STUDIES ON THE PRESERVATION OF BHADRI LEMON (*Citrus limon* (L.) Burm.) JUICE AND ITS UTILIZATION FOR BEVERAGE MAKING

By

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Thesis submitted to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of :

MASTER OF SCIENCE

in

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Hisar

1984

DEDICATED

TO

MY PARENTS

WHOSE UNFAILING LOVE HAS ALWAYS BEEN

A SOURCE OF INSPIRATION TO ME

CERTIFICATE I

This is to certify that the thesis entitled "Studies on the preservation of Bhadri lemon (*Citrus limon* (L.) Burm.) juice and its utilization for beverage making" submitted for the degree of Master of Science, in the subject of Horticulture, of the Haryana Agricultural University, is a bonafide research work carried out by Shri Murli Dhar Bansal under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled "Studies on the preservation of Bhadri lemon (*Citrus limon* (L.) Burm.) juice and its utilization for beverage making" submitted by Shri Murli Dhar Bansal to the Haryana Agricultural University, in partial fulfilment of the requirements for the degree of Master of Science, in the subject of Horticulture, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

27/12/89

Major Advisor

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Murli Dhar Bansal
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ABBREVIATIONS

KMS	Potassium metabisulphite
SB	Sodium benzoate
SO_2	Sulphur dioxide
K	Potassium
ha	Hectare
wt.	Weight
g	Gram
kg	Kilogram
mg	Milligram
ml	Millilitre
l	Litre
%	Per cent
ppm	Parts per million
ppb	Parts per billion
v	Volume
N	Normality
@	At the rate of
rpm	Revolutions per minute
nm	Nenometer
^oC	Celsius
^oF	Fahrenheit
$\angle$	Less than
7	More than
HDPE	High Density Polyethylene
GLC	Gas Liquid Chromatography

CHAPTER I

INTRODUCTION

In India, citrus occupies an area of 100,000 ha, next only to mango and banana. It is a native of the Himalayan region and China and has spread to various tropical and sub-tropical parts of the world. There are several hundred varieties of citrus species under cultivation in different parts of the world, over a hundred of which are found in India. In India, Maharashtra has the largest area under citrus, followed by Andhra Pradesh and Karnataka (Hirevenkangoudar, 1984).

Among the citrus species, the lemon (*Citrus limon*) is undoubtedly the most ubiquitous in its area of application and quite appropriately has been called "the fruit of many uses". Although not edible in the same manner as other citrus species, the lemon and its juice probably have had a greater variety of culinary, beverage, industrial and medicinal uses than any other fruit.

Lemon is one of the important citrus fruits appreciated not only for its beautiful appearance and pleasing flavour but also for its excellent food qualities. It is a good source of vitamin C and like other fruits it supplies other vitamins, fruit acids, minerals and alkaline salts which are needed in diet as health promoting substances (Singh and Singh, 1980).

The success that has attracted the cultivation of lemon in India is very dismal despite the suitable climatic conditions, existing for its cultivation. Many lemon varieties like Kagzi Kalan, Eureka, Seedless, Villa-franca, Baramasi and Galgal etc. are grown in different parts of India, but very little is known about the commercial cultivation of Bhadri lemon - a variety which has shown a great promise at the experimental orchard of Haryana Agricultural University, Hisar. When other lemons are scare in the market this giant lemon fruit can meet the demands thus showing its worth (Dhingra and Gupta, 1980).

The lemon may be utilized in many ways. The average weight of Bhadri lemon fruit is about 1.5 kg and average yield is about 250 fruits/tree. The lemon contains nearly 20 per cent juice and the rest portion goes waste. The waste portion includes albedo (34 %), flavedo (20 %), seed (2 %) and rag (24 %). The juice can be utilised for beverage making. From albedo portion of the fruit, candy can be prepared.

The peel can be utilised for extraction of oil. Wilson and Young (1917) obtained 8.1 to 15.3 pounds of oil per ton of lemon fruits. The substantial amount of the better quality citrus oils are consumed by the food and beverages industry (Swisher and Swisher, 1977). These are also used in soft drinks, Sharbets, ices, confectionary and bakery products, as well as household extracts. The principal uses of citrus oils in soft drinks include carbonated beverages, syrups, ades and drinks, emulsions and extracts.

Among several speciality products from citrus there is current interest in the oils from citrus seeds, not only to recover valuable oils for industrial use, but also as a means of reducing problem of waste control and pollutions. Nolte and Loesecke (1940) described the first commercial production of citrus seed oil from grapefruit seeds. Citrus seed oils are generally suitable for use in leather and textile field. They also have application in the preparation of fatty acid derivatives.

Therefore, keeping in view the important characteristics of Bhadri lemon fruit and a need for its suitable utilisation, investigations were undertaken with the following objectives to:

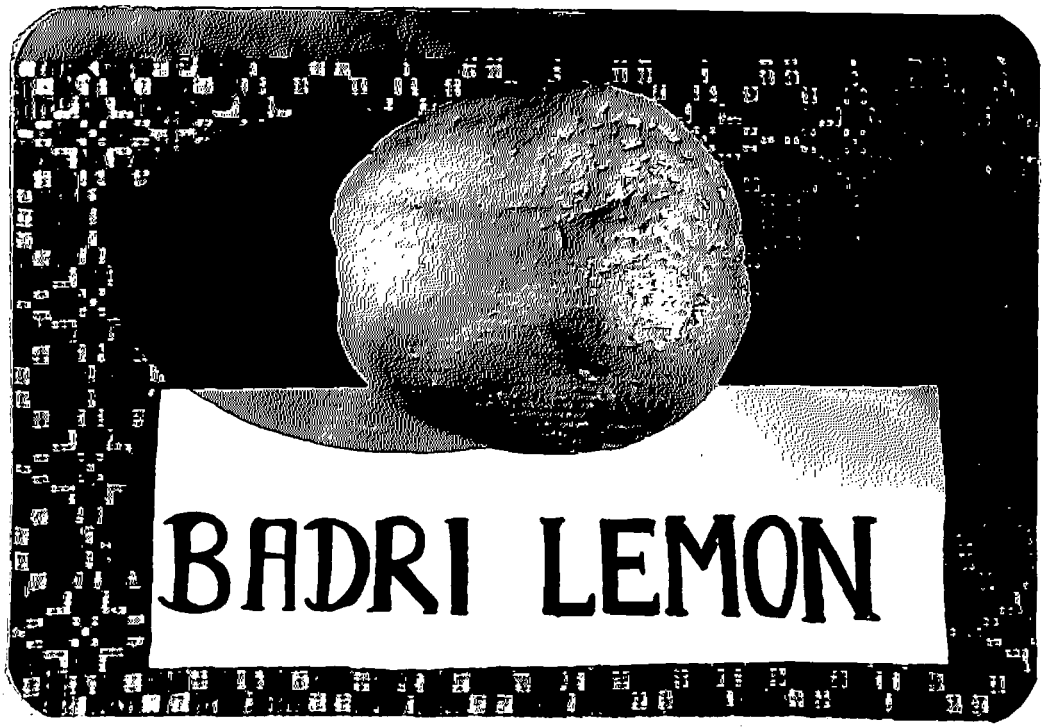
i) standardise the method for preservation of juice,

ii) study the keeping quality of the preserved juice,

iii) utilise the preserved juice for beverage making and evaluation of its quality,

iv) utilise the peel and the seed for extraction of oil, and

v) utilise the albedo portion for candy making.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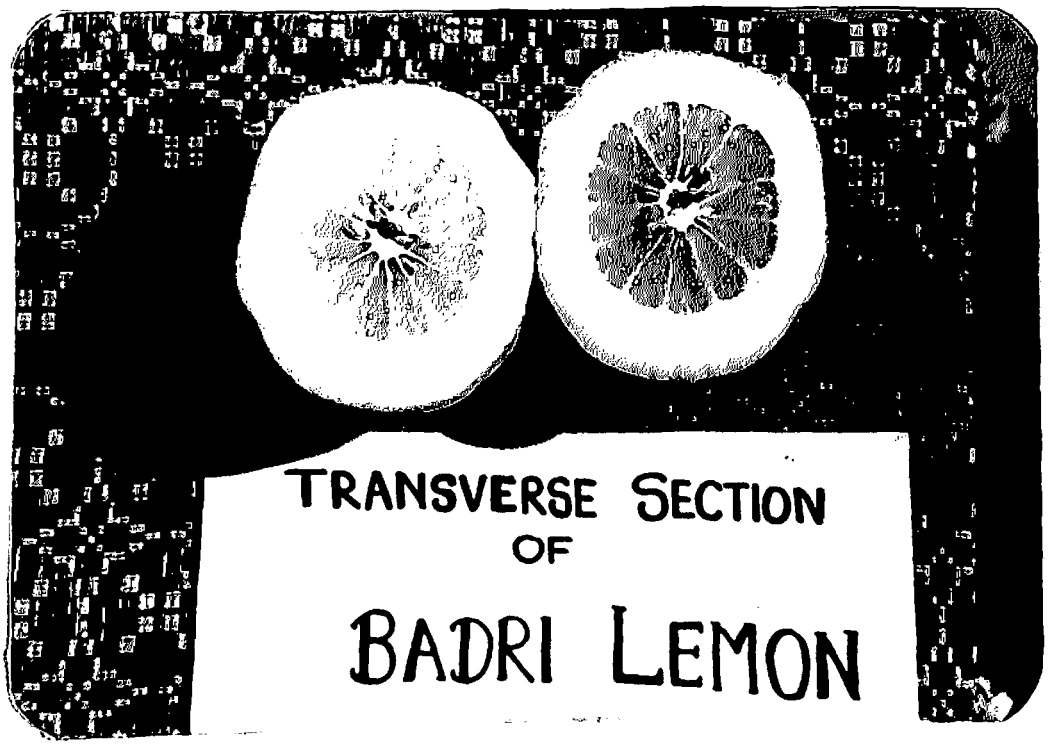
TRANSVERSE SECTION
OF
BADRI LEMON

REVIEW OF LITERATURE

An appreciable amount of work has been done on the utilisation of citrus fruits for the preparation of various products and different methods have been adopted for their processing. Various publications have appeared on the processing of citrus for jams, jellies, marmalades, beverage making, oil extraction from peel and seed etc. However, the information regarding the preservation of Bhadri lemon juice as such and the utilisation of its waste material is not available. Therefore, the results reported by various workers and other relevant information on the processing of fruits have been grouped under the following heads:

2.1. Composition of fruit

2.2. Processing factors

2.2.1. Heating and pasteurization

2.2.2. Chemical preservatives

2.3. Biochemical changes during processing and storage

2.4. Utilisation of waste material

2.4.1. Extraction of peel oil

2.4.2. Extraction of seed oil

2.5. Organoleptic evaluation

2.1. Composition of fruit:

Working with the analysis of lemon juice Benk (1980) reported that it contained total acids 83.3 g/l, nitrogen

compounds 7.7 g/l, phosphate 797 mg/l and calcium 279 mg/l. In the case of self pressed lemon juices, ash content was 2.1 to 3.9 g/l, K was 1030 to 1670 mg/l and calcium content was 70-160 mg/l. The important amino acid of lemon juice were alanin 5.4-8.9 per cent, prolin 14.0-25.6 per cent and glutamic acid 10.3-17.4 per cent. Ascorbic acid content of whole fresh fruit of bitter oranges (C. aurantium L.), peel, juice, pickles a,b,c and Gojju (Sauces) were 15.8, 15.5, 23.15, 14.2, 10.6, 5.55 and 13.33 mg/100 g respectively (Kalpana and Nath, 1981).

Kuber Ram and Chaudhary (1974) reported the ascorbic acid content of fruit juices of different genera and species of family Rutaceae. Maximum ascorbic acid was 68.39 mg in the juice of mandarin oranges followed by Kagzi lime (62.95 mg), Sweet orange (54.29 mg), Trifoliate orange (50.43 mg), Lemon (39.46 mg), Grapefruit (32.08 mg), Kumquat (29.87 mg) and lowest with Pummelo juice (21.56 mg/100 ml). Twenty six samples of laboratory produced juice from summer flowering lemon were analysed by Calvarano and Giacomo (1977) and found that the acidity (citric anhydride) was 47.36-66.56 g/l, isocitric acid was 0.181-0.393 g/l and citrate: isocitrate ratio was 160-287.

Lemon juice contained lower concentration of minerals, especially K (mean 102.7 mg/100 ml) vs. 158.6 for oranges, 150.2 for mandarins and 127.2 for grapefruit (Galoppini et al., 1974).

Flavinoids were estimated by Carpena and Tomas (1970) from citrus peel and found that yields of flavonoids were 2.2-4.6 per cent on fresh peel basis and 6.6-13.8 per cent on dried peel basis. Yield of hesperidin was 1.06-1.76 g/kg fresh peel basis from Satsuma oranges, 2.84 g/kg from Primofiore lemons. Mandarin peel had the greatest tangertin concentration (183 ppm) while lemon peel had the least (0.06 ppm). Orange peel contained an average, slightly greater amounts of tangertin content of any other citrus peel (Rouseff and Tings, 1979).

