

Effect of temperature and removal of amino acids on non-enzymatic browning of lemon juice concentrates during storage

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Browning of lemon juice concentrates during storage especially at higher temperatures, not only causes loss of sensory appeal but also reduces the nutritional value of the product. The effect of removal of amino acids from lemon juice by cation exchange resin treatment is evaluated for reduction of browning of prepared concentrates (45, 60 and 71°B) during nine months storage at two temperatures, i.e., ambient and low temperatures. The storage of concentrates prepared both from untreated and treated lemon juices brings about some increase in furfural, HMF and browning with consistent loss of sugars, ascorbic acid, amino acids, and phenols. However, during storage, the removal of amino acids by cation exchange resin treatment of lemon juice is highly effective to reduce furfural, HMF, and browning of concentrates by about 7.54, 42.99, and 3.81 - folds as compared to their untreated counterparts. Also the retention of ascorbic acid, amino acids, and phenols is higher in concentrates of treated juice as compared to those from untreated juice. The changes in various quality characteristics of concentrates stored at refrigerated temperatures is lesser as compared to those stored at ambient temperatures. Further the retention of quality attributes is better in concentrates of 45 and 60°B than in the concentrate of 71°B.

Keywords: Lemon, Concentrate, Non-enzymatic browning, Storage, Amino acids, Cation exchange resins

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Introduction

Browning of lemon juice and concentrates during heat processing as well as storage has been a problem through the history of processing industry. Maintaining the product at low temperature has been and still is the only means to reduce colour and flavour deterioration of processed citrus juice and concentrates in long-term storage¹. But, as soon as the temperature increases during storage, the rate of these deteriorative reactions increases remarkably. Further the application of heat is almost indispensable during processing, therefore causes browning and colour deterioration in fruit juice. The removal of browning substrates thus seems to be a better option to reduce quality deterioration during processing and storage of fruit juices and concentrates, although a compromise is to be made for the removal of one or a few of the nutritional components. Lemon juice is not consumed for its amino acid contents but these compounds, although present in small amounts cause considerable browning, colour deterioration, and reduce aesthetic

appeal of the product. Although a few reports are available on the removal of amino acids from kiwifruit juice for reducing colour deterioration of prepared concentrates during storage, the available information on such studies in citrus juices, especially lemon juice is hardly available^{2,3}. Keeping this in view the present investigations were carried out to remove amino acids, one of the browning reaction substrates, from lemon juice to reduce the colour deterioration and loss of nutritional quality of the prepared concentrates during storage.

Materials and Methods

The juice of lemon cv. "Hill Lemon" (*Citrus pseudolimon* Tan.) fruits harvested at optimum maturity from local orchards in district Sirmour, Himachal Pradesh, India was extracted by using semi-automatic motor operated rosin machine (Bajaj Maschinen Pvt Ltd, New Delhi, India). The extracted juice was then strained through muslin cloth and heat pasteurized at 90° C for 10 s followed by quick cooling to room temperature and preservation with 500 ppm sulphur dioxide⁴. Hill lemon juice was clarified by using "Pectinase CCM" enzyme (Biocon India Ltd, Bangalore, India) @ 0.2 per cent for 2 h at

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50 ± 2°C, followed by filtration through a bed of filter paper pulp under vacuum to get a sparkling clear juice⁵. For removal of amino acids, 228 mL of juice was passed under gravity through an acidic cation exchange resin, Dowex-50W (Fluka Chemie GmbH, Switzerland), packed in a glass column (3 cm internal diameter) up to a height of 5.5 cm. The treated juice was collected in a beaker and evaluated for any changes in chemical quality. The column after use was washed with 0.2 N HCl solution (3 to 4 -times the volume of resin) and regenerated with 0.2 N NaOH solution (2 to 3 -times the volume of resin) with EDTA⁶. The excess of the alkali was removed by washing the cation exchange resin with 3 to 4 volumes of distilled water and dried under suction at room temperature. Properly washed and regenerated column was repeatedly used for separation of amino acids without any practical loss in its activity.

