Chapter III

EXPERIMENTS

To study the preservation of lime juice using additives, effects of some additives during the storage of lime juice were investigated. Additives used in this experiment were potassium metabisulfite, potassium sorbate, ascorbic acid and butylated hydroxyanisole. Firstly single effect of potassium metabisulfite and potassium sorbate was studied by adding various concentration of each additive into lime juice and followed by the studies on quality changes during storage. Then, combined effect of the additives were studied whether it would result in better preserving action than the single effect or not. So that various combinations of these additives were chosen for treating lime juice and its qualities were determined periodically during storage test. Besides these, effect of changing temperature and air corporating was preliminary investigated because this condition might occur when it was distributed to consumers.

3.1 Preparation of Lime Juice

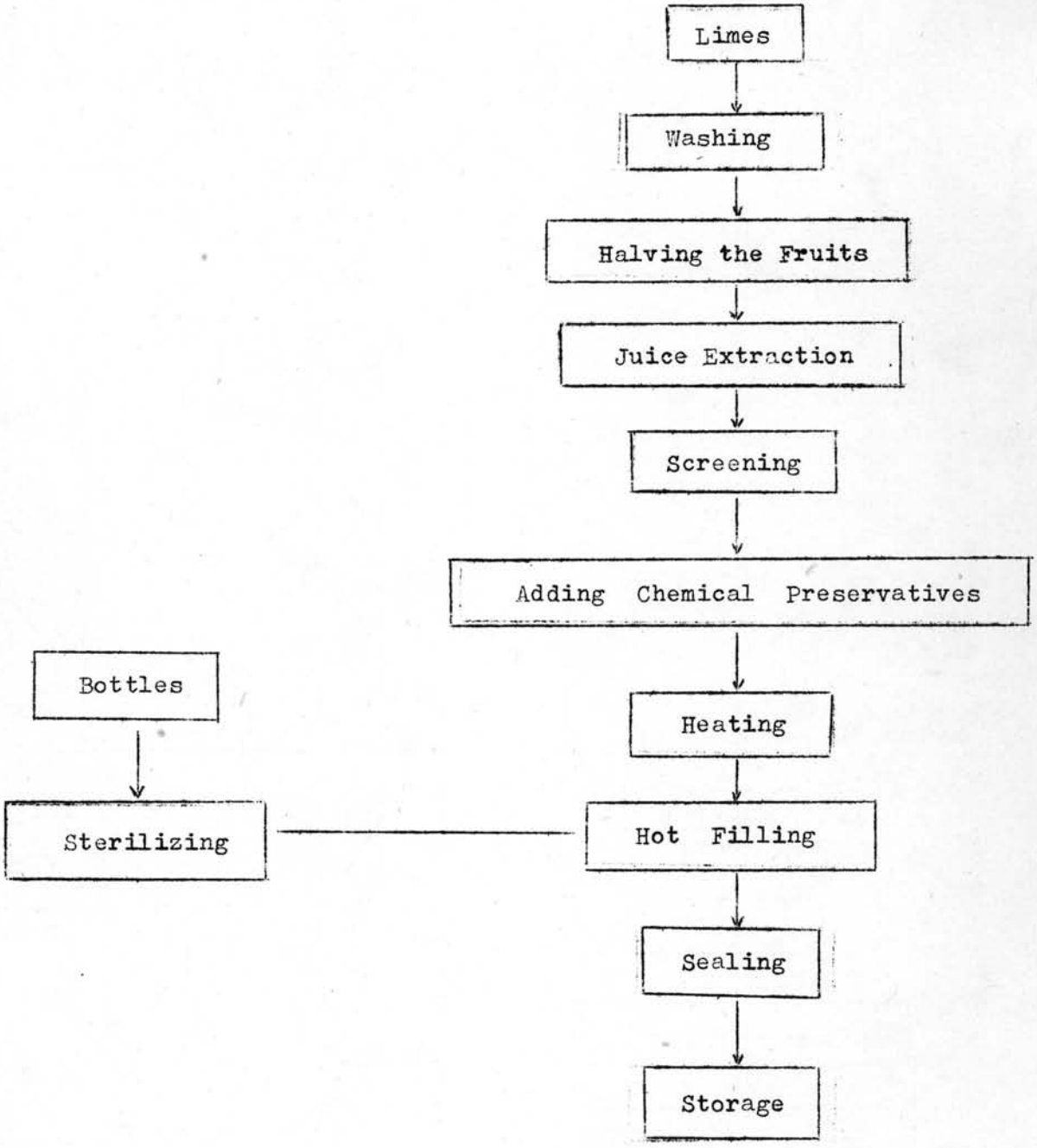
To obtain treated single strength lime juice, the limes were processed as follows:

The limes were immersed in tap water for 10 minutes and then washed and rinsed thoroughly to remove all dirt and contamination. It was then halved and the juice was extracted by using hand-pressed

extractor. The juice was screened by using cheese-cloth for removing rag and seeds; this step should be done as quickly as possible to minimise the dissolving of the limonoid bitter principles from the rag and seeds. Chemical preservatives were added and then the juice was heated to 85°C in order to inactivate enzymes and destroy any microbial that would cause the deterioration of the juice. The heated juice was hot filled into 250 ml. sterilized bottles. The bottles were then caped tightly and stored at room temperature or refrigerator temperature according to the experimental design.

Then the qualities of the juice were investigated periodically in terms of ascorbic acid content, pH, °Brix, % acidity, % transmittance, colour, and sensory test.