2.2. Processing factor:

2.2.1. Heating and pasteurization:

Citrus juices are sensitive to heat. Their delicate fresh aroma and flavour may be lost or damaged by undue exposure to heat, so they are usually pasteurized as rapidly as possible. Atkins et al. (1956) and Kew et al. (1957) observed that heating to about 71°C prevented fermentation, but 86° to 99°C was required to stabilize cloud in orange juice. The temperature required for cloud stability depend upon time of heating and pH of the juice. Kew and Veldhuis (1950) proposed a rapid method for checking effectiveness of pasteurization. A complex series of enzyme reactions, brought about primarily by the enzyme pectinesterase, is responsible for cloud loss, and processing is directed to the conditions necessary to deactivate this enzyme.

Kew et al. (1957) conducted extensive tests on the effect of time and temperature of pasteurization on cloud

stability of canned grapefruit juice. Cloud was stabilized in a high speed pasteurizer at 98.9°C in 1.75 seconds; at 90°C in about 13 seconds and at 85°C in about 43 seconds. They also determined that the temperature at which cloud stabilized increased, on the average, a little less than 0.5°C with each decrease of 0.1 pH unit.

The use of flash pasteurization to stabilize cloud in citrus juices in a controlled and reproducible manner was first described by Stevens (1940). Later Stevens et al. (1950) discussed this technique and its application to citrus juices stored at either normal temperatures or frozen. It is usually desirable to inactivate substantially all of the pectic enzyme (pectinesterase) in such juice products as natural strength lemon juice, concentrate for lemonade and bottlers bases that will be stored at ordinary temperatures.

Ranote and Bains (1982) reported that the reducing sugar content of kinnow juice increased more in heat processed sample irrespective of the season of picking. They further reported that the retention of ascorbic acid in heat processed preserved juice was less than in sulphur dioxide preserved juice. There was also a slight browning in heat processed kinnow juice after 8 weeks of storage as compared to the juice preserve with SO_2 which showed no change under similar conditions. The colour of the juice was also not better when it was preserved by heating.

Mehta and Bajaj (1983) conducted an experiment on the effect of storage and methods of preservation on the physico-chemical characteristics of citrus juices. With pasteurization (at 80°C for 20 minutes) kinnow juice had retained its original colour during storage but slight change in colour of Blood-red juice after four months was observed. They further reported that loss in ascorbic acid was more in juice preserved with pasteurization than the juice preserved with potassium metabisulphite and Sodium Benzoate. Acidity was also decreased in the juice preserved with pasteurization.

2.2.2. Preservatives:

The advent of heat sterilization, refrigerated storage and drying has given a great impetus to modern method of food preservation. Chemical preservatives, however, play 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. Although many preservatives have been recommended for use in foods, sulphur dioxide and benzoic acid dominate the scene, and find wide spread applications.

In India, fruit juice beverages, squashes, cordials, barley waters, crushes, synthetic and fruit juice syrups sold commercially are preserved with chemical preservatives. Generally, sulphur dioxide is used because, India being at high ambient temperatures, these products are prone to

browning which SO_2 inhibits. In addition to these products, sulphur dioxide is also used in bulk storage of juices and pulps for subsequent use in the manufacture of beverages, jam, jellies etc. SO_2 is also widely used in wine making (Ough et al.,1960) and in controlling non-enzymatic browning during drying of foods (Jacobs,1951). Sulphur dioxide also protects ascorbic acid in citrus juices against oxidation, but it accelerates the oxidation of orange oil (Beard et al., 1972).

When SO_2 alone is used for the preservation of citrus juice in bulk, the level required depend upon the pH of the juice. Typical recommendations (Charley,1963) are for orange juice 1500 to 1800 ppm, for grapefruit juice, 1200 to 1500 ppm, and for lemon and lime juices 500 to 800 ppm. Martin (1961) suggested to add benzoic acid at a levels of 1000 ppm, together with 100 to 250 ppm of sulphur dioxide to preserve a light, bright colour, especially in lemon and grapefruit juices. Mehta and Bajaj (1983) observed comparatively lower losses in citrus juice samples preserved with potassium metabisulphite than those preserved with pasteurization. Similar observation was reported by Ranote and Bains (1982). Lower losses of acidity were observed in citrus juices preserved with sodium benzoate (Mehta and Bajaj, 1983). Ranote and Bains (1982) further reported that sulphur dioxide prevents the browning in kinnow juice and the retention of the natural colour of the juice seemed to have been better with sulphur dioxide method of preservation

than heat processing. Sulphur dioxide also prevents browning reaction in stored mango pulp and syrup (Ghosh et al.,1982). The kinnow juice preserved with sulphur dioxide had superior colour, flavour and higher retention of ascorbic acid as compared to heat processed juice over a period of 28 weeks storage.

2.3. Biochemical changes during processing and storage:

2.3.1. Sugars:

The change in sugar content of the fruit products were affected by the temperature of processing and storage. Ranote and Bains (1982) reported that the reducing sugar content of kinnow juice increased more in heat processed samples irrespective of the season of pickking. The changes were even more pronounced in the juices with natural pH (3.5) in contrast to with pH adjusted to 4.0. Processing of bottled juice in boiling water contributed to hydrolysis of sugars by acidity in the juice as compared to sulphur-dioxide preserved bottled juice.

Total sugars and reducing sugars of bael fruit increased and non-reducing sugars decreased. Roy and Singh (1979) explained that this could be due to hydrolysis of polysaccharides and inversion of non-reducing sugars. However, accumulation of reducing sugars was found to be more in sulphur dioxide preserved pulp compared to canned one and in squash compared to nectar. This could be attributed due to higher acidity at the end of storage in sulphur dioxide preserved pulp and squash due to formation of sulphurous

acid which caused the rapid inversion of non-reducing sugars.

Increase in reducing sugars were correlated with the decrease of non-reducing sugar content. Breakke et al. (1976) while lining on some quality attributes of papaya nectar reported that decrease in sucrose content correlated with an increase in reducing sugars. 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. Similar trend was observed in value of toned juice. The amount of non-reducing sugars decreased considerably with storage being more in those samples kept at room temperatures than at the low temperature. Storage temperature affect the reducing sugars, total sugars and non-reducing sugars of stored product. A slight decrease in total soluble solids of dried ber juice during storage for 9 months at room temperature was observed by Khurdiya (1980). However, a gradual increase in reducing sugar content from 8.58 to 11.48 per cent was noticed during 20 days storage at room temperature in Phalsa beverage. The rate of increase in reducing sugars was much slower at 20°C and 30°C upto 100 days storage. Temperature does not effect the total soluble solids and total sugars of phalsa beverage during storage (Khurdiya and Anand, 1981).

Shrestha and Bhatia (1982) observed practically no change in total soluble solids of apple juice during storage.

The statistical analysis of the data showed significant difference among the varieties. The reducing sugar content increased during storage. The increase of reducing sugars was more at 37°C than at room temperature indicating that further temperature during storage could lead to gradual inversion of non-reducing to reducing sugars by hydrolysis. Total sugars and specific gravity remained practically unchanged. El-Sherbiny and Rizk (1981) studied on storage stability of orange concentrates prepared by different techniques. There was no loss in total sugars. However, values for reducing sugars increased,while the corresponding values for non-reducing sugars decreased.

Singh and Mathur (1953) stored cashew apples at various temperatures ranging from 82° to room temperature (82°-92°F) and they concluded that there was an increase in the percentage of total soluble solids and reducing sugars. These increase was greater at higher storage temperature.

2.3.2. Ascorbic acid:

The demand for fruit beverages is largely based on their nutritive value, flavour, aroma and colour. They are a source of vitamins, minerals, carbohydrates, amino acids, flavonoid compound and probably still unidentified constituents.

Pruthi and Lal (1951) advocated canning of citrus juice for better retention of ascorbic acid. Similarly Ranote and Bains (1982) found that ascorbic acid content of

kinnow juice decrease with processing and storage. The retention of ascorbic acid in So_2 preserved juice was higher than in the heat processed bottled juice. The pH has no effect on ascorbic acid retention.

Sarmah et al.(1981) reported that there are clear evidence of the significantly higher loss of ascorbic acid in heat processed single strength and toned kinnow mandarin juice stored at room temperature over a period of 28 weeks to the losses suffered by the low temperature.

The sulphur dioxide treatment was more effective in the retention of ascorbic acid apart from inhibiting browning reaction. Ascorbic acid retention was the highest in frozen concentrate (95-98 %) and quite high (90-97 %) in sulphited orange juice concentrates stored at 41°F and 86°F (Pruthi, 1962). Singh and Mathur (1953) found that the ascorbic content decreased at all the storage temperature of cashew apples.

The losses of ascorbic acid was higher at higher temperature compared to lower temperature. A loss of 19-25 per cent of ascorbic acid in orange juice and 18-24 per cent in grape juice when stored for 6 months at 26.7°C as compared to 4-12 per cent and 7-9 per cent respectively when stored at 4.4°C (Stadtman, 1948). The study further showed that at the end of 9 weeks the loss of total ascorbic acid ranged from 3 per cent (at 10°C) to 75 per cent (at 50°C). Similarly Fennema (1977) observed 1 and 5 per cent of ascorbic acid losses in orange juice concentrate and grapefruit concentrate

at 42°F and stored at 18°C for 9 month, respectively. During the study of 17 brands of reconstituted orange juice, Squires and Hanna (1979) noticed about 2 per cent decrease of ascorbic acid per day during storage.

Sethi et al. (1980) reported 29.95 to 17.64 mg ascorbic acid per 100 ml lost when stored at room temperature for 9 months. The ascorbic acid content of freeze dried orange juice was linear with increased storage time at room temperature but at 30 ± 3°C losses of ascorbic acid during storage for 4 months were negligible. At 25°C, losses were slight, whereas at 37°C losses were considerable.

Massaioli and Hadded (1981) reported the ascorbic acid content of orange juice was found to decrease slightly during storage over a 14 days period with the loss of ascorbic acid being dependent on the handling method used. Six of brand tested contained sufficient ascorbic acid at the end of the study to approximately meet the minimum requirement for unopened juice.

Palainswamy and Muthukrishan (1974) prepared the squash from 8 lemon varieties and studied the chemical characters of lemon juice and squashes during storage. They found that ascorbic acid content of both juices and squashes decreased. Nagy and Smoot (1977) showed that the log percentage vitamin C retention was linearly related with time at 29°C but not at 38 or 46°C.

The orange stored in December (27°C for 7 days) showed 5 per cent increase in ascorbic acid but in February, a

decrease of 7.5 per cent was observed (Mudambi,1977). At lower temperature (0°C and 5°C) no change in ascorbic acid content during storage was observed. Peeled orange when exposed to sun showed 40 per cent reduction in ascorbic acid as compared to marked stability in ascorbic acid content shown by unpeeled orange when both are exposed for 9 hour.

Commercially canned single strength grapefruit juices were stored at various temperature by Smoot and Nagy (1980). Their total active vitamin C (TAVC) contents (L-Ascorbic acid (AA)) plus dehydro-ascorbic acid (DHA) and diketogulonic acid (DKA) contents were evaluated at 3 week intervals. The loss of TAVC ranged from 3 per cent at 10°C to 68 per cent at 50°C. AA was continuously lost during storage, and the rate of loss increased as storage temperature increased. Levels of DHA and DKA remained essentially unchanged during the 12-week storage period.

The sum of ascorbic acid and dehydroascorbic acid (physiologically available ascorbic acid) in reconstituted orange juice was remarkably stable over a 2-week period, both at 4°C and at room temperature (Hortan and Dickman, 1977). Stability was only partially due to pH of orange juice, since available ascorbic acid declined more rapidly in a phosphate buffer solution at the same pH.

Ascorbic acid content decreased considerably when orange concentrates prepared by different techniques stored for 9 months at -10°C by El-Sherbiny and Rizk (1981).

2.3.3. Acidity:

In general acidity of the fruit products reported to be increased with duration of storage but when product was cooked to a higher consistency a decrease in acidity was observed.

Palainswamy and Muthukrishnan (1974) studied the chemical characters of lemon juices and squashes during storage. They found that the titratable acidity decreased in both juices (range 2.312 - 3.060 %) and squashes (range 0.540 - 0.650 %). Titrable acidity slightly increased in case of orange concentrates prepared by different techniques (El-Sherbiny and Rizk, 1981).

The total titrable acidity of amla juice increased more rapidly in samples stored for 10 weeks at room temperature than those stored in refrigeration (Mehta and Rathore, 1976). The increase was from initial 2.07 to 3.22 per cent at room temperature and to 2.8 per cent in refrigerator. Similarly Sethi et al. (1980) reported an increase in acidity from 1.35 to 2.35 per cent after 9 months of storage of orange juice.

The acidity and pH of dried ber juice remain constant during storage at room temperature for 90 days (Khurdiya, 1980). Similarly Khurdiya and Anand (1982) observed not much changes in acidity in Phalsa juice beverage during storage at all the three temperatures.

Storage temperature affects the acid content of product. The decrease in acidity was higher at room

temperature than at 37°C. Nagi and Manjrekar (1976) while studying storage of apple cider found that acidity decreased from 0.50 to 0.44 per cent at room temperature and to 0.463 per cent at 37°C when stored for 4 months. Acids were selectively lost during freeze concentration of apple juice (Tannous and Lawns, 1981). But contradictory results are reported by Shrestha and Bhatia (1982) that changes in pH of stored apple juice occurred at both temperature. Reduction of acidity was more at 37°C than at room temperature.