Both types of juices, i.e., one from which amino acids had been removed (treated) and untreated one, were used for the preparation of concentrates of 45, 60 and 71° B using a rotary type vacuum evaporator (Jain Scientific Glass Works, Ambala, India) at 50 ± 2°C under 28 ± 2" Hg vacuum. In order to achieve fast condensation of vapours the temperature of water circulating through the condenser was maintained between -15 to -5°C by using a circulatory water bath (Vikrant Scientific works (Pvt) Ltd, Bahadurgarh, Haryana). The freezing point of water was lowered by addition of 20 per cent salt to it. All the concentrates were packed in glass bottles (200 mL capacity) with air-tight HDPE caps, after uniform addition of 250 ppm SO₂. All the concentrates were kept at two different temperatures, i.e. ambient (12.20-31.77°C) and low temperature (3-7°C) for nine months and analysed for their chemical quality at periodic intervals of 3 months (data presented is only for the values obtained at the beginning and completion of storage studies).

Standard analytical procedures were followed for estimation of titratable acidity, non-enzymatic browning, and hydroxymethyl furfural (HMF) of samples⁷. Sugars were estimated by rapid colorimetric method of simultaneous determination of total reducing sugars and fructose⁸, ascorbic acid by direct colorimetric method⁷, total amino acids were determined by Ninhydrin colorimetric method⁹ and total phenols by colorimetric method¹⁰. Davis method was used for determination of flavonoids (naringin and hesperidin)¹¹, while furfural was measured by

colorimetric method given by Dinsmore and Nagy¹². The data on chemical characteristics was analysed statistically by CRD¹³.

Results and Discussion

There was some reduction in the TSS and total solids of clarified lemon juice after treatment by cation exchange resin, which was probably due to the removal of some of the soluble and insoluble juice constituents by the resin (Table 1). The titratable acidity, total sugars and ascorbic acid also suffered about 5.37, 6.43 and 4.54 per cent losses, respectively, but the corresponding higher values for these constituents on dry weight basis (dwb) in the treated juice might be attributed to the removal of insoluble solids from lemon juice after cation exchange resin treatment. These insoluble suspended solids must have contributed their weight to the total solids of untreated juice but not to the acidity, sugars and ascorbic acid, as all these compounds are water

Table 1—Effect of cation exchange resin treatment on the physico-chemical characteristics of clarified lemon juice

Parameter	Before cation exchange resin treatment (Mean ± SE)	After cation exchange resin treatment (Mean ± SE)
TSS (°B)	9.05 ± 0.04	7.99 ± 0.03
Total solids, (per cent)	9.74 ± 0.08	8.47 ± 0.06
Titratable acidity, (per cent)	5.40 ± 0.08 (55.44)	5.11 ± 0.05 (60.33)
Sugars: Glucose, (per cent)	1.20 ± 0.04 (12.32)	1.11 ± 0.03 (13.10)
Fructose, (per cent)	1.29 ± 0.03 (13.24)	1.20 ± 0.02 (14.17)
Total, (per cent)	2.80 ± 0.08 (28.74)	2.62 ± 0.06 (30.93)
Ascorbic acid, (mg per cent)	38.08 ± 0.82 (390.96)	36.35 ± 0.39 (429.16)
Total amino acids, (mg per cent)	143.02 ± 2.45 (1468.38)	4.59 ± 0.48 (54.19)
Total phenols, (mg per cent)	4.15 ± 0.12 (42.61)	3.43 ± 0.08 (40.49)
Flavonoids: Naringin, (mg per cent)	10.06 ± 0.05 (103.28)	5.27 ± 0.06 (62.21)
Hesperidin, (mg per cent)	8.98 ± 0.06 (92.20)	4.55 ± 0.04 (53.72)
Furfural, (ppb)	2.17 ± 0.06 (22.28)	1.03 ± 0.02 (12.16)
Hydroxymethyl furfural, (ppm)	0.06 ± 0.006 (0.61)	0.01 ± 0.002 (0.12)
Non-enzymatic browning, (OD _{440 nm})	0.021 ± 0.002	0.010 ± 0.001

Figures in the parenthesis are on dry weight basis (dwb)

soluble. About 96.79 per cent of the amino acids and 17.35 per cent of the total phenols were also removed on treatment of clarified lemon juice with cation exchange resin Dowex-50W. A remarkable reduction of amino acids and phenolics on treatment of kiwifruit with Dowex 50W and XAD-4 has been reported³. Further, some loss of flavonoids (39.76 per cent naringin, 41.73 per cent hesperidin, on dwb) were also observed on treatment of lemon juice by cation exchange resin, which were probably due to adsorption of these compounds by the resin. Further, some desirable changes were also observed in the furfural, HMF and non-enzymatic browning (OD_{440nm}) of lemon juice. Thus the cation exchange resin was very effective in removal of amino acids (browning reaction substrate) from lemon juice and caused only minor loss of other nutritional and therapeutic juice compounds.

