and vegetables is more than 80% (wb); they are classified as highly perishable commodities. The overall world's fruits production was about 609,213,509 metric ton in 2010 (FAO, 2010). According to the estimates, nearly 30% of the fruits are lost due to spoilage, mishandling, during transportation and lack of cold storage and processing techniques (Singh *et al.*, 1994). Fruit juice preservation has an important role in the conservation and better utilization of fruits and vegetables in order to avoid the

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glut and utilize the surplus during the off-season. It is necessary to employ modern methods to extend storage life for better distribution and also processing techniques to preserve them for utilization in the off-season. The fruit can be preserved by converting it into products like jam, jelly, fruit bar, juice, pickle and murabba to prolong their utilizable lifespan. Fruit juice preservation has an important role in the conservation and better utilization of fruits and vegetables in order to avoid the glut and utilize the surplus during the off-season. It is necessary to employ modern methods to extend storage life for better distribution and also processing techniques to preserve them for utilization in the off-season. The fruit can be preserved by converting it into products like jam, jelly, fruit bar, juice, pickle and murabba to prolong their utilizable lifespan. Fruit juicing is one of the easiest way to preserve fruit, (Vidhya and Narain, 2011; Gonzalez-Molina *et al*., 2010; Mulero *et al*., 2012; Opera, 2015).

The production of fruit and vegetable juices is important both from the human health and commercial standpoints. The availability of nutritious components from fruits and vegetables to a wide range of consumers is thus facilitated throughout the year by the marketing of their juices. The production process of fruit and vegetable juices includes steps like extraction, clarification, and stabilization (Bhat, 2000).

Citrus fruits are among the most important horticultural crops, lemon (*Citrus limon* L.) being the third most important citrus crop species (Gonzalez-Molina *et al*., 2010). Several studies have pointed out that lemon is a rich source of nutrients and phytochemicals, including flavonoids, citric acid, vitamin C, and minerals (Gonzalez-Molina *et al*., 2008), which have numerous health promoting properties (Gonzalez-Molina *et al*., 2010; Mulero *et al*., 2012).

For this reason, lemon juice is an interesting food matrix for designing new beverages, as well as being a suitable source of value-added products since overproduction and non-marketable fruits lead to a serious environmental problem of un-used agro waste on a yearly basis. In this regard, lemon juice represents an alternative for the conversion of a bio-burden into a food product. Lemon juice has a high antioxidant capacity due to the presence of citrate, vitamin C, vitamin E and flavonoids such as eriocitrin, hesperetin (Miyake *et al*., 1998) and limonoids (Yu *et al*., 2005). The main objective of this study was to observe the effect of different storage conditions on the bio-chemical quality, especially on ascorbic acid content of the lemon drink.

MATERIAL AND METHODS

The fresh lemon fruits, *Citrus limon* of Sarbati variety were purchased from market. The fully ripened, healthy and uniform size fruits were selected. The fruits were washed, peeled and cut into two equal parts. The juice was extracted manually and filtered through muslin cloth to remove rags and seeds (Fig 1).

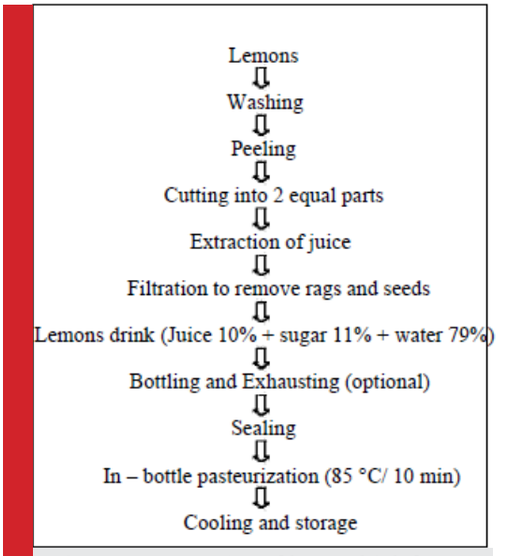

FIGURE 1: Preparation of lemon drink from lemons

In lemon drink preparation, levels of lemon juice, sugar and water have been optimized by preliminary trials. Citric acid was also added as preservative in lemon drink. Lemon drink was bottled with and without exhausting. After bottling, bottles were pasteurized at 85 °C for 15 minutes. Pasteurized bottles were cooled and stored (6°C and 37°C) up to the storage period of 150 days. The bio-chemical parameters of the samples evaluated after each 30 days interval. The obtained data were analysed for statistical significance. Four types of lemon drink samples have been prepared. For samples T_1 and T_3 exhausting has been used before bottling and stored at 6°C and 37°C temperature respectively and samples T_2 and T_4 bottled without exhausting and stored 6°C and 37°C temperature respectively.