Sharma et al. (1974) reported that there was no perceptible change in colour, flavour but there was a progressive increase in acidity of mango milk powder stored at room temperature (30°C). Rao and Roy (1980) also found that the acid content of mango sheet increased with increase in storage temperature. But a progressive decrease in acidity on storing at room temperature (25 ± 5°C) was observed by Dabhade and Khedkar (1980).

Acidity of mango pulp was also affected by the different type of containers. Dan and Adsule (1979) reported that upto storage of six months, the increase in acidity was the same in all the samples but during subsequent storage period of six months, this increase was comparatively minimum in glass containers and maximum in black HDPE Pouches. Increase in acidity from 0.36 to 6.64 per cent in glass containers after 12 months has also been reported.

2.3.4. Tannins:

Many fruits contain tannins which while eating, react with the protein of mucous membrane to give an astringent taste which is a negative point for acceptability of a fruit as fresh. These tannins also affect the fruit processing by giving colouration to the products.

Astringency and bitterness are the major taste effects assigned to the phenols. Small polymeric yet soluble tannins, and in foods the leucoanthocyanins, are responsible for the astringency (Bate-Smith and Swain, 1953). Grünhut (1898) noted that tannins decrease in wine from 2310 to 2090 mg/litre made a noticeable difference in bitterness. The astringency of tannin is proportional to its protein-binding capacity. Tannins in the absence of sugar had a considerable effect in interfering with the recognition acid, but sugar minimized the effect of tannin (Hinreiner et al., 1955 a,b).

Three white ports, prepared with a range of total phenol content overlapping the range of red wine, dropped in 10 days at about 20°F from 1047 to 713, 711 to 533 and 186 to 175 mg/litre (Okalelov and Kotlyarenko, 1951). The decrease was 32 per cent for the high tannin wine and only 6 per cent for the low-phenol wine, indicating that the smaller juice phenols were less susceptible to precipitation than the larger flavonoids condensed tannins from grape solids.

The effect of storage on anthocyanin and their colour has been studied in grape juice and a number of other red, tart fruit juices, as well as in wine. Sastry and Tischer (1952 a,b) found that the tannins of grape juice conferred important protective action on anthocyanin against the destructive effect of ultraviolet irradiation. Ponting et al. (1960) studied heat degradation of grape juice anthocyanin and found a linear relation between the logarithum of the time and temperature necessary for a 10 per cent destruction of anthocyanin colour.

Luthi (1957) reported that apple and pear juices with excess tannin often ferment poorly, but that fermentation is usually rapid if this tannin is removed by gelatin fixing or if an extra nitrogen source is added.

2.3.5. Browning:

Sugaramine interactions of the maillard type (Reynolds, 1963,1965) and the resultant flavour changes (Hodge,1967) are not as prominent in juices as in dried fruits,because of their aqueous nature and low pH. Organic acids and ascorbic acid are more involved in interactions with the sugars and amino acids. Kapoor et al. (1958) studied the effect of four sugars and 10 organic acid in combination and observed that fructose caused maximum and glucose the minimum browning, oxalic and pyruvic acids caused maximum browning with fructose and sucrose. 5-hydroxymethyl-2 furaldehyde (HMF) is produced in fruit juices from sugar, particularly ketones by heating during processing and can

give rise to browning reactions with amino compounds (Diemair and Jury, 1965) and sugars and undergo further polymerization and rearrangement both in the presence and absence of oxygen (Koch and Kleesat, 1961).

Addition of sulphur dioxide during the preparation delays the browning and thereby extended the storage life of the products. Ranote and Bains (1982) reported a slight browning in heat processed kinnow juice after 8 weeks of storage as compared to the juice preserved with sulphur dioxide which showed no change under similar conditions. The retention of natural colour of the juice seemed to have been better with the sulphur dioxide method of preservation than the heat processing.

Hydroxymethyl furfural (HMF) formation in the phalsa juice increased with lowering of pH, increasing time and temperature of storage (Khurdiya and Anand, 1982). Addition of cane sugar to the juice was found to have a protective influence on the colour stability. Formation of HMF decreases with increase in the sugar concentration in the juice at 37°C. Loss of pigment in the juice increased with increase in temperature of processing. The adjustment to different pH value were found no avail on colour stability in phalsa juice.

Browning was more pronounced at higher temperature. A gradual increase in browning and hydroxylmethyl furfuryl formation in phalsa beverage was observed with increasing storage period.

This was more pronounced at room temperature than at 20°C (Khurdiya and Anand, 1981).

Berk and Braveman (1958) studied the effect of pH on browning of straight and concentrated citrus juice. They found that in the range of pH 2.0-3.5 the extent of browning during storage at 30°C for 30 days was inversely proportional to the pH.

Siddappa et al. (1959) found that the intensity of browning in Coorg orange juice and squash increased with the concentration of added ascorbic acid upto level of 1 per cent. According to Brekke et al. (1970) the browning of guava puree concentrate was apparent after 2 months and became more pronounced with continued storage. The browning may be related to a rapid loss of ascorbic acid that was virtually complete after one month.

Addition of sodium or calcium chlorides delays the browning of crushed apples (Piox et al., 1980) and the inhibitory action is due to the chloride anion and not to the sodium or calcium cations. Chloride slows down the enzymatic activity, while ascorbic acid merely delays the browning without altering the enzymatic activity.

2.4. Utilisation of waste:

2.4.1. Essential oils:

Essential oils from citrus are a major by-product of the citrus processing industry abroad. Each year large amounts of oils are produced and the outlook is for production to be maintained in the years ahead. These oils are used as

flavouring materials for not only citrus products, but a wide range of foodstuffs throughout the world. Orange oil accounts for about 90 per cent of the oil produced with the other 10 per cent being made up of grapefruit, lemon, lime, tangerine and mandarin (Dougherty and Petrus,1971).

Shankaracharya et al. (1977) studied the physico-chemical characters of some citrus essential oils. The variation in the physico-chemical properties of the citrus oils attributed to the differences in the varieties,season of harvest, mode of oil extract and treatments given. In general, the cold pressed oils were highly coloured while the distilled oils were almost colourless. They showed that the values for sp.gr., aldehydes, esters and evaporation residue of deterpenated/concentrated oils increased with the increase in the content of oxygenated compounds while the optical rotation decreased. This is because the terpenes which show high optical rotation are either reduced or totally eliminated in the deterpenated oils. The terpenless orange, lemon and lime oils were 20, 6 and 4 times concentrated respectively in their flavour as compared with their cold pressed oils.

Thirty seven components from lemon cold, pressed lemon oils were determined in a single chromatographic run by using the internal standard method and a computing integration (Staroscik and Wilson,1982). Six of these components, L-thujene, 3-carene, octanol, nerol, geraniol and nonyl acetate had not previously been quantitated in lemon oils.

Figueired and Tango (1969/1970) found that the mean oil contents in 3 varieties of ripe orange (batch of 20) were 0.45, 0.62 and 0.79 per cent. The essential oil of 4 varieties of orange and 1 of lemon were found to contain 15 and 7 components respectively. Approximately 95 per cent of the components of orange was limonene, vs 61 per cent of lemon oil (in which B-pinene (18 %) and terpenene (16 %) were the other main constituents).

Salib et al. (1978) extracted the peel oil from the peels of 8 different Egyptian citrus fruit. The peels contained 1.0-3.5 per cent essential oil. Quantitative analysis of badadi and sweet orange peel oil showed that citronellal was the major component in baladi, while citral predominated in sweet lemon and orange peel. Limonene was the major constituent of lime oil, bitter orange and grapefruit contained citronellal in equal proportion with limonene and citral, respectively. Mandarin and Navel orange contained nearly the same proportion of citronellal and citronellol, but mandarin was characterized by the presence of approximately 13 per cent limonene, while Navel orange peel oil contained approximately 10 per cent citral.

Sano and Calvarano (1982) studied the composition of essential oils of Tahiti lemon. They identified 28 compounds; 83-88 per cent terpenes, 3-4 per cent aldehyde and ketones, 3-4 per cent alchols and esters, 2-3 per cent sesquiterpenes.

Reduction in optical density of essential oil increased with the temperature and time of treatment. The peroxide index increased with the temperature and duration of heat

treatment (Tateo and Nulli,1980).

Analysis of lime juice essence volatiles led to the identification of 27 compounds comprising of 13 alcohols, four ketones, four aldehydes, three hydrocarbons, two ethers and one ester (Moshonas and Shaw,1972). From lime essence volatiles,however,34 compounds were identified as 18 alcohols, 6 aldehydes, two ketones, 3 hydrocarbons,two esters and three oxides.

The aqueous orange essence on analysis by GLC afforded 37 identified components including seven newly bound orange essence components (Moshonas and Shaw,1972 a). One of these, trans-2-pentenal, is a new citrus constituents with a fruity aroma with a flavour threshold of 100-150 ppb in synthetically prepared sample. Shaw and Wilson (1981) showed that Nootkatone exerted more effect on aroma of grapefruit oil than on the flavour of juice flavoured with it. Taste and aroma panel studies have shown that in addition to nootkatone, several other compounds present in oil influence the good grapefruit aroma and flavour.

2.4.2. Seed oils:

The characteristics and composition of the crude oil extracted from Citrullus colocy seeds were examined by Sawaya et al.(1983). The oil showed relatively low peroxide values that were within the acceptable limits set for other vegetable oils. Kinsella (1974) showed that grape seed oil is a very rich source of linoleic acid (68.2 to 72.5 %) considered an essential fatty acid for man.

Nolte and Loesecke (1940) described the first commercial production of citrus seed oil from grapefruit seeds in florida. Based upon the weight of the whole fruit, they estimated that the average yield of seeds from seeded grapefruit varieties was about 4.7 per cent. The oil content of air-dried seeds was in the 30-40 per cent range. The seedy varieties of citrus fruit, such as the pine apple orange and Duncan grape fruit contain about 3.5 per cent seeds on the wet basis (U.S.Dep.Agric.1962). The seed composition approximates 55-60 per cent moisture and 15 per cent seed oil.

Braddock and Kesterson (1973) showed that all varieties of citrus seed oils contains significant amounts of poly-unsaturated fatty acids.

2.5. Organoleptic evaluation:

Fruit beverages are a source of vitamins,minerals, carbohydrates, amino acids, flavonoid compounds and many other constituents. Many fruit juices are either too acidic or too strongly flavoured to pleasant beverage without diluting or blending or both (Pederson and Beattie,1943). Often, these strong, tart juices are delicious after dilution with thin syrup or blend juice (Tressler,1961; Luh,1971).

A ready-to-serve beverage of ber having 33.3 per cent juice and 20.8^{0} Brix and an acidity of 0.5 per cent was liked moderately by the panel of ten judges (Khurdiya,1980).

Sulphur dioxide helps in maintaining good organoleptic quality of various products probably because it acts as a preservative and checks the oxidation of fruit constituents and growth of microorganism which may produce off flavour. Ranote and Bains (1982) reported that the kinnow juice preserved with sulphur dioxide secured consistently maximum scores. Adjustment of pH of different juices to 4.0 had a marginal improvement in the flavour scores. Potassium metabisulphite (350 ppm sulphur dioxide) preserved juice had superior flavour and colour retention as compared to heat processed bottled juice which developed browning.

Temperature affects the retention of colour of ready-to-serve beverage during storage. Khurdiya and Anand (1981) found that the colour in phalsa beverage was best retained at 3°C followed by 20°C and at room temperature (31-36°C). The beverage can be best stored upto 100 days. The acceptability of the beverage goes down when stored at 20°C at room temperature. Similar findings are reported from bael fruit. Practically no changes in organoleptic quality in frozen pulp of bael fruit after six months and in case of other products stored at 37°C, the organoleptic quality remained much above the acceptable point (Roy and Singh, 1979).

Blending of different juice effect the organoleptic quality of the product. According to Rao et al. (1979), the blends containing Rangpur lime and acid lime juice in ratio of 12.5:12.5 indicated a clash in flavour, resulting

in poor scoring. Whereas 15:10 and 20:5 were rated high both for flavour and consistency. Tannous and Lawn (1981) reported that blends of American and Maharaji in ratio of 1:2 with 2 per cent added sugar had the highest organoleptic score. It was noteworthy that the blending acidic Maharaji juice with sweet juice resulted in higher organoleptic scores. There was a little effect of storage on quality of blended juice while significant effect of storage was seen in Ambri juice after 3rd month due to changes in colour and appearance. Addition of ascorbic acid improved both the colour and flavour of reconstituted apple juice. The test panel showed preference for the clarified apple juice.

CHAPTER III

MATERIALS AND METHODS

The present investigation on the processing technology of Bhadri lemon (Citrus limon (L.) Burm.) were carried out in Fruit Technology Laboratory, Department of Horticulture, Haryana Agricultural University, Hisar. The various experiments conducted were preservation of fresh juice, preparation of squash from preserved juice, extraction of oil from seed and peel of the fruit and the preparation of candy from albedo portion.