The nine months storage of lemon juice concentrates brought about only slight reduction in their acidity i.e. 0.74 per cent at ambient temperatures and 0.52 per cent at refrigerated conditions (Figure 1). The overall higher acidity (dwb) observed throughout the storage period, in concentrates prepared from treated juice as compared to those prepared from untreated juice might be attributed to the higher initial acidity of the treated juice. The slight decline in

acidity during storage might be due to the chemical interactions between organic constituents of lemon juice concentrates. A loss of about 0.87 per cent total sugars was registered after nine months storage of concentrates at ambient temperatures, while this loss was only 0.37 per cent at refrigerated temperatures (Figure 2), which could be attributed to the involvement of sugars in browning reactions and formation of HMF which are of course accelerated at higher temperatures. Further the decline in total sugars after nine months of storage was only 0.29 per cent in the concentrates prepared from treated juice as compared to 0.97 per cent loss in those prepared from untreated juice, which was probably due to less availability of amino acids in the treated juice to react with sugars and cause browning via Maillard reactions.

The net reduction of amino acids after nine months storage of concentrates were as high as 9.13 per cent in the concentrates stored at ambient temperatures as compared to only 2.90 per cent at refrigerated temperatures (Figure 3). The reaction of amino acids with sugars, ascorbic acid, and other juice constituents during storage of concentrates probably caused the reduction in their total amino acid contents. Further, during nine months storage the changes in amino acid contents of concentrates from treated juice were less

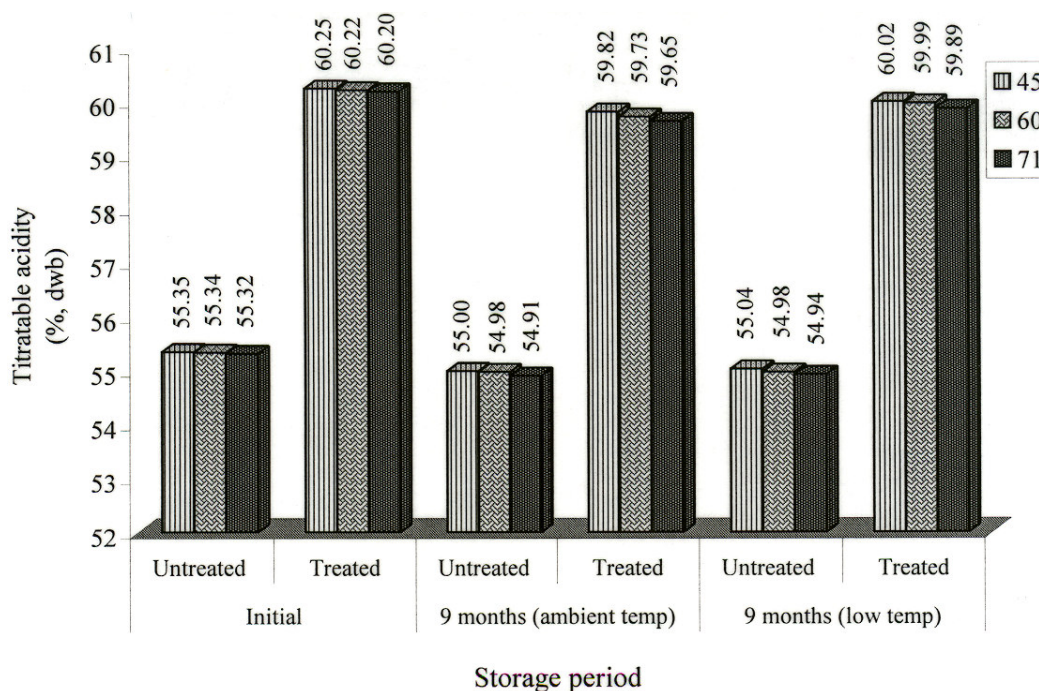


Fig. 1—Effect of cation exchange resin treatment and temperature on titratable acidity of lemon juice concentrates during storage