The titratable acidity of fresh lemon drink samples was determined as per the procedure described by Ranganna (1986). Aliquot of the samples were diluted with distilled water and then titrated with 0.1 N NaOH using 1 % phenolphthale in solution as indicator. The percent acidity (as citric acid) as was calculated using following formulae.The pH of lemon drink was determined by using digital pH meter (Make: Elico LI 610). The pH meter was standardized with distilled water of pH 7.0 and standards at pH 4.0 and pH 9.0.

The total soluble solids content of the lemon drink was directly measured by using digital hand refractom-

eter (Make: ATAGO hand refractometer). Ascorbic acid in lemon drink at different stages of storage was determined according to AOAC method (AOAC, 1995). Lemon drink of 2 mL was taken in a conical flask and blended with 20 mL of metaphosphoric-acetic acid solution to extract ascorbic acid. The mixture was filtered using a Whatman filter paper and transferred to a volumetric flask. 2 mL of the filtrate extract with 5 mL metaphorsphoric- acetic acid solution was rapidly titrated with 2, 6 dichlorophenol indophenol solution until light distinct rose pink colour persisted for more than 5 sec.

Reducing sugar content of lemon drink samples was also determined using Lane and Eynon method (Ranganna, 1986) in which 5 mL of clarified lemon drink was transferred in to a 250 mL flask. About 100 mL of distilled water was added and solution was neutralized with 1N NaOH solution using phenolphthalein as the indicator. Two ml of lead acetate solution was added to the flask and shaken. It was made to stand for 10 min. Necessary amount of potassium oxalate solution was then added to remove the excess lead present in the sample. The solution was then made up to 250 mL using distilled water, filtered and used for the estimation of total reducing sugar. A solution containing equal volume of Fehling reagents A and B was titrated against the sample solution and the amount of reducing sugar present in 100 ml of juice was estimated as per the procedure described by Ranganna (1986).

Total sugar contents of the lemon drink samples were determined using Lane and Eynon method (Ranganna, 1986). For total sugar determination, five ml of clarified aonla juice was taken in a 250 ml conical flask and 50 ml distilled water was added to it. About 5 g of citric acid was then added and boiled for 10 minutes for the inversion of sugar. The resulting solution was then neutralized with 1 N NaOH solution using phenolphthalein as the indicator. The chemical used for the titration was a mixture of Fehling solution A and B. This solution was titrated against the sample solution and the amount of total invert sugar present in 100 ml of juice was estimated as described by Ranganna (1986). Data were analyzed statistically (ANOVA) using analysis of variance and differences among the means were determined for significance at $P<0.01$ using commercial statistical package, Design Expert – version 8.0.7.1.

RESULTS AND DISCUSSION

Acidity is also an important attribute because tartness is a major factor in the acceptability of lemon drink. Acid gives the characteristic sourness to the product. Citric acid is the major acid in the lemon juice that enhances the characteristic flavour of the lemon drink. The data regarding acidity of different treatments of lemon drink is presented in Table 1. Highest acidity i.e. 0.389 was recorded in sample T_4 (bottled without exhausting and stored at 37°C), while lowest i.e. 0.315 was observed in T_1 (bottled with exhausting and stored at 6°C). There was a gradual increase in acidity in all treatments during storage up to 150 days (Fig 2). However significant variation in acidity was observed during the storage of 150 days. This increase in acidity was attributed to the degradation of sugar into carboxyl acids.

The pH has great importance to maintain self stability of the lemon drink. pH can also influence the flavour and processing requirements of the beverage. The data regarding pH of different treatments of lemon drink is presented in Table 1. Storage intervals also influenced the pH of the beverage. A decline in pH towards acidic region was noticed as the storage of the lemon drink increased. However, significant variation in pH was observed during the storage of 150 days (Fig 3). This decrease in pH was attributed to formation of acidic compounds by degradation of reducing sugars, as discussed by Zia (1987). Similar trend of decrease pH was also reported by Saleem (1980).