3.1. Materials:

3.1.1. Fruits:

Bhadri lemon fruits for processing were harvested at colour turning stage from experimental orchard of the Department of Horticulture, Haryana Agricultural University, Hisar.

3.1.2. Chemicals:

Most of the common chemicals used were either the product of Analar quality of BDH Chemicals, India or E.Mereck, India.

3.2. Methods:

3.2.1. Preservation of Bhadri lemon juice:

3.2.1.1. Preparation of fruits:

After harvesting the fruits were washed in running tap water to remove dirt and dust particles. Then the fruits were cut into two halves.

3.2.1.2. Extraction of juice:

The juice was extracted through Rosing Machine (Horizontal type). The whole of the juice was then filtered to obtain a clear juice devoid of any pulp etc. The juice was then kept in cold storage (4°C) for 24 hrs for sedimentation. The clear juice was siphoned off.

3.2.1.3. Treatments:

i) Heating the juice to 85°C

ii) Heating the juice to 85°C and pasteurization

iii) Pasteurization

iv) Potassium metabisulphite @ 0.05 %

v) Potassium metabisulphite @ 0.1 %

vi) Sodium benzoate @ 0.05 %

vii) Sodium benzoate @ 0.1 %

viii) Control (untreated)

3.2.1.4. Bottling:

The treated juice was poured into hot, well sterilized crown bottles of 200 ml capacity and corked air-tight.

3.2.1.5. Pasteurization:

Where pasteurization was required, the filled bottles were pasteurized in boiling water for 20 minutes till the temperature of the product reaches 85°C.

3.2.1.6. Storage:

The juice filled bottles were kept at ambient temperature (15 ± 2°C) for storage studies. Samples were taken out in duplicate at 15 days interval and analysed for the following constituents:

i) Reducing sugars

ii) Total sugars

iii) Total soluble solids

iv) Ascorbic acids

v) Total acidity

vi) Browning

vii) pH

viii) Tannins

3.2.2. Preparation of squashes:

3.2.2.1. Preparation:

The squash was prepared from fresh as well as stored Bhadri lemon juice. The squash prepared from fresh juice was stored at room temperature for 3 months and analysed for the following constituents at 15 days interval.

i) Reducing sugars

ii) Total sugars

iii) Total soluble solids

iv) Acidity

v) Ascorbic acids

vi) pH

vii) Browning

viii) Tannins

After 90 days the squash prepared from stored juice and stored squash prepared from fresh juice was evaluated organoleptically.

3.2.3. Extraction of volatile oil from peel(Flavedo):

3.2.3.1. Preparation of sample:

The peel of the fruit was taken and the flavedo and albedo portion was separated from the peel completely. The flavedo portion was chopped into small pieces and utilised for extraction of oil.

3.2.3.1. Extraction of volatile oil:

The volatile oil content of citrus peel was determined by steam distillation method and the oil was collected in a specially designed glass apparatus (Fig.2) in the Fruit Technology Laboratory of the Department of Horticulture, which consists of a 500 ml volumetric flask to which a graduated tube with stop cork was attached at its neck for measuring the amount of oil.

Took 500 g of chopped flavedo portion and added 1 litre of distilled water into a round bottom flask. The whole mass was boiled for 2 hours and collected the distillate in specially designed flask. The distillate was collected until the distilled oil comes within the graduated scale. Noted the volume of oil collected and the recovery was calculated on the basis of following formula:

$$\text{Volatile oil content (\%)} = \frac{\text{ml of oil} \times \text{Sp. gravity of oil}}{\text{Wt. of sample taken}} \times 100$$

3.2.4. Extraction of seed oil:

The oil from dried lemon seeds was extracted by the method of Folch et al.(1957).

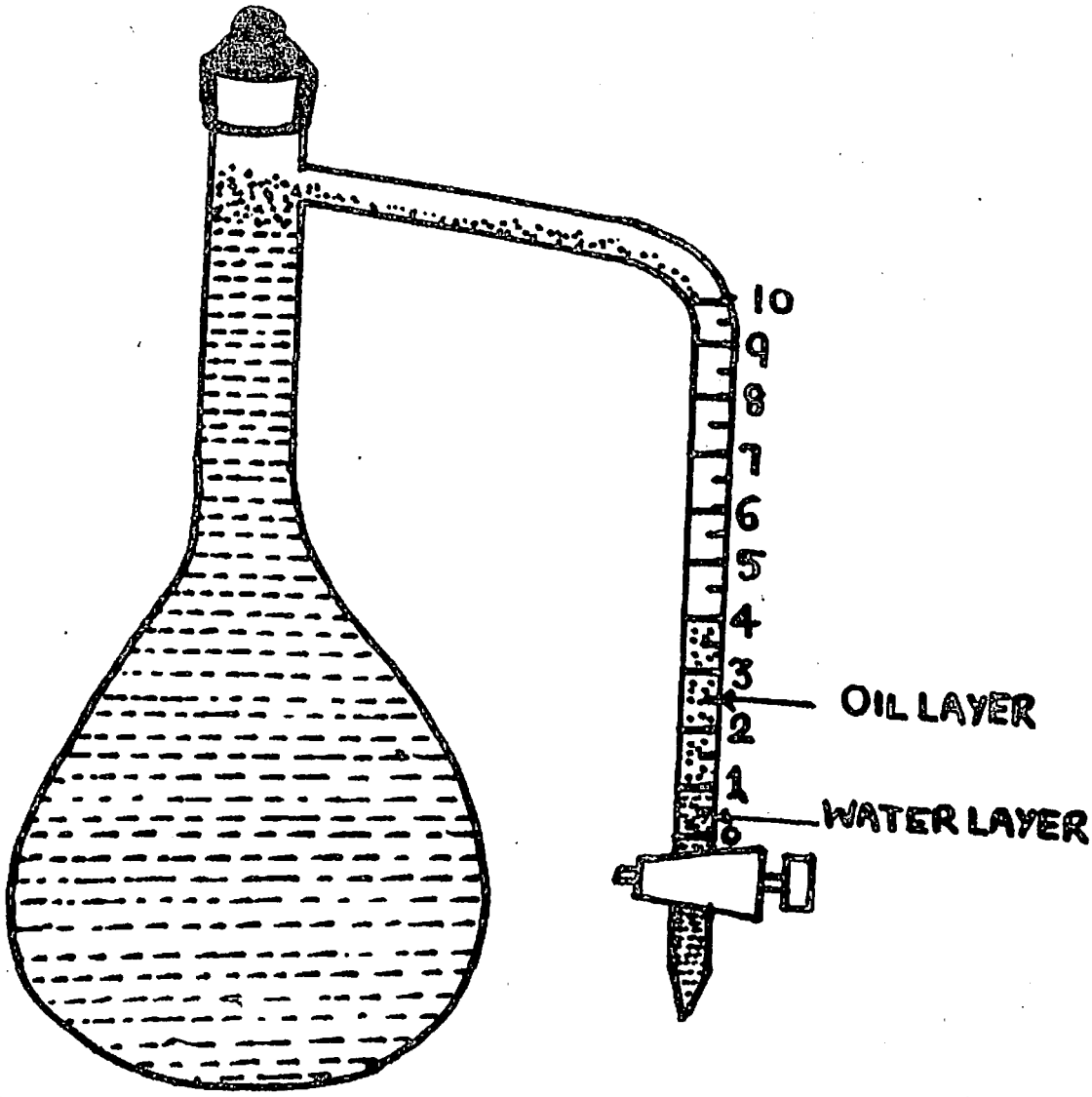

FIG 2. ESSENTIAL OIL COLLECTING FLASK AS DESIGNED BY DHAWAN, S.S. AND GUPTA, S.K.

Procedure:

5 g of the dried seeds were taken and crushed thoroughly in pestle and mortar. 100 ml of chloroform: methanol (2:1 v/v) was added and the whole material was put into an air tight glass stoppered iodometeric flask. The contents of the flask were shaken for one hour on an electric shaker and filtered through the glass sintered funnel. The extraction was repeated twice, all the extractions were pooled and solvent was distilled off under vacuum from the filterate till the oils were completely free of the solvents.

3.2.5. Utilization of albedo for candy making:

Albedo comprises about 34 per cent of the fruit portion which is not utilised and goes waste. A delecious candy from albedo, preserved in sugar syrup was made by slow cooking process. The process followed for candy making has been briefly outlined in flow sheet (Fig.1.).

3.2.5.1. Preparation of fruit:

Big size lemon fruits were selected and washed in tap water and peeled.

3.2.5.2. Separating of albedo:

Thick white portion from the peel was removed and round slices of albedo were cut with slicer.

3.2.5.3. Brining:

The slices were kept for 3 days in 4 per cent Brine solution to extract bitter principles. The brine solution was changed daily. The slices were removed from the solution

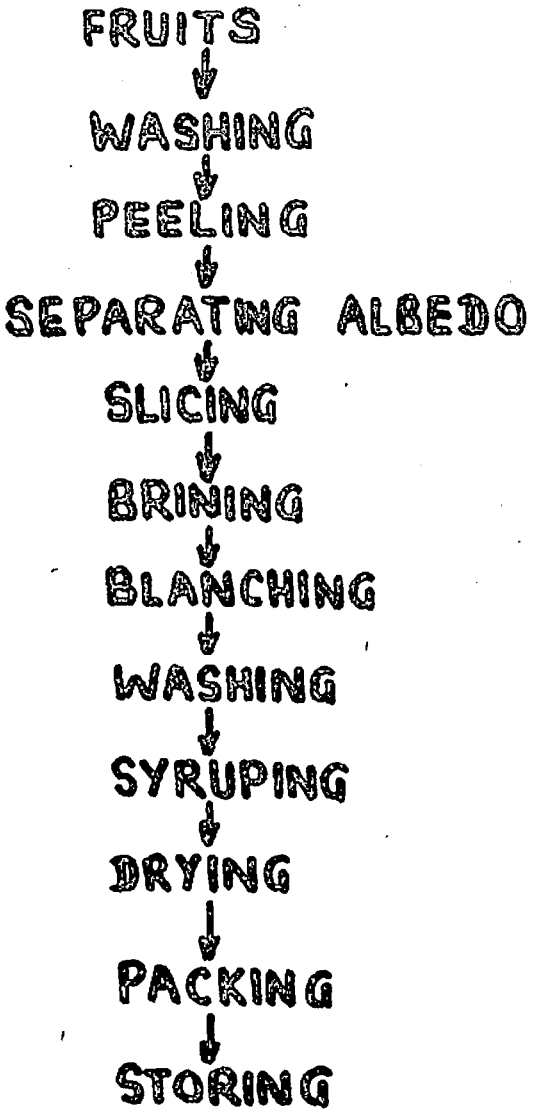

Fig 1. FLOW SHEET FOR CANDY MAKING

and washed in tap water to remove any traces of salt.

3.2.5.4. Blanching:

Cut slices were blanched in hot boiling water for 5 minutes to inactivate the enzymes and reduce bitterness.

3.2.5.5. Syruping:

Sugar syrup of 40 per cent concentration was prepared by dissolving 665 g of cane sugar in 1 litre of water. Citric acid was added to the syrup @ 0.5 per cent. Sugar was dissolved completely by boiling and scum was removed. The syrup was filtered through muslin cloth and its concentration was checked by Abbe Refractrometer. The treated slices were placed in a glass container and sugar syrup was added @ 1.5 l/kg of slices. Next day the syrup was decanted and added back after mixing 300 g of additional sugar by boiling. This was again kept overnight and addition of sugar was repeated on 4th and 6th day and subsequently allowed to stand for a week. Finally sugar syrup was heated to bring the concentration of sugar syrup to 75°B. After 4 weeks the slices were removed from the syrup and dried at 50°C in the oven.

3.2.5.6. Packing and storing:

The candied slices were packed in glass jar and stored at room temperature.

3.3. Chemical analysis:

3.3.1. Sugars:

Sugars were estimated by the method of Hulme and Narain (1931).

Reagents:

A. Potassium ferricyanide solution:

Potassium ferricyanide	= 8.25 g
Anhydrous sodium carbonate	= 10.6 g
Volume	= 1 litre

B. Potassium iodide solution:

Potassium iodide	= 12.5 g
Zinc sulphate	= 25.0 g
Sodium chloride	= 125.0 g
Volume	= 1 litre

C. 5 % Acetic acid solution (v/v):

Glacial acetic acid	= 50 ml
Volume	= 1 litre

D. Sodium thiosulphate (N/100) solution:

Sodium thiosulphate	= 2.419 g
Volume	= 1 litre

E. Starch solution (Indicator):

Soluble starch	= 1.0 g
Sodium chloride	= 20.0 g
Volume	= 1 litre

Estimation:

Five ml of Bhadri lemon juice and squash was diluted to appropriate concentration and was taken for estimation. Similarly sugar solution of candy and albedo was diluted to appropriate concentration.

a) Reducing sugars:

Five ml of sugar solution and five ml of potassium ferricyanide solution was taken in a test tube (1" wide 7"

long). The tubes were covered and kept for 15 minutes in boiling water bath. The tubes were cooled under the tap water and 5 ml of iodide-zinc solution followed by 3 ml of acetic acid solution (5 % v/v) were added in each tube. The liberated iodine was titrated with sodium thiosulphate (N/100) using starch as an indicator. The end point was the disappearance of blue colour and appearance of milky white colour. A blank with 5 ml of distilled water was also run simultaneously. The results were calculated by the following formula and expressed in mg sugar/100 ml.

$$\left\{\begin{array}{l}\text{ml of sodium}\\ \text{thiosulphate}\\ \text{used in blank}\end{array} - \begin{array}{l}\text{ml of sodium}\\ \text{thiosulphate}\\ \text{used in unknown}\end{array}\right\} + 0.05 \times 0.34 = \begin{array}{l}\text{mg of}\\ \text{sugar}\end{array}$$

b) Total sugars:

To 25 ml of sugar solution added 4 ml of hydrochloric acid in flask and kept for 10 minutes at 68°C. After hydrolysis the volume was made to 50 ml. The acidity was neutralized by adding anhydrous sodium carbonate till the effervesence stopped. Total sugars were then determined by the method as described in reducing sugars.