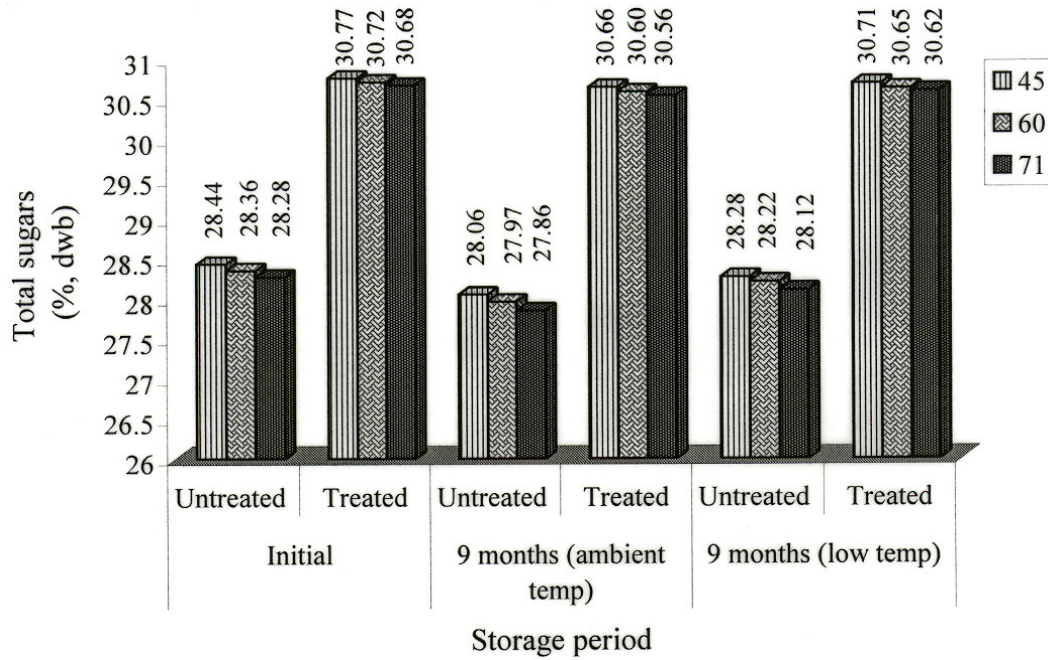


Fig. 2—Effect of cation exchange resin treatment and temperature on total sugars of lemon juice concentrates during storage