The data on total soluble solids (TSS) for all treatments has been presented in Table 1. Total soluble solids of all the samples of lemon drink increased during the storage period of 150 days. However, significant variation in TSS was observed during the torage period of 150 days. Maximum TSS i.e. 13.9° brix was found in the sample T_4, that was bottled without exhausting and stored at 37°C. Whereas, minimum TSS i.e. 13.4° brix was found in sample T_2 that was bottled without exhausting and stored at 6°C (Fig 4). This increase in TSS in all the samples of the lemon drink during storage period of 150 days, might be due to formation of pectic substances from protopectin and monosaccharides from disaccharides i.e. degradation of sucrose into glucose and fructose. Similar results have been reported by Sarolia and Mukherjee (2002) in their studies on the lime juice. These results are also connecting with previous studies of Kaunjoso and Luh (1967), while studying on the canning and storage of oranges and in canned peaches.

Ascorbic acid content of all samples of lemon drink decreased during the storage period of 150 days. Ascorbic acid content decreased at faster rate for sample T_3 (bottled with exhausting and stored at 37°C) and T4 (Bottled without exhausting and stored at 37°C) then samples T_1 (bottled with exhausting and stored at 6°C) and T2 (bottled without exhausting and stored at 6°C). After storage period of 150 days, the retention of the ascorbic acid was 0.468, 0.421, 0.411 and 0.394 mg/100 gm for samples T_1, T_2, T_3 and T_4 respectively (Fig 5). Statistical analysis showed that the results are highly significant