3.3.2. Total soluble solids:

Total soluble solids were observed at ambient temperature by using Hand Refractometer and readings were corrected for 20°C.

3.3.3. Ascorbic acid:

The ascorbic acid was determined by the method described by Rangana (1977).

Reagents:

i) Metaphosphoric acid (3 %):

Metaphosphoric acid	= 15 g
Glacial acetic acid	= 40 ml
Volume	= 500 ml

ii) 2,6-Dichlorophenol-indophenol solution:

2,6-Dichlorophenol indophenol	= 50 mg
Sodium bicarbonate	= 42 mg
Volume	= 200 ml

Estimation:

A suitable sample was titrated against 2,6-dichlorophenol indophenol solution till the light pink colour appeared. The results were expressed as mg ascorbic acid per 100 ml sample.

3.3.4. Total acids:

Total acids were estimated by titrating 5 ml of juice against standard solution of 0.1 N sodium hydroxide using phenolphthlein as an indicator. The end point appear as light pink colour. The acidity was expressed as g citric acid per 100 ml.

3.3.5. Browning:

Browning was observed by taking transmittance of the solution in spectronic-20. The samples for browning were centrifuged for 15 minutes at 4000 rpm. To 10 ml of centrifugate added 15 ml of alcohol to make 60 per cent aqueous solution and kept for half an hour. It was, then filtered through Whatman filter paper No.1 to obtain clear

solution. The colour was measured at 440 nm using 60 per cent aqueous alcohol as blank. The increase in absorbance of a sample at 440 nm was taken as a measure of non-enzymatic browning.

3.3.6. pH:

The pH of the juice was taken on Systronic Digital pH meter using standard buffers.

3.3.7. Tannins:

The tannins were determined by the method of A.O.A.C. (1980).

a) Reagents:

i) Folin ciocaltau reagent

ii) Sodium carbonate saturated solution: To 100ml of water added 35 g of anhydrous sodium carbonate, dissolved at 70-80°C, allowed to cool overnight. Decent the clear liquid before use.

iii) Tannic acid standard solution: Dissolved 100 mg tannic acid in 1 litre of water. Prepared fresh solution for each determination.

b) Preparation of standard curve:

Pipetted 0 to 10 ml aliquots of the standard tannic acid solution into 100 ml volumetric flask containing 75 ml of water, added 5 ml folin ciocaltau reagent and 10 ml Na_2Co_3 solution, and diluted to volume with water. Mix well and det.A after 30 minutes at 760 nm. Plot A against mg tannic acid/100 ml.

c) Determination:

Using 1 ml sample, det.A as in (b) and obtain mg tannic acid/100 ml from standard curve. Samples treated as above may be compared in Nessler tubes against freshly prepared tannic acid standards treated in same manner.

3.3.8. Specific gravity:

The specific gravity of the volatile oil and seed oil was determined by taking the ratio of mass/volume.

3.3.9. Refractive index:

Refractive index of volatile oil and seed oil was determined by Abbe-Refractrometer (Erma, Japan) at 20°C.

3.3.10. Iodine value:

The iodine value was determined by the Wiz's method (A.O.A.C., 1960).

Reagents:

i) 0.1 N sodium thiosulphate

ii) Starch solution (indicator)

iii) Wiz's iodine solution N/10

iv) Potassium iodide (10 %)

Procedure:

Added 10.0 ml of petroleum ether to dissolve 500 mg of seed oil and 25 ml of Wiz's solution in 250 ml conical flask. Allowed it to stand for atleast one hour in a dark place. A blank was also run simultaneously containing 10 ml of petroleum ether and 25 ml of Wiz's solution.

Now added 5.0 ml of 10 per cent potassium iodide and 50 ml water to each flask and titrated against 0.1 N sodium thiosulphate using starch as an indicator. Disappearance of blue colour was the end point. The iodine value was calculated by the following formula:

$$\text{Iodine value} = \frac{100}{\text{wt. of oil}} \times 0.0127 \times (V_1 - V_2)$$

V_1 = Vol. of 0.1N sodium thiosulphate used in the blank.

V_2 = Vol. of 0.1N sodium thiosulphate used in case of oil.

3.3.11. Estimation of free fatty acids in seed oil:

The fatty acid were estimated by the method of A.O.A.C. (1960).

Reagents:

i) Phenolphthalin 1 % solutions in alcohol neutralized with N/10 NaOH

ii) Denatured alcohol (Neutral): Mix 10 volumes of ethyl alcohol with 1 volume of methyl alcohol and neutralized with N/10 NaOH using phenolphthalin as an indicator.

iii) N/10 NaOH

Procedure:

Weighed 1 g of oil in 250 ml conical flask and added 10 ml of denatured alcohol (neutral) and shaken well. Added 0.4 ml of phenolphthalin as indicator and titrated against N/10 NaOH with vigorous shaking after each addition till a permanent light pink colour was appeared which persists for atleast 1 min.

Calculations:

$$\% \text{ of oleic acid in oil} = \frac{100}{W} \times \frac{282}{10} \times \frac{V}{1000}$$

where, W is the weight of oil used in g,

V is the volume of N/10 NaOH used in ml, and

282 is the equivalent weight of oleic acid.

3.3.12. Determination of fatty acid composition:

Preparation of methyl esters (Luddy et al., 1968):

Reagents:

Reagent-A: Sodium methylate 0.4N.

The reagent was prepared by the method of Luddy et al. (1960). Metallic sodium was cut into small bright pieces under petroleum ether and added to a known volume of redistilled obsolute methanol in amounts slightly in excess of that required for the desired normality (only small piece of metal was added at a time). When the addition was complete the normality of sodium methylate was adjusted to 0.4.

Reagent-B: Carbon disulphide (CS_2)

Reagent-C: Mixture of silica gel and calcium chloride (1:1).

Procedure:

The sample was taken in a centrifuge tube. Added 0.25 ml of 0.4N sodium methylate. The vial was flushed with nitrogen gas and was covered with tin foil. The vial was immersed in water bath at 65°C to a depth of 1/2 inch and was shaken vigorously for 30 seconds. The mixture of sample and reagent became homogenous indicating that the estrification

of the sample was complete. The heating was continued without shaking for an additional 1½ minute. The vial was removed from the bath and was cooled to room temperature. It was opened and 0.6 g of mixture of silica gel and calcium chloride (1:1) was quickly added and stirred vigorously with glass rod. 3 ml of CS_2 was added, the cap was replaced and was shaken for 1-2 minutes by hand. The vial was centrifuged for 1-2 minutes at 1200 rpm. A clear layer of CS_2 containing all the methyl ester of the fatty acid was decanted into small test tubes. Fatty acid methyl esters were further separated by GLC.

3.3.12.1. Fractionation of methyl esters by gas liquid chromatography:

Methyl esters of fatty acids were separated in a Hewlett Packard Model No.5730A gas chromatograph equipped with flame ionization detector. Stainless steel column (10'x1/8") was packed with 20 per cent diethylene glycol succinate (DEGS) on 60-80 mesh chromosorb W. The column temperature was 190°C and the flow of the nitrogen carrier gas was maintained at 35 ml/min. The peaks were identified by comparison of their retention times with those of standard fatty acids. The area under the individual peak was calculated by the formula, $\frac{\text{base}}{2} \times \text{height}$, and converted directly into relative percentage.

3.3.13. Crude fibre:

The crude fibre from albedo and candy was determined by the method of A.O.A.C. (1970).

Reagents:

A. 0.255 N sulphuric acid solution (1.25 g H_2SO_4/ 100 ml).

B. 0.313 N sodium hydroxide solution (1.25 g NaOH/ 100 ml).

Determination:

Extracted 2 g of dry material with petroleum ether. Transferred the residue to the digestion flask. Added 200 ml of the boiling sulphuric acid solution, immediately connected the digestion flask with condenser and heated. Rotated the flask frequently until the sample was thoroughly wetted. During digestion care was taken to keep the material from remaining on the sides of the digestion flask without contact with the solution.

After 30 min. removed the flask and filtered through muslin cloth in a fluted funnel. Washed with boiling water until the washings were no longer acid. Added 200 ml NaOH solution. Connected the flask with reflux condenser and boiled for exactly 30 min.

After 30 min. of boiling, removed the flask and immediately filtered through filtering cloth in a fluted funnel. Washed with water. Returned the residue to the digestion flask thoroughly. Washing all residue from cloth with hot water. Filtered into the Gooch crucible.

After thorough washing of the residue in the Gooch crucible with boiling water, washed with approximately 15 ml of alcohol. Dried the crucible and the contents at 110°C

to constant weight. Cooled in a desicator and weighed. Ignited the contents of the crucible in an electric muffle furnace at dul red heat until carbonaceous matter was destroyed (approximately 20 min). Cooled in a desicator and weighed. The loss in weight represents crude fibre:

$$\text{Per cent crude fibre} = \frac{\text{Loss in wt. noted}}{\text{Wt. of sample taken}} \times 100$$

3.3.14. Crude protein:

The crude protein from albedo and candy was determined by the Micro-Kjeldahl method. The procedure consists of three steps.

i) Digestion:

A weighed amount of substance (0.1 g) was taken in a Kjeldahl's flask, 0.5 g of catalytic mixture (K_2SO_4:$CuSO_4$ (10:1) was added and 10 ml sulfuric acid was added. The mixture was heated till it becomes clear. After cooling the final volume of the digested clear solution was made to 100 ml with distilled water.

ii) Distillation:

Took 10 ml of the digested solution in the assembly and added 10 ml of 40 per cent NaOH, immediately air tight the assembly, then passed a current of steam. Ammonia gas begins to evolve gradually alongwith steam, which after condensing through a condenser collected in the standard (N/100) 10 ml sulfuric acid solution, having two drops of methyl red indicator.

iii) Estimation of ammonia:

Titrated this N/100 sulfuric acid against standard N/100 NaOH solution run a blank simultaneously. Difference between volume of NaOH (N/100) used for blank and sample solution will indicate the amount of ammonia evolved. Calculated the percentage nitrogen as follows:

Calculation:

Suppose 'W' g of the sample is taken and that V_1 ml of acid of normality N_1 is required for the neutralisation of ammonia evolved.

$$\text{Percentage of nitrogen} = \frac{14 \times V_1 \times N_1}{1000 \times 100} \times \frac{100}{W}$$

$$\text{Percentage of protein} = \frac{14 \times V_1 \times N_1}{1000} \times 6.25$$

CHAPTER IV

RESULTS AND DISCUSSION

During the course of investigation the effect of various treatments on the preservation, biochemical changes of lemon juice during storage and the utilisation of stored juice for beverage making was studied. Efforts were made to utilise the waste material like peel and seed for oil extraction and candy making. The presentation of results and discussion has been arranged under the following heads:

4.1. Composition of fresh fruit.

4.2. Effect of various treatments on the preservation of juice and its utilization.

a) Biochemical changes in the juice during storage.

b) Biochemical changes in the squash during storage.

c) Organoleptic evaluation of squash.

4.3. Utilisation of waste material.

a) Composition of albedo and candy.

b) Oil and oil characteristics of seed and peel.

4.1. Composition of fresh fruit:

The results on the proximate composition of fresh fruits of Bhadri lemon were presented in Table 1.

The fruit contained 18.45 per cent juice and the rest of the portion included the albedo (34.48 %), the flavedo (20.89 %), the seed (1.82 %) and the rag (24.34 %). The quality of the juice was determined and total soluble

Table 1: Proximate composition of fresh Bhadri lemon fruit

T.S.S. (%)	6.65
Acidity (g/100 ml)	4.13
Ascorbic acid (mg/100 ml)	47.6
Reducing sugars (g/100 ml)	0.80
Total sugars (g/100 ml)	1.25
pH	1.99
Browning (O.D. at 440 nm)	0.022
Tannins (mg/100 ml)	42.5
Albedo (%)	34.48
Flavedo (%)	20.89
Seed (%)	1.82
Rag (%)	24.34
Juice (%)	18.45
Average weight of the fruit (kg)	1.5

solids, acidity, ascorbic acid, reducing sugars, total sugars, tannins, pH and browning were estimated.