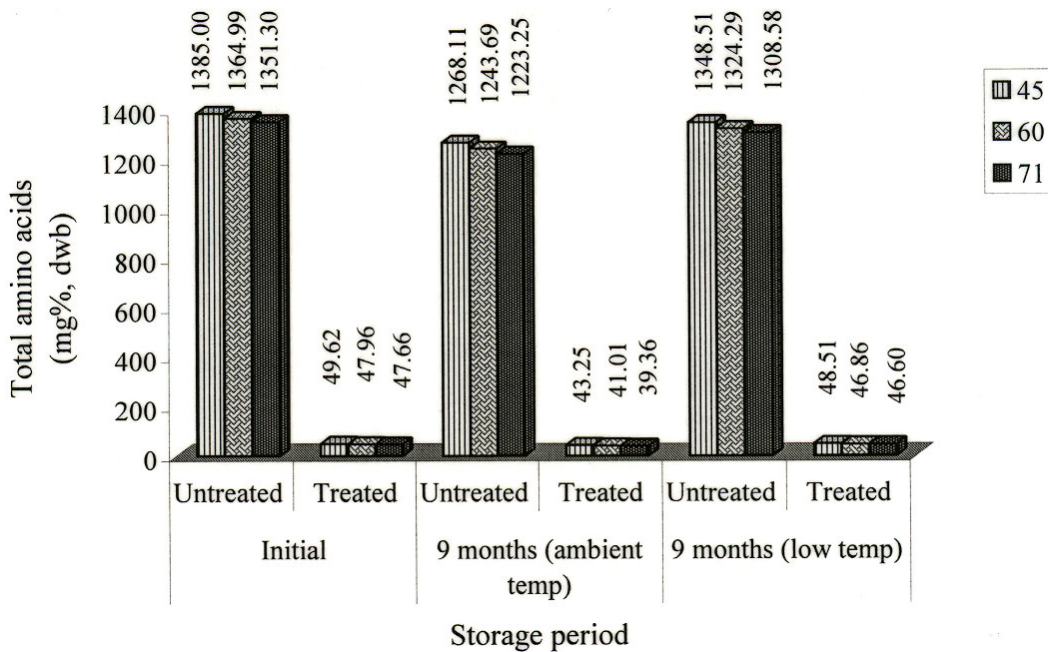


Fig. 3—Effect of cation exchange resin treatment and temperature on total sugars of lemon juice concentrates during storage

as compared to that of those prepared from untreated juice, which was probably due to the reduced efficiency of amino acids at low concentrations to react with other juice constituents during the course of browning reactions. The decline in total phenols observed after nine months of storage were 31.99 per

cent in concentrates stored at ambient temperatures than only 12.88 per cent at refrigerated temperatures (Figure 4). These changes might be attributed to their involvement in browning reactions. The formation of quinones from phenolic substances, their further reaction with amino acids, proteins and their

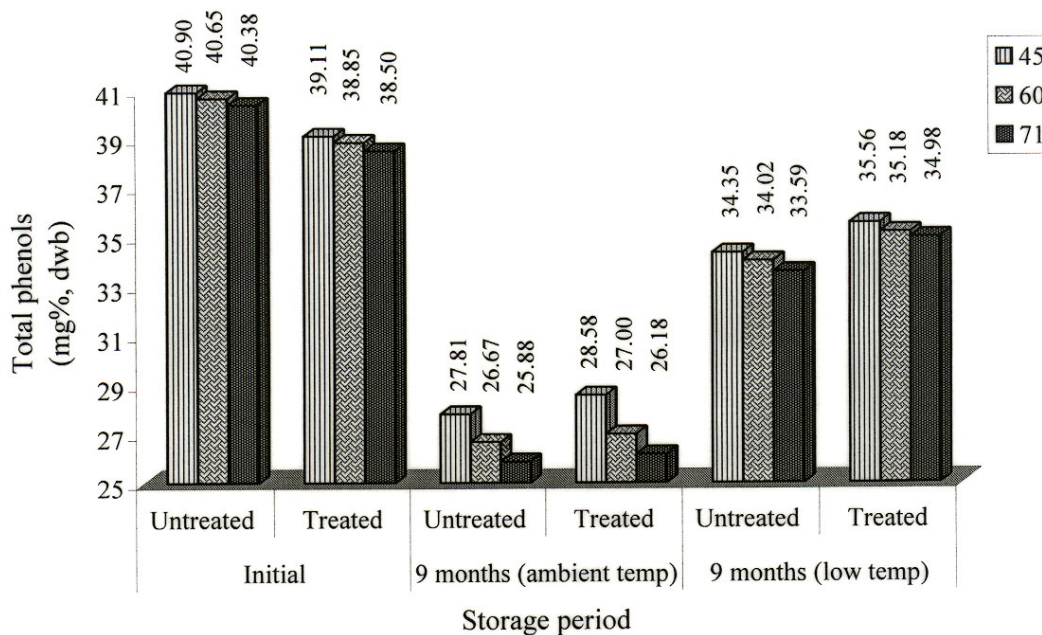


Fig. 4—Effect of cation exchange resin treatment and temperature on the total phenols of lemon juice concentrates during storage