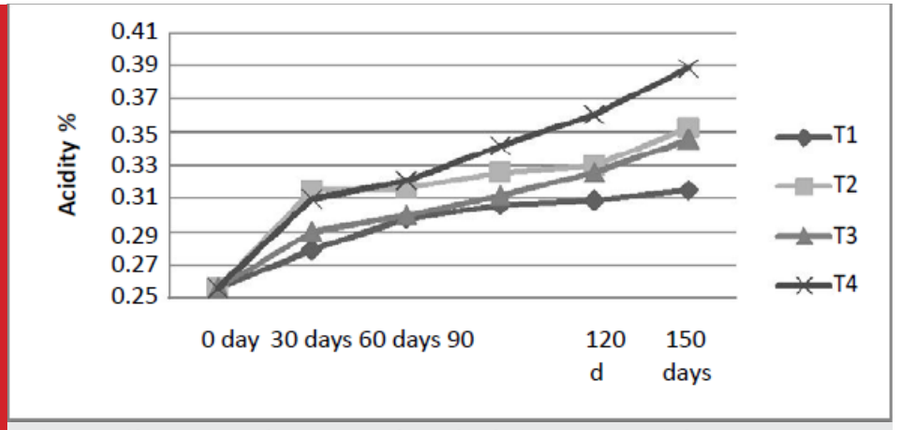

FIGURE 2: Changes in acidity content of samples during storage period

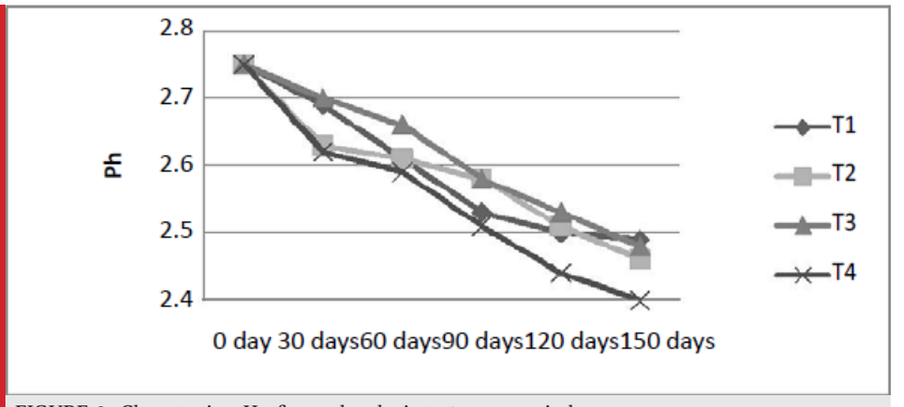

FIGURE 3: Changes in pH of samples during storage period

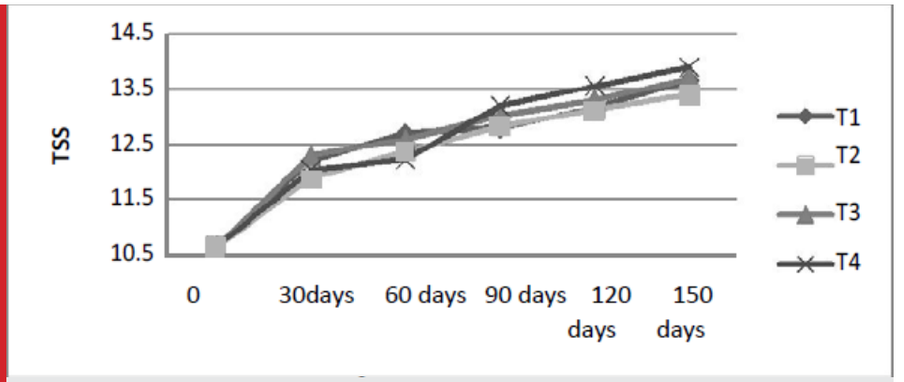

FIGURE 4: Changes in TSS (o Brix) of samples during storage period

Table 1: Changes in Biochemical composition of lemon drink during storage in different treatments

Treatments	Storage (Days) Parameters	0	30	60	90	120	150
	Acidity	0.254	0.277	0.297	0.308	0.307	0.312
	pH	2.76	2.67	2.62	2.55	2.52	2.46
	TSS (° Brix)	10.61	12.4	12.6	12.6	13.13	13.64
T1	Ascorbic acid	2.62	2.56	0.495	0.484	0.468	0.466
	Reducing Sugar	2.02	2.07	2.18	2.21	2.23	2.32
	Total Sugar	2.33	2.34	2.41	2.47	2.51	2.52
	Acidity	0.256	0.315	0.316	0.326	0.33	0.353
	pH	2.75	2.63	2.61	2.58	2.51	2.46
	TSS (° Brix)	10.64	11.9	12.37	12.83	13.13	13.4
T2	Ascorbic acid	2.63	1.58	0.459	0.453	0.443	0.421
	Reducing Sugar	2.05	2.07	2.11	2.18	2.22	2.26
	Total Sugar	2.31	2.36	2.38	2.38	2.43	2.49
	Acidity	0.256	0.29	0.3	0.312	0.326	0.346
	pH	2.75	2.7	2.66	2.58	2.53	2.48
	TSS (° Brix)	10.64	12.3	12.6	13.03	13.3	13.7
T3	Ascorbic acid	2.63	2.213	0.551	0.435	0.417	0.411
	Reducing Sugar	2.05	2.06	2.13	2.18	2.21	2.26
	Total Sugar	2.31	2.34	2.42	2.46	2.49	2.52
	Acidity	0.256	0.31	0.321	0.342	0.361	0.389
	pH	2.75	2.62	2.59	2.51	2.44	2.4
	TSS (° Brix)	10.64	12.03	12.23	13.2	13.56	13.9
T4	Ascorbic acid	2.63	2.51	0.54	0.424	0.401	0.394
	Reducing Sugar	2.05	2.12	2.16	2.21	2.25	2.30
	Total Sugar	2.31	2.46	2.48	2.52	2.56	2.59