The total soluble solids of the juice were 6.65 per cent whereas the acidity was 4.13 g/100 ml. The juice contained 0.80 g/100 ml reducing sugars and 1.25 g/100 ml total sugars. The ascorbic acid content in the juice was 46.7 mg/100 ml. The juice contained 42.5 mg/100 ml of tannins. The pH of the juice was 1.99 and the browning expressed in terms of optical density at 440 nm was 0.22. The average weight of the fruit was found to be 1.5 kg.

The composition of lemon fruit has been determined by various workers. The California Fruit Grower Exchange (1946) found an average of 2.15 g total sugars per 100 g of fresh lemon juice. McCready et al. (1950) observed 0.94 per cent reducing sugars in filtered single strength lemon juice.

While analysing several varieties of mature lemons Stahl (1935) observed that their pH ranged from 2.1 to 2.5. The California Fruit Grower Exchange (1946) also found in the juice to have an average pH of 2.30. Nelson (1928) reported that lemon juice contained 3.84 per cent citric acid.

Ascorbic acid in citrus juices is the main source of vitamin and it has been observed that Bhadri lemon contains a good amount of ascorbic acid. Dhingra and Gupta (1980) reported that the Bhadri lemon juice contained 40.0 mg/100 ml ascorbic acid. They further reported that the juice

contained 3 g/100 ml citric acid and 7 per cent total soluble solids, whereas Singh and Dhawan (1983) observed 42.5 mg/100 ml ascorbic acid, 3.2 g/100 ml citric acid and 6.8 per cent total soluble solids in Bhadri lemon juice.

Singh and Singh (1980) evaluated four varieties of lemon for total soluble solids, acidity and ascorbic acid. The values they observed for the total soluble solids of the juice were 7.6, 7.3, 7.7 and 6.5 per cent for Baramasi, Eureka Oblong, Kagzi Kalan and Lucknow Seedless, respectively. The corresponding values for acidity were 3.9, 3.8, 3.9 and 3.4 per cent and for ascorbic acid the values were 54, 28, 48 and 23 mg/100 ml, respectively.

4.2. Effect of various treatments on the preservation of juice and its utilisation:

4.2.1. Biochemical changes in the juice during storage:

In this experiment various heat treatments and chemical preservatives were applied for the preservation of juice and biochemical changes during storage were studied at 15 days interval upto 90 days. The results obtained are discussed below:

4.2.1.1. Total sugars:

The total sugar content of the Bhadri lemon juice was estimated at various intervals of storage and the results obtained are presented in Table 2.

A perusal of the data given in Table 2 indicates that the total sugar content of the juice was significantly affected by the treatments and storage period. On the first

Table 2: Effect of different treatments and storage period on total sugar content in Bhadri lemon juice

(g/100 ml)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	1.25	1.20	*	*	*	*	*	-
Heating	1.27	1.45	2.15	2.99	3.48	3.69	3.77	2.69
Heating + Pasteurization	1.27	1.35	2.12	2.90	3.52	3.75	3.84	2.68
Pasteurization	1.27	1.32	2.22	2.88	3.35	3.63	3.88	2.65
KMS @ 0.05 %	1.25	1.50	2.30	3.00	3.55	3.80	3.92	2.76
KMS @ 0.1 %	1.29	1.45	2.28	2.95	3.40	3.55	3.65	2.65
SB @ 0.05 %	1.27	1.40	2.21	2.98	3.50	3.75	3.90	2.72
SB @ 0.1 %	1.27	1.38	2.16	2.90	3.45	3.72	3.80	2.67
Mean	1.27	1.41	2.21	2.94	3.46	3.70	3.82	-

* Not recorded due to fermentation

C.D. (P = 0.05) Treatments = 0.01
Storage = 0.01
Treatments x Storage = 0.04

day of storage the highest total sugars were observed in KMS @ 0.1 per cent treatment (1.29 g) whereas the contents were lowest in control and KMS @ 0.05 per cent (1.25 g) treatments. Not much differences were observed in all other treatments.

Period of storage also affected the total sugar content of juice. The total sugars increased significantly with the increased period of storage in all the treatments except in control where it decreased on 15th day ofstorage. The highest total sugars were observed in KMS @ 0.05 per cent (2.76 g) treatment and this lead was maintained throughout the storage period except on 1st day of storage where it was less, whereas the sugars were lowest in pasteurization and KMS @ 0.1 per cent (2.65 g) treatment. The total sugar content in control was found to be decreased as estimated only upto 15 days of storage due to fermentation of juice.

The results showed a progressive and marked increase in total sugar content throughout the storage period. The increase in total sugars might be due to the hydrolysis of polysaccharides like pectin, celluloses, starch etc. and its conversion into simple sugars. Roy and Singh (1979) also supported this fact for the increase in sugars during processing in bael beverage and later on it was also supported by Ranote and Bains (1982) while processing of the kinnow juice in heat processed samples. Shrestha and Bhatia (1982) also suggested maximum changes in stored apple

juice at room temperature than at lower temperature which indicate that temperature during storage could lead to a gradual inversion of polysaccharides into sugars.

The results are also in confirmation with the findings of Palainswamy and Muthukrishnan (1974) on preservation of lemon juice during storage and in juices of Mandarin, Sweet orange and Lemon, it was observed an increase in total sugars content during storage (Mehta and Bajaj, 1983). Moreover, it can be stated that the increase in total sugars coincides with the increase in total soluble solids.

4.2.1.2. Reducing sugars:

The reducing sugar content of the Bhadri lemon juice was estimated at various intervals of storage and the results obtained are presented in Table 3.

The data given in Table 3 indicates that the reducing sugars of the juice were significantly increased by various treatments and storage period. On the 1st day of storage, the highest reducing sugars were observed in both the treatments i.e., heating and KMS @ 0.1 per cent (0.83 g) closely followed in heating + pasteurization, KMS @ 0.05 per cent and SB @ 0.05 per cent where it were 0.82 g in all these treatments. The sugars were lowest in control, pasteurization and SB @ 0.1 per cent (0.80 g) treatments. During storage there was a gradual increase in reducing sugars with prolonged storage period in all the treatments except in control where it were reduced on 15th day of storage. The mean values showed the highest increase in

Table 3: Effect of different treatments and storage period on reducing sugar content in Bhadri lemon juice

(g/100 ml)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	0.80	0.76	*	*	*	*	*	-
Heating	0.83	0.95	1.06	1.18	1.35	1.62	1.83	1.26
Heating + Pasteurization	0.82	0.93	1.09	1.20	1.41	1.59	1.78	1.26
Pasteurization	0.80	0.89	1.13	1.25	1.47	1.71	1.91	1.31
KMS @ 0.05 %	0.82	0.98	1.10	1.28	1.46	1.70	1.92	1.32
KMS @ 0.1 %	0.83	0.92	1.12	1.32	1.49	1.75	1.96	1.34
SB @ 0.05 %	0.82	0.90	1.17	1.34	1.39	1.68	1.89	1.31
SB @ 0.1 %	0.80	0.89	1.16	1.29	1.37	1.65	1.80	1.28
Mean	0.82	0.92	1.12	1.27	1.42	1.67	1.87	-

* Not recorded due to fermentation

C.D. (P = 0.05) Treatments = 0.01

Storage = 0.01

Treatments x Storage = 0.04

reducing sugars in KMS @ 0.1 per cent (1.34 g) treatment, whereas the content was lower where heating and heating + pasteurization (1.26 g) was done. Further, it was observed that the reducing sugar content was significantly lower in heating and heating + pasteurization treatments as compared to the other treatments except SB @ 0.1 per cent, where it was statistically at par. The reducing sugars in control could not be estimated beyond 15 days of storage as the juice was fermented.

The increase in reducing sugars with the increased period of storage in all the treatments could be attributed to gradual inversion of non-reducing sugars into reducing sugars in acidic medium. These results are in confirmation with the findings of Palainswamy and Muthukrishnan (1974) where they observed a gradual increase in reducing sugars content in lemon juice during storage. Similar increase was also observed in juices of Mandarin, Sweet orange and Lemon by Mehta and Bajaj (1983), in Kinnow juice by Ranote and Bains (1982) and in Amla juice by Shrestha and Bhatia (1982) when stored at ambient temperature. They further reported that conversion of non-reducing sugars was higher at room temperature than at low temperature.

4.2.1.3. Total soluble solids:

The total soluble solids of Bhadri lemon juice were estimated at various intervals during storage and the effect of different treatments on keeping quality were recorded and presented in Table 4.

Table 4: Effect of different treatments and storage period on total soluble solids content in lemon juice

(per cent wt/wt)

Treatments	Storage period (days)						
	1	15	30	45	60	75	90
Control	6.65	5.82	*	*	*	*	*
Heating	6.65	6.71	6.71	6.82	7.15	7.65	7.76
Heating + Pasteurization	6.65	6.71	6.71	6.82	7.15	7.65	7.76
Pasteurization	6.65	6.71	6.71	6.82	7.15	7.65	7.76
KMS @ 0.05 %	6.65	6.71	6.71	6.82	7.15	7.65	7.76
KMS @ 0.1 %	6.65	6.71	6.71	6.82	7.15	7.65	7.76
SB @ 0.05 %	6.65	6.71	6.71	6.82	7.15	7.65	7.76
SB @ 0.1 %	6.65	6.71	6.71	6.82	7.15	7.65	7.76

* Not recorded due to fermentation

C.D. (P = 0.05) = N.S.

A perusal of the data given in Table 4 indicates that there was an increase in total soluble solids with the increased period of storage in all the treatments which ranged from 6.65 to 7.76 per cent except in control where the total soluble solids were decreased on 15th day of storage. The decrease in total soluble solids were perhaps due to initiation of fermentation in the juice. Since the juice got fermented, no observation was recorded after 15 days of storage. Among the treatments, no differences were observed during the period of storage.

The reason for the gradual increase in the total soluble solids with increased period of storage could be explained by the fact that the polysaccharides which were present in the juice might be converted into simple sugars by hydrolysis. A similar increase in total soluble solid content with the increase in storage period was observed in the juices of Mandarin, Sweet orange and Lemon (Mehta and Bajaj, 1983) and by Palainswamy and Muthukrishnan (1974) in lemon juice.

Irving (1961) also observed similar type of increase in total soluble solids in lemon juice when stored at higher temperature.

4.2.1.4. Acidity:

The acidity of Bhadri lemon juice expressed in terms of citric acid was estimated at various intervals of storage period and the results obtained are presented in Table 5.

Table 5: Effect of treatments and storage period on acidity of Bhadri lemon juice

(g citric acid/100 ml)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	4.12	3.94	*	*	*	*	*	-
Heating	4.12	4.09	4.00	3.93	3.82	3.81	3.76	3.93
Heating + Pasteurization	4.13	4.12	4.09	4.09	4.01	3.89	3.87	4.03
Pasteurization	4.12	4.09	3.94	3.90	3.86	3.80	3.68	3.91
KMS @ 0.05 %	4.09	4.04	3.87	3.84	3.82	3.76	3.63	3.86
KMS @ 0.1 %	4.10	4.08	3.97	3.88	3.76	3.65	3.60	3.86
SB @ 0.05 %	4.08	3.93	3.90	3.77	3.75	3.61	3.60	3.80
SB @ 0.1 %	4.11	3.98	3.84	3.80	3.76	3.74	3.68	3.84
Mean	4.11	4.05	3.94	3.89	3.83	3.75	3.69	-

* Not recorded due to fermentation

C.D.(P = 0.05) Treatments = 0.05

Storage = 0.05

Treatments x Storage = 0.14

The acidity of the juice was significantly affected by the different treatments and storage period. On the 1st day of storage, the highest acidity was observed in heating+ pasteurization treatment (4.13 %) closely followed by heating, pasteurization and in control (4.12 %), whereas the lowest acidity was observed in treatment where SB @ 0.05 per cent was added (4.08 %). However, the differences among the treatments were not significant.

During storage a significant decrease in acidity was noticed with the increase in period of storage in all the treatments including control. As indicated by mean values the highest acidity was observed in heating + pasteurization treatment (4.03 %) as compared to other treatments and this lead was maintained throughout the storage period, whereas the values were lowest in treatment SB @ 0.05 per cent and KMS @ 0.1 per cent throughout the period of storage.

The interaction between days of storage and various treatments were also found significant. No significant differences were observed in heating + pasteurization treatment upto 90 days except at two storage periods i.e. 45 and 60 days where the values were significant. Similarly, no significant differences were observed in KMS @ 0.1 per cent upto 30 days and in other treatments upto 15 days of storage as compared to heating and pasteurization, after that the reduction in acidity was found significant. The acidity in control could not be estimated beyond 15 days of storage as the juice was fermented.

The decrease in acidity observed during storage could be attributed to the chemical interaction between the organic constituents of the juices induced by the temperature and action of enzymes. The decrease in acidity during storage was also observed in lemon juice by Palainswamy and Muthukrishnan (1974) and by Mehta and Bajaj (1983) in Mandarin, Sweet orange and Lemon juices. Similarly the decrease in acidity in apple cider at room temperature from 0.48 to 0.46 per cent than at 37°C when stored for 4 months was noticed by Nagi and Manjrekar (1976). The highest acidity levels maintained by the treatments where the juice was either heated or pasteurized might be due to the inactivation of enzymes which might play part in the reactions responsible for decreasing acidity.