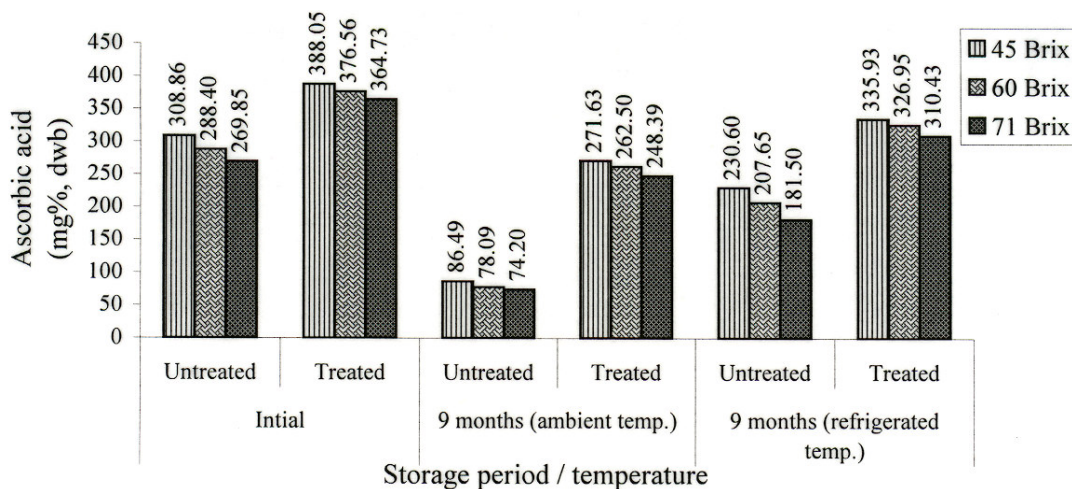


Fig. 5—Effect of cation exchange resin treatment and storage temperatures on the retention of ascorbic acid in lemon juice concentrates

subsequent polymerization during browning reactions is also evident as reported^{14,16}. The concentrates prepared from treated juice experienced merely 19.51 per cent (dwb) decline in their total phenols during nine months storage while, the corresponding reduction was as high as 25.24 per cent in the concentrates prepared from untreated juice. The less availability of amino acids for reacting with phenols in the concentrates prepared from treated juice might have caused lesser changes in their phenol contents.

The ascorbic acid contents of lemon juice concentrates experienced some loss during nine months of storage due to their light and heat sensitivity and involvement in browning reactions. Loss of ascorbic acid (dwb) in the concentrates stored at ambient temperatures was 48.84 per cent while at refrigerated temperatures only 20.20 per cent after storage of nine months (Figure 5). Further, this loss was more pronounced in the concentrates prepared from untreated juice (50.49 per cent) as compared to

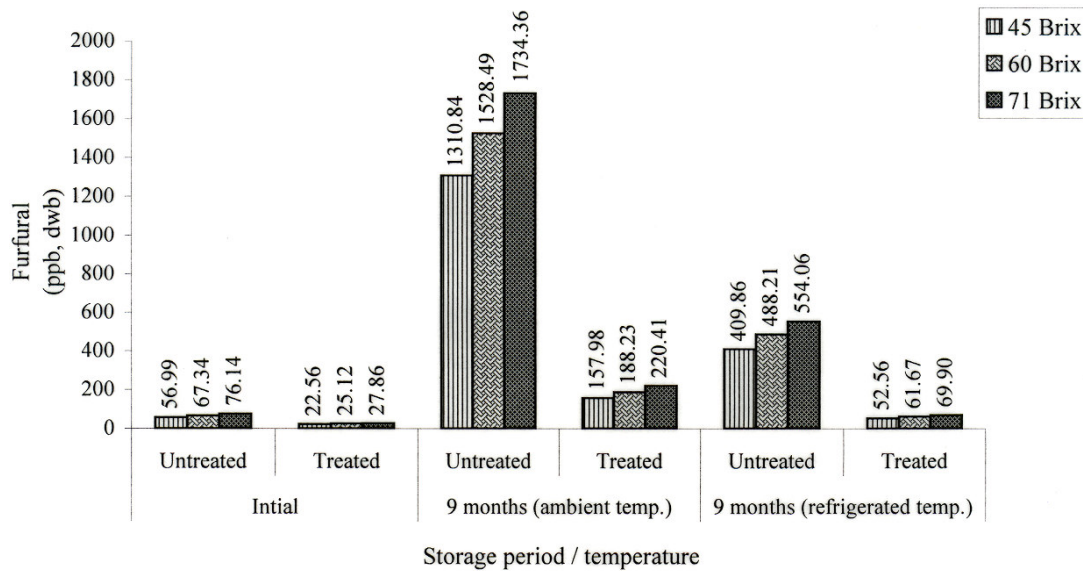


Fig. 6—Effect of cation exchange resin treatment and storage temperatures on development of furfural in lemon juice concentrates