All data are significant at 0.01% confidence level

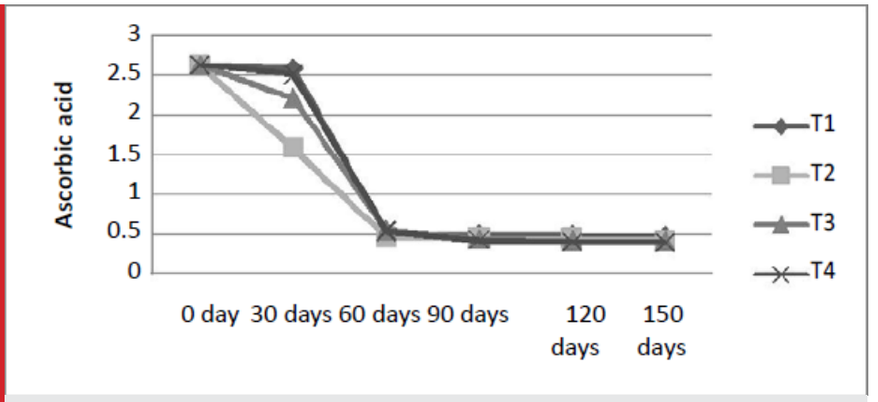

FIGURE 5: Changes in ascorbic acid content of samples during storage period

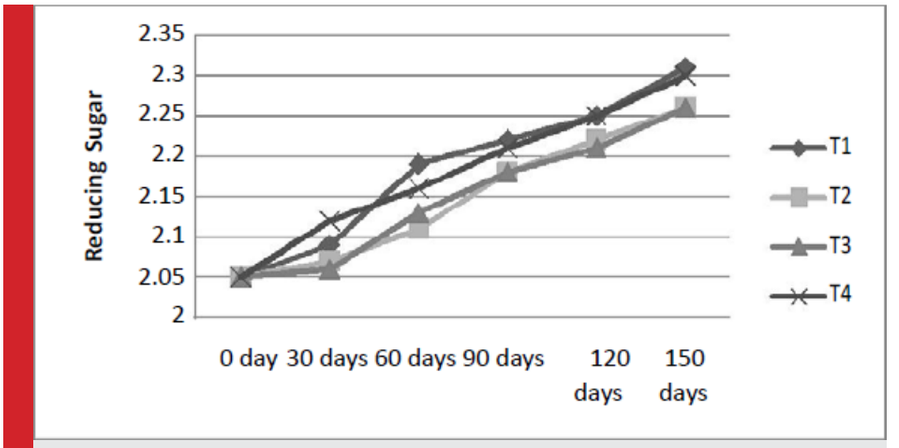

FIGURE 6: Changes in reducing sugar content of samples during storage period

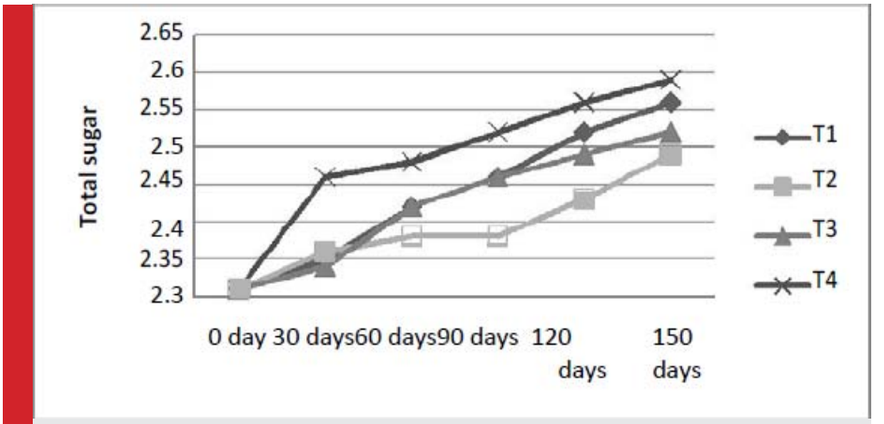

FIGURE 7: Changes in total sugar content of samples during storage period

during the storage of 150 days. These losses of ascorbic acid were attributed to the effect of processing, storage time and exposure to light. The degradation of ascorbic acid in lemon drink may follow ascorbic acid and anaerobic pathways (Moshonas and Shaw, 1989). Similar decreasing trend for ascorbic acid contents in different fruit beverages were also reported by the Ranote and Bains (1982).

The data on reducing sugar for all treatments has been presented in Table 1. For reducing sugars a significant difference was observed during the storage period of 150 days. Reducing sugars of all the samples T_1, T_2, T_3 and T_4 of lemon drink increased during storage period of 150 days. Reducing sugar tend to increased from initially 2.05 to 2.31, 2.26, 2.26 and 2.3% for samples T_1, T_2, T_3 and T_4 respectively during 150 days (Fig 6). This gradual increase in reducing sugar during storage for all samples might be due to the hydrolysis of non reducing sugar which continue to increase while non reducing sugar showed decreasing trend in fruit beverages during storage, rise in level of reducing sugar might be due to inversion process of sucrose to glucose and fructose by the acid of the diet drink. Similar observations were also reported by Babsky *et al.,* (1986) and pruthi *et al.,* (1984) that non reducing sugars of drinks is converted in to reducing sugar during storage. The data regarding total sugars revealed that total sugars of all samples of lemon drink increased during the storage period of 150 days. Total sugar tends to increased from initially 2.31 to 2.56, 2.49, 2.52 and 2.59% for the samples T_1, T_2, T_3 and T_4 respectively during 150 days (Fig 7). A gradual increasing trend in acidity, total soluble solids, reducing sugar and total sugar and a decreasing trend in pH and ascorbic acid content was noted in all four treatments during storage period of 150 days. The overall results showed that samples without presence of gasses (sealed with vacuum) at 6°C gave best results for ascorbic acid retention.

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