Contradictory results have also been reported by various workers during storage of various products. Uprety et al. (1963) found an increase in acidity in stored lime juice. Similarly, Mehta and Rathore (1976) also observed a more rapid increase in total titrable acidity in amla juice in samples stored at room temperature than stored in refrigerator for 10 weeks.

4.2.1.5. pH:

The pH of the Bhadri lemon juice was estimated at various interval of storage and the results obtained are presented in Table 6.

Table 6: Effect of different treatments and storage period on 'pH' of Bhadri lemon juice

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	1.99	2.10	*	*	*	*	*	-
Heating	2.02	2.09	2.19	2.48	2.64	2.65	2.68	2.39
Heating + Pasteurization	2.01	2.22	2.39	2.41	2.65	2.67	2.69	2.43
Pasteurization	2.05	2.19	2.35	2.40	2.65	2.69	2.76	2.44
KMS @ 0.05 %	2.03	2.14	2.24	2.42	2.60	2.66	2.68	2.40
KMS @ 0.1 %	2.06	2.20	2.23	2.39	2.65	2.68	2.73	2.42
SB @ 0.05 %	2.05	2.18	2.25	2.42	2.59	2.63	2.69	2.40
SB @ 0.1 %	2.08	2.23	2.28	2.45	2.61	2.67	2.72	2.43
Mean	2.04	2.18	2.28	2.42	2.63	2.66	2.71	-

* Not recorded due to fermentation

C.D. (P = 0.05) Treatments = 0.03
Storage = 0.03
Treatments x Storage = 0.07

The pH of the juice was significantly affected by various treatments and storage period (Table 6). On the 1st day of storage, the lowest pH was observed in control (1.99) closely followed by heating + pasteurization (2.01) and heating (2.02) treatment, which was significantly lower than the other treatments. The highest pH (2.08) was observed in SB @ 0.1 per cent treatment.

During storage there was an increase in pH with the increased period of storage. The lowest increase was observed in heating, KMS @ 0.05 per cent and SB @ 0.05 per cent treatments whereas in pasteurization the highest increase was observed as compared to initial values. The pH of the juice was significantly lower in heating (2.39) as compared to heating + pasteurization (2.43), pasteurization (2.44) and SB @ 0.1 per cent (2.43) treatments but remained statistically at par with all other treatments.

The interaction between storage period and various treatments were also found significant. There was a significant increase in pH in all the treatments during storage upto 60 days except in heating where no significant differences were observed upto 15 days of storage. The pH in control could not be taken after 15 days of storage as the juice was fermented.

The results indicated that the pH of the juice increased with the increased period of storage in all the treatments which might have resulted to corresponding decrease in acidity. Various workers have also observed

the changes in pH during storage. Shrestha and Bhatia (1982) observed the change in pH in stored apple juice at various temperatures. However, the pH of dried ber juice remained constant during storage at room temperature for 90 days (Khurdiya, 1980). Rao and Krishnamurthy (1982) showed negligible change in pH during storage of tomato crush and tomato juice concentrate packed in different containers and stored at room temperature or low temperature.

4.2.1.6. Ascorbic acid:

The ascorbic acid content in the juice of Bhadri lemon was determined at various interval of storage and the results are presented in Table 7.

A perusal of the data given in Table 7 indicates that the ascorbic acid content of the juice was significantly affected by the treatments and storage period. On the 1st day of storage, among the treatments the pasteurization treatment retained the highest ascorbic acid content (47.60 mg) closely followed by SB @ 0.05 per cent (47.25 mg) and KMS @ 0.05 per cent (47.0 mg) treatments whereas the retention of ascorbic acid was the lowest in heating (45.0 mg) treatment.

A gradual and significant decrease in ascorbic acid content was noticed with the increased period of storage in all the treatments. Significant differences were observed among various treatments. The highest retention of ascorbic acid was observed in KMS @ 0.1 per cent (39.61 mg) closely followed by SB @ 0.05 per cent (38.45 mg) treatment, however,

Table 7: Effect of different treatments and storage period on ascorbic acid content in Bhadri lemon juice

(mg/100 ml)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	46.50	30.40	*	*	*	*	*	-
Heating	46.00	34.80	30.10	28.90	25.10	21.90	20.80	29.81
Heating + Pasteurization	45.75	32.20	29.80	27.40	22.10	18.40	17.60	27.61
Pasteurization	47.60	35.40	32.50	30.40	27.50	23.80	22.00	31.31
KMS @ 0.05 %	47.00	38.60	37.40	34.30	32.10	30.50	28.20	35.44
KMS @ 0.1 %	46.75	41.00	40.20	39.30	38.50	36.80	34.70	39.61
SB @ 0.05 %	47.25	40.20	39.20	37.70	36.30	35.40	33.10	38.45
SB @ 0.1 %	46.50	36.50	35.10	33.20	32.90	30.10	28.40	34.67
Mean	46.84	36.96	34.90	33.03	30.64	28.13	26.41	-

* Not recorded due to fermentation

C.D. (P = 0.05) Treatments = 0.45

Storage = 0.45

Treatments x Storage = 1.20

the retention was lowest in heating + pasteurization (27.61 mg) treatment. The trend remained same throughout the storage period. The loss of ascorbic acid was at a faster rate upto 15 days of storage where about 20.6 per cent of ascorbic acid was lost. Treatment where heating + pasteurization was done, the loss was at a much faster rate as compared to all other treatments. The ascorbic acid in control could not be estimated after 15 days of storage due to fermentation.

From the results, it is revealed that there was a significant loss in ascorbic acid content with the increase in storage period. This loss of ascorbic acid might be due to heat processing and the presence of air at the head space of glass bottles during storage. Such losses can be minimized by eliminating air during filling and handling as suggested by Johnson and Toledo (1975).

Losses in ascorbic acid content were also noticed by various workers. Palainswamy and Muthukrishnan (1974) observed that the ascorbic acid losses in lemon juice during storage varied between 14.76 to 28.07 mg/100 ml. Similarly Mehta and Bajaj (1983) observed a decrease in ascorbic acid content during storage of Mandarin, Sweet orange and Lemon juices. The decrease in ascorbic acid in lemon juice (Irving, 1961), in lime juice (Uprety et al., 1963) and in cashew apple juice (Sastry et al., 1963) was also observed during storage. The increased loss of ascorbic acid with increasing time has also been reported in grape-

fruit juice by Smoot and Nagy (1979).

In the present investigation, the maximum losses in ascorbic acid was observed where heating was done. Similarly, Sarmah et al. (1981) reported that there were clear evidence of the significantly higher loss of ascorbic acid in heat processed single strength and toned Kinnow mandarin juice stored at room temperature over a period of 28 weeks to the losses suffered by the low temperature.

4.2.1.7. Tannins:

The tannin content of Bhadri lemon juice was estimated at various intervals of storage period and the results obtained are presented in Table 8.

The results given in Table 8 indicate that the tannin content of the juice was significantly affected by the various treatments and storage period. On the 1st day of storage, the highest tannin content was observed in KMS @ 0.1 per cent (42.5 mg) treatment closely followed by KMS @ 0.05 per cent and heating (42.0 mg) treatments, whereas the tannins were lowest in SB @ 0.1 per cent (39.5 mg) treatment closely followed by heating + pasteurization (39.75 mg). No significant differences between the heating+ pasteurization and pasteurization were observed.

In all the treatments the tannins were found to be decreased with increased period of storage. The highest tannins were observed in KMS @ 0.1 per cent (36.07 mg) treatment as compared to all other treatments and this

Table 8: Effect of different treatments and storage period on the tannin content in Bhadri lemon juice

(mg/100 ml)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	41.50	30.50	*	*	*	*	*	-
Heating	42.00	31.25	23.75	21.25	18.25	15.75	13.75	23.71
Heating + Pasteurization	39.75	30.00	26.50	21.00	18.00	14.75	12.25	23.18
Pasteurization	40.00	30.25	23.50	23.25	22.75	21.00	11.25	24.57
KMS @ 0.05 %	42.00	35.00	33.75	26.75	25.00	20.75	16.25	28.50
KMS @ 0.1 %	42.50	41.25	38.75	37.50	35.00	34.00	23.50	36.07
SB @ 0.05 %	41.75	35.00	31.25	21.25	21.00	20.00	12.50	26.11
SB @ 0.1 %	39.50	23.75	22.50	19.75	18.00	14.75	11.50	21.39
Mean	41.07	32.35	28.57	24.39	22.57	20.14	14.43	-

* Not recorded due to fermentation

C.D. (P = 0.05) Treatments = 0.47

Storage = 0.47

Treatments x Storage = 1.26

lead was maintained throughout the storage period whereas the lowest tannin content was observed in SB @ 0.1 per cent (21.39 mg) treatment. The rate of decrease was much higher upto 15 days and in between 75-90 days of storage. The tannin content in control could not be estimated after 15 days of storage as the juice got fermented.

The results revealed that the tannins in the juice were decreased significantly with the increase in storage period. This decrease in tannin content might be due to formation of precipitates with organic constituents. The decrease in tannins could also be attributed to their condensation into brown pigments which was evidenced by increase in browning of the juice during storage.

Various workers have reported the changes in tannin content during processing and storage. Tressler and Pederson (1936) and Pederson et al. (1941) stored the port wine for 6 months. The total phenols were decreased from 1330 to 1180 mg/litre, off flavour and other changes were also observed. The decrease of 32 per cent of tannins in white ports during storage was also observed by Okolelov and Kotlyarenko (1951).

4.2.1.8. Browning:

Storing of fruit products often results deterioration in colour due to enzymatic or and non-enzymatic browning which impare the quality of the products. In the present studies, juice of Bhadri lemon preserved by using different treatments were stored at ambient temperature and browning

was observed at 15 days interval upto 90 days. Optical density of the samples was taken as an index of browning and the results obtained are presented in Table 9.

The results given in Table 9 indicate that the browning of the juice was significantly affected by the treatments and storage period. On the 1st day of storage the browning was found to be highest in SB @ 0.1 per cent (0.032) treatment and was lowest in all the treatments i.e. control, pasteurization, KMS @ 0.05 and 0.1 per cent and SB @ 0.05 per cent (0.022).

The browning in juice was also increased significantly with the increased period of storage. The maximum browning was observed in SB @ 0.05 per cent (0.053) treatment and it was minimum in KMS @ 0.1 per cent (0.037) treatment. In control samples the browning was much higher as compared to all other treatments when compared upto 15 days of storage, beyond this the data could not be taken due to fermentation of juice.

A gradual increase in browning in lemon juice with increase in storage period at room temperature might be due to the enzymatic and non-enzymatic reactions in the juice. The possibility of browning due to enzymes is ruled out because at such a high temperature enzymes get inactivated. Therefore, in this case the browning might have taken place through the non-enzymatic reactions and oxidation of various phenolics and other compounds which lead to the formation of brown pigments.

Table 9: Effect of different treatments and storage period on the browning of Bhadri lemon juice

(Optical density at 440 nm)

Treatments	Storage period (days)							
	1	15	30	45	60	75	90	Mean
Control	0.022	0.036	*	*	*	*	*	-
Heating	0.025	0.032	0.036	0.043	0.046	0.056	0.063	0.043
Heating + Pasteurization	0.027	0.034	0.036	0.039	0.046	0.046	0.046	0.039
Pasteurization	0.022	0.027	0.027	0.041	0.046	0.056	0.056	0.039
KMS @ 0.05 %	0.022	0.029	0.036	0.046	0.048	0.061	0.068	0.044
KMS @ 0.1 %	0.022	0.022	0.029	0.043	0.046	0.046	0.051	0.037
SB @ 0.05 %	0.022	0.032	0.046	0.056	0.058	0.076	0.081	0.053
SB @ 0.1 %	0.032	0.041	0.051	0.051	0.051	0.051	0.051	0.047
Mean	0.025	0.031	0.037	0.046	0.048	0.056	0.059	-

* Not recorded due to fermentation

C.D. (P = 0.05) Storage = 0.005

Treatments = 0.005

Treatments x Storage = 0.012

The present results of browning are in agreement with those of Brekke et al. (1970) who noticed browning in guava concentrates after 2 months of storage and this became more pronounced with continued storage. Khurdiya and Anand (1981) also reported a gradual increase in browning and HMF formation in phalsa beverage with increasing storage period and it was more pronounced at room temperature than at low temperature.

Results of increased browning during processing and storage have also been reported in citrus juices. Ranote and Bains (1982) reported increased browning in heat processed kinnow juice after 8 weeks of storage as compared to the juice preserved with sulphur dioxide which showed no change under similar condition. Decline in ascorbic acid content in the juice could also be a possible reason for the development of browning in the juice during storage (Stadtman, 1948).

4.2.2. Biochemical changes in the squash during storage:

The biochemical changes in squash prepared from fresh juice was estimated at various interval of storage and the results obtained are presented in Table 10.