those prepared from treated juice (22.26 per cent). The presence of considerable quantities of amino acids in the concentrates prepared from untreated juice could be responsible for the accelerated breakdown of ascorbic acid during storage of these concentrates. On the other hand the removal of about 96.79 per cent of the amino acids by cation exchange resin treatment helped in better retention of ascorbic acid in lemon juice concentrates during storage. The accelerated breakdown of ascorbic acid in the presence of amino acids has also been reported¹⁷. Thus the treatment of lemon juice with cation exchange resin (Dowex-50W) prior to concentration and storage of prepared concentrates at refrigerated temperatures resulted in more retention of ascorbic acid than that of those prepared from untreated juice and stored at ambient temperatures. The low temperature storage of concentrates showed significantly lower furfural accumulation, (4.86 -folds increase), whereas the corresponding increase under ambient storage was as high as 17.42 -folds from the initial values (Figure 6), which was probably due to more degradation of ascorbic acid under these conditions. Further the concentrates prepared from treated lemon juice experienced only 3.97 folds increase in their furfural contents as compared to 14.03 folds increase in concentrates prepared from untreated juice which might be attributed to the non-availability of sufficient amino acids to react with ascorbic acid and cause its degradation. Thus the

treatment of lemon juice with cation exchange resin was successful to reduce furfural accumulation by 7.54-folds during nine months storage.

The nine months storage of concentrates brought about considerable increase in HMF contents probably due to the reactions of amino acids and sugars to form HMF and cause browning of juice concentrates. Since, these reactions are influenced by temperature the changes were only 5.62-folds in concentrates stored at refrigerated temperatures as compared to 22.42 -folds at ambient temperature (Figure 7). Further the concentrates prepared from treated lemon juice exhibited only 1.07-times increase in their HMF contents as compared to 16.07-times increase in concentrates prepared from untreated lemon juice, after nine months of storage, which might be attributed to the removal of amino acids (a reactive substrate for HMF formation) by cation exchange resin treatment. Thus the treatment of lemon juice with cation exchange resin, prior to concentration, was successful to reduce HMF development by 42.99-folds, during storage up to nine months. The storage temperature exerted its influence on browning with comparatively lesser increase in browning observed in the concentrates stored at refrigerated temperatures (6.09-folds) as compared to those stored at ambient temperatures (11.94-folds) during nine months of storage (Figure 8). The increase in browning of concentrates during storage was due to reactions of amino acids with sugars,

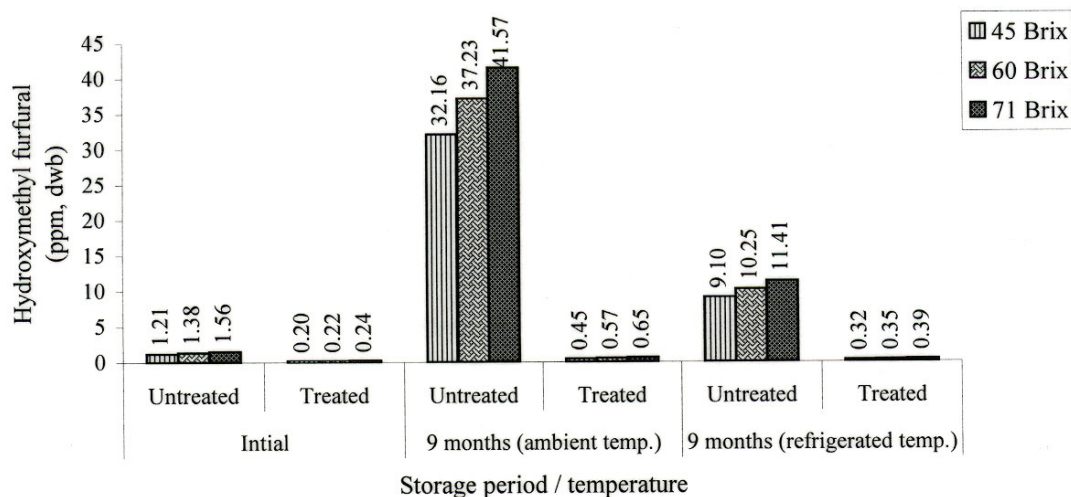


Fig. 7—Effect of cation exchange resin treatment and storage temperatures on development of hydroxymethyl furfural in lemon juice concentrates

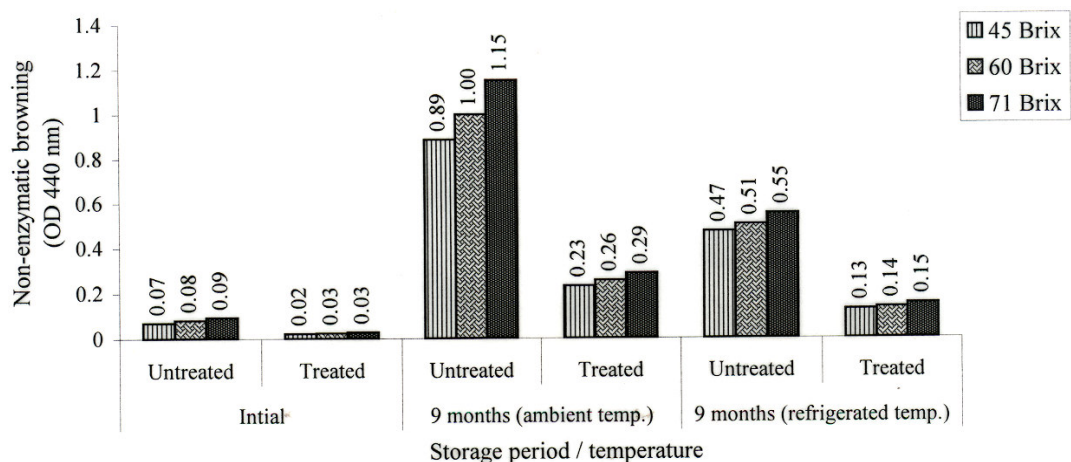


Fig. 8—Effect of cation exchange resin treatment and storage temperatures on non-enzymatic browning of lemon juice concentrates

degradation of ascorbic acid and reactions of amino acids with other juice constituents i.e. phenol etc., all resulting in formation of brown pigments. However, after nine months of storage the non-enzymatic browning of concentrates prepared from treated juice was 3.81-times less as compared to that of their untreated counterparts. Highly significant reduction in browning of concentrates prepared from treated juice might be attributed to the absence of sufficient amino acids to react with sugars, ascorbic acid, and phenols and cause browning. On the other hand the presence of amino acids in the untreated juice concentrates caused its reaction with other browning substrates leading to their darkening during storage. Further the amino acids below 0.66 per cent do not significantly

affect browning of the product¹⁸. Thus the treatment of lemon juice with cation exchange resin (Dowex-50W) prior to juice concentration and subsequent storage at refrigerated temperatures successfully reduced the browning by about 7.24-times when compared with untreated juice and their subsequent storage at ambient temperatures. Further the browning rates were accelerated in the concentrate of 71°B as compared to 45 and 60°B suggesting the unsuitability of preparation and storage of lemon juice concentrates beyond 60°B.

Conclusions

In summation, it emerges that the lemon juice concentrates stored at either of the temperatures

experienced some increase in their furfural, hydroxymethyl furfural, furfural, and non-enzymatic browning and a consistent decline in acidity, total sugars, ascorbic acid, amino acids, and phenols during storage up to nine months. Further, these changes were comparatively very less in the concentrates stored at low temperature than that of concentrates stored at ambient temperatures. The treatment of lemon juice with cation exchange resin prior to concentration also successfully reduced the non-enzymatic browning, development of furfural and HMF and helped in better retention of nutritional and pharmaceutical juice constituents such as ascorbic acid, sugars and phenols. during storage. Further the concentrates of 45° and 60°B retained the quality better as compared to concentrates of higher folds, at both the temperatures of storage.

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