A perusal of the data given in Table 10 indicates that the overall composition of the squash varied with the time during storage upto 3 months. The total soluble solids of the squash increased from 46.65 per cent to 49.76 per cent. This slight increase in total soluble solids might be due to the hydrolysis of polysaccharides in acidic medium. The

Table 10: Effect of storage period on squash prepared from fresh juice

Parameters	Storage period (days)						
	1	15	30	45	60	75	90
TSS (%)	46.65	46.71	47.71	47.82	48.65	48.65	49.76
Acidity (%)	1.20	1.18	1.17	1.15	1.13	1.12	1.10
pH	2.10	2.13	2.18	2.21	2.25	2.26	2.30
Ascorbic acid (mg/100 ml)	11.60	10.80	9.86	9.11	7.87	7.20	6.25
Tannins (mg/100 ml)	11.50	11.00	10.60	10.10	9.80	9.10	8.50
Browning (O.D.at 440 nm)	0.022	0.032	0.041	0.051	0.056	0.058	0.076
Total sugars (g/100 ml)	30.25	35.29	39.00	39.51	39.98	40.20	40.38
Reducing sugars (g/100 ml)	9.10	9.30	11.99	13.60	16.81	16.98	18.33

Juice percentage in squash = 25.

total and reducing sugars had also been found to be increased during storage. Increase in reducing sugars were almost twice as compared to the initial values. The reason for the increase in total and reducing sugars during storage had already been discussed earlier. The acidity of the squash was decreased during storage, correspondingly the pH of the squash was found to be increased. The increase in pH ranged from 2.10 to 2.30 during 3 months storage. Tannins were also decreased from 11.50 to 8.50 mg/100 ml. The decrease in tannins during storage might be due to their condensation into brown pigments, which is evidenced by the increase in browning of squash during storage. The tannins also decreased due to precipitation with organic acids. The retention of ascorbic acid in the squash was 6.25 mg whereas it was 11.60 mg/100 ml in fresh squash prepared from fresh juice. The loss of about 50 per cent ascorbic acid in squash during storage might be due to slow oxidation of ascorbic acid into dehydroascorbic acid.

Biochemical changes in squash during storage have also been observed by various workers. Palainswamy and Muthukrishnan (1974) reported that there was an increase in total soluble solids, reducing sugars and total sugars in lemon squash during storage. They further noticed that ascorbic acid content and acidity of the lemon squash decreased during storage. An increase in reducing sugars content of phalsa beverage during storage was also reported by Khurdiya and Anand (1981), but they did not observe much

changes in acidity.

4.2.3. Organoleptic evaluation of squash:

The squash prepared from fresh and stored juice was evaluated organoleptically by a panel of judges (Table 11) by following Headonic Rating Test.

Regarding the colour of the squash the highest scores (8.25/9.00) were given to the squash prepared from fresh juice which was evaluated just after preparation. The colour of the squash evaluated after 3 months and of squash prepared from 3 months stored juice having different treatments scored less as compared to the squash prepared from fresh juice evaluated soon after its preparation. The squash prepared from 3 months stored juice having KMS @ 0.1 per cent scored the highest scores (6.25/9.00), although the values were less than the squash prepared from fresh juice evaluated immediately.

Regarding the flavour of the squash the highest scores (8.5/9.0) were given to the squash prepared from fresh juice. The squash prepared from 3 months stored juice having SB @ 0.05 per cent treatment scored highest scores (6.75/9.0) among all other treatments.

When consistency and taste was taken into account it was observed that the squash prepared from fresh juice was preferred over squash which was prepared from 3 months stored juice having different treatments. No differences could be observed in the consistency of the squash prepared from the juice treated with KMS @ 0.1 per cent, SB @ 0.05

Table 11: Organoleptic evaluation of squash prepared from fresh and stored juice

Treatments	Colour	Flavour	Consistency	Taste	Total marks (out of 36)
1. Squash prepared from fresh juice					
i) After preparation	8.25	8.50	7.00	8.25	32.00
ii) After 3 months	4.25	4.50	5.25	5.00	19.00
2. Squash prepared from 3 months stored juice					
i) Heating	4.50	6.00	6.00	5.75	22.25
ii) Heating + Pasteurization	4.00	5.00	5.25	5.50	19.75
iii) Pasteurization	4.25	6.00	5.75	5.50	21.50
iv) KMS @ 0.05 %	6.00	6.00	6.00	6.50	24.50
v) KMS @ 0.1 %	6.25	6.00	6.25	6.25	24.75
vi) SB @ 0.05 %	5.50	6.75	6.25	8.00	26.50
vii) SB @ 0.1 %	5.00	6.25	6.25	7.25	24.75

per cent, SB @ 0.1 per cent. The squash prepared from the juice treated with SB @ 0.05 per cent was preferred in taste as compared to any other sample, however, the scores were less than the scores obtained by the squash prepared from fresh juice.

The highest total scores (32/36) were given to the squash prepared from fresh juice and the least (19/36) to the squash prepared from fresh juice, which was evaluated after 3 months. Among the squashes prepared from juices having various treatments the highest scores (26.5/36) were given to the squash prepared from juice having SB @ 0.05 per cent treatment. The results also revealed that the squash prepared from juice preserved by preservatives was better than the squash prepared from the juice preserved by heating treatments because of development of bitter taste.

4.3. Utilisation of waste material:

4.3.1. Composition of albedo and candy:

The albedo and candy prepared from albedo portion of the peel was analysed for moisture, crude protein, crude fibre, total and reducing sugars. The results obtained are presented in Table 12.

Table 12: Composition of albedo and candy

Parameters	Albedo	Candy
Moisture (%)	86.24	3.50
Crude protein (%)	3.71	1.32
Crude fibre (%)	6.12	1.52
Total sugar (%)	21.62	73.5
Reducing sugar (%)	12.00	27.0

The moisture was 86.24 per cent in case of albedo whereas it was 3.5 per cent in candy. The albedo contained 3.71 per cent protein and candy contained 1.32 per cent. The crude fibre content was 6.12 per cent in albedo and 1.52 per cent in candy. In albedo the total sugars were 21.62 per cent whereas in candy it were 73.5 per cent. The reducing sugar content of the albedo and candy was 12.0 and 27.0 per cent, respectively.

From the data it has been observed that except an increase in sugars in candy due to absorption, not much changes in composition of other constituents were observed. The reduction in moisture is obvious, because it has been replaced by the sugars and drying of candy.

4.3.2. Composition of seed and peel oil:

The oil and oil characteristics of seed and peel of Bhadri lemon were studied and the results are presented in Table 13.

From the results it was found that seed contained 31.8 per cent of oil. The specific gravity of the oil was 0.916, while refractive index, iodine value and free fatty acids were 1.471, 99 and 0.35 per cent, respectively. The peel of the fruit (flavedo) was found to contain 0.3 per cent of essential oils, while specific gravity and refractive index of oil were 0.970 and 1.476 respectively.

Since the fatty acid composition of an oil largely determines the oil stability, nutritional and quality and also it varies with variety and environment. Therefore, the

Table 13: Oil and oil characteristics of seed and peel of Bhadri lemon

Oils	Oil (%)	Specific gravity	Ref. Index	Iodine value	Free fatty acid (%)
Seed	31.8	0.916	1.471	99	0.35
Peel (essential oil)	0.3	0.970	1.476	-	-

fatty acid composition of seed oil was studied by Gas Liquid Chromatography.

The data given in Table 14 showed that the seed oil of Bhadri lemon contained 25.65 per cent palmitic acid, 0.72 per cent Palmitoleic acid, 2.21 per cent stearic acid, 26.92 per cent oleic acid, 39.59 per cent linoleic acid and 4.91 per cent linolenic acid. Linoleic acid which is considered as an essential fatty acid is present in good amount and is quite comparable with the groundnut oil (Gupta et al., 1983). Hence, it can be utilised for human consumption.

Table 14: Fatty acid composition

Fatty acids	%
Palmitic acid	25.65
Palmitoleic acid	0.72
Stearic acid	2.21
Oleic acid	26.92
Linoleic acid	39.59
Linolenic acid	4.91

The physiochemical properties of citrus oils have been discussed by various workers. Kesterson et al. (1971) observed that the specific gravity of peel oil was 0.8398 to 0.8433 and refractive index was 1.4713 to 1.4721. Hendrickson and Kesterson (1965) found that the sp. gravity of lemon seed oil was 0.914 - 0.917, refractive index was

1.471 to 1.472 and iodine value was 103 to 110. Nolte and Loesecke (1940) observed that the air-dried seeds of grapefruit contained 30-40 per cent oil. Hendrickson and Kesterson (1965) analysed the fatty acid of citrus seed oils. They observed that the lemon seed oil contained 26 per cent pamitic acid, 2.8 per cent stearic acid, 27 per cent oleic acid, 33 per cent linoleic acid and 11.2 per cent linolenic acid. Braddock and Kesterson (1973) also analysed the fatty acids of citrus seed oils. They found that the lemon seed oil contained 20 to 24 per cent palmitic acid, 0.1 to 0.3 per cent palmitoleic acid, 2 to 4 per cent stearic acid, 26 to 31 per cent oleic acid, 31 to 38 per cent linoleic acid and 8 to 12 per cent linolenic acid.

It is, therefore, concluded from the present study that the Bhadri lemon juice can not be stored for more than a week without giving any treatment. The juice can, however, be preserved with either giving heat treatment or by use of chemical preservatives. Since, the heat preserved juice develop bitterness during processing and storage, therefore, the use of chemical preservatives have been found most effective. A good quality squash can be prepared from juice preserved with chemical preservatives. Lemon peel i.e. albedo can be utilised successfully for making a good quality candy and the flavedo can be utilised for extracting essential oils. Lemon seeds have also been found to be a good source of a oil which can be used in a food industry.

CHAPTER V

SUMMARY

The present investigations were undertaken with a view to standardise the method of preserving the Bhadri lemon juice, to study the biochemical changes in juice and squash during storage and to utilise the lemon waste material i.e., the peel and the seeds. Experimental work was carried out in the Fruit Technology Laboratory of the Department of Horticulture, Haryana Agricultural University, Hisar. Fresh lemon fruits were harvested at colour turning stage and analysed for proximate components like acidity, pH, ascorbic acid, total sugars, reducing sugars, total soluble solids, tannin and browning. The juice after giving different treatments was filled in crown bottles and stored at ambient temperature (15 ± 2°C) upto 90 days. Simultaneously, the squash prepared from fresh juice was also stored and evaluated organoleptically. During storage the juice and squash were analysed periodically at 15 days interval. The albedo was utilised for making candy and the seed and the peel (flavedo) of the fruit were utilised for oil extraction.

Total sugars of the juice showed an increasing trend during storage. The increase in total sugars were maximum in the juice preserved by KMS @ 0.05 per cent and the sugars were minimum in the juice where pasteurization was done.

Maximum increase in reducing sugars were noticed in juice treated with KMS @ 0.1 per cent whereas minimum increase in reducing sugars were observed when heating and heating + pasteurization treatments were given.

Total soluble solids (TSS) were found to be increased during storage but no differences were observed among various treatments.

The acidity of thejuice showed a downward trend during storage. The loss in acidity was, however, maximum in the juice preserved by SB @ 0.05 per cent whereas it was minimum in the juice preserved by heating + pasteurization treatment. Simultaneously a gradual increase in pH of the juice was observed throughout storage period.

Ascorbic acid content of the juice decreased during storage. Maximum retention of ascorbic acid was observed in the juice preserved by KMS @ 0.1 per cent whereas the retention was minimum in heating + pasteurization treatment.

The tannins of the juice showed a decreasing trend during storage. The maximum decrease was observed in juice treated with SB @ 0.1 per cent, whereas the maximum retention was observed in juice preserved by KMS @ 0.1 per cent.

Storage of juice at ambient temperature caused browning. The browning was more in juice preserved by SB @ 0.05 per cent and minimum was observed in juice treated with KMS @ 0.1 per cent.

Biochemical constituents of squash during storage showed an upward trend in total soluble solids, total sugars, reducing sugars, pH and browning. There was a continuous decrease in acidity, tannings and ascorbic acid content of squash during storage.

As regards organoleptic evaluation, the squash prepared from fresh juice and evaluated after preparation scored the highest scores, and got the least scores when evaluated after 3 months. Among the squashes prepared from juices preserved by different treatments, the squash prepared from juices preserved by preservatives scored higher than the squash prepared from juices preserved by heat treatments.

A good quality candy could be prepared from albedo portion of the peel by keeping in sugar syrup of 75^{o} Brix and drying after four weeks.

The seed could be utilised for extracting the oil.The oil contained a good amount of essential fatty acid,linoleic acid. From the flavedo portion,essential oils could be recovered upto 0.3 per cent which may be utilised for making essences for fortification.

It is observed from present studies that the Bhadri lemon can be utilised for juice, candy and extracting oils from peel and seeds. Lemon juice can be preserved by using chemical preservatives i.e. KMS @ 0.1 per cent and SB @ 0.05 per cent. The squash prepared from preserved juice stands better on the basis of consumer's acceptance.

Hence, it is,therefore, recommended that it is better to preserve juice and make squash as and when it is required rather than to store squash as such for long time.

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APPENDIX

HEDONIC RATING TEST

Name_____ Date_____

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by assigning points that best describes your feeling about the sample. Remember you are the only one who can tell what you like. An honest expression of yours personal feeling will help us.

S.No.	Colour	Flavour	Consistency/ Texture/Feel	Taste	Total	Remarks
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						

Rating	Organoleptic score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1