Processing Schemes



3.2 Method of Analysis

In order to study the changes in quality during storage, several analytical methods were used. Most of these methods are well described in either the A.O.A.C. Method of Analysis (1975) or The Chemical Analysis of Food by Pearson, which can be applied to lime juice practically.

3.2.1 Determination of ascorbic acid

The volumetric determination using 2:6 dichlorophenolindophenol, as described by Cox and Pearson (1962), was used.

Standard indophenol solution

0.05g of 2:6 dichloro-phenolindophenol was dissolved in water and diluted to 100 ml. and the solution was filtered. To standardise, 0.0500g of pure ascorbic acid was dissolved in 60 ml of 20 percent metaphosphoric acid and was diluted with water to exactly 250 ml. Then 10 ml of this solution was pipetted into a small flask and was titrated with the indophenol solution until a faint pink colour persists for fifteen seconds. The concentration was expressed as mg ascorbic acid equivalent to 1 ml of the dye solution.

Sample analyses

50 ml of juice was pipetted into a 100 ml volumetric flask, 25 ml of 20 percent metaphosphoric acid was added as stabilising agent and the solution was made up to the mark with water 10 ml of the solution was pipetted into a small flask, 2.5 ml of acetone was added and the solution was titrated with the standardised indophenol solution until a faint pink colour persisted for fifteen

seconds. The Vitamin C content in the sample was calculated as mg per 100 ml of juice. The acetone may be omitted if sulphur dioxide is known to be absent. Its function is to form the acetone-bisulphite complex with sulphur dioxide which otherwise interferes with the titration.

3.2.2 Determination of acidity

The titration with 0.1N NaOH, having phenolphthalein as an indicator, was used.

10 ml of juice was pipetted into a small flask, 20 ml of distilled water was added and the mixture was mixed thoroughly. 10 ml of the solution was pipetted into a small flask, 2 drops of phenolphthalein indicator was added and the solution was titrated with 0.1N NaOH solution. The acidity was calculated as percentage citric acid as it was the predominant acid in the lime juice.

3.2.3 Determination of total soluble solids (^{o}Brix)

Total soluble solid of lime juice was determined by means of Abbe' Refractometer.

3.2.4 pH Measurement

The pH value of lime juice was determined by using pH meter.

3.2.5 Determination of percentage of transmittance

To compare the cloudiness of treated lime juice to fresh lime juice, percentage of transmittance of these samples were determined by using densitometer having distilled water as blank. The lower value of percent transmittance indicates the more cloudy juice.

3.2.6 Colour measurement

Colour of lime juice was measured by using Munsell Disck Colorimeter. Due to the lack of coloured plates that used for measuring the colour of lime juice some of the coloured plates used for measuring milk and apricots were selected for measuring the colour of lime juice in this experiment. The coloured plates which were used are as follows:

Yellow	5 Y 8/12
White	N 9.2/
Grey	N 7/
Orange	10 YR 8/6
Green	5 G 8/6

The result was expressed as percentage of each colour which would result in the colour similar to the lime juice after blending all the colours with Munsell Disck Colorimeter.

Besides the colour measurement, the browning of the lime juice was observed by visual inspection. The brown colour was ranked as follows:

Browning	0	Fresh lime juice colour.
	+	The changing colour cannot be distinguished sharply.
	++	
	+++	
	++++	Light brown, still to be acceptable.
	+++++	Slightly un-acceptable.
	++++++	Unacceptable.
	+++++++	Brown colour
	++++++++	Deep Brown colour.

Increases in the numbers of plus signs indicate the increase of brown colour of juice.

3.2.7 Sensory test

Treated lime juice as well as fresh lime juice were submitted to sensory examination to provide information about acceptability and preference during the storage test. All the samples were tested by untrained consumers and the Hedonic Scaling Method was used.

One part of lime juice, 3 parts of water, and one part of 50% (w/v) sucrose solution were mixed together. This sample was tested for ranking by the method of Hedonic Scaling.

Hedonics relates to the psychology of pleasurable and unpleasant states of consciousness. In hedonic scaling, a numerical rating scale as follows was used.

9 Like extremely

8 Like very much

7 Like moderately

6 Like slightly

5 Neither like nor dislike

4 Dislike slightly

3 Dislike moderately

2 Dislike very much

1 Dislike extremely

The result of sensory test for each sample was determined by average value of the scales.

3.3 Effect of Potassium Meta-bisulfite

To study the effect of various concentration of potassium meta-bisulfite on the preservation of lime juice stored at room temperature and refrigerator temperature (7.5°C), samples were prepared by adding various concentration of potassium meta-bisulfite as indicated in Table 1

Table 1

Treatment	ppm of potassium meta-bisulfite
I 1	0
I 2	100
I 3	200
I 4	300
I 5	400
I 6	500

All of the samples were prepared as described before and then were stored at refrigerator temperature and room temperature for 4 months. Changes of the lime juice qualities during storage were determined periodically.

3.4 Effect of Potassium Sorbate

To study the effect of various concentration of potassium sorbate on the preservation of lime juice stored at room temperature and refrigerator temperature (7.5°C), samples were prepared by adding various concentrations of potassium sorbate as indicated in Table 2

Table 2

Treatment	ppm of potassium sorbate
II 1	0
II 2	100
II 3	200
II 4	300
II 5	400
II 6	500

All of the samples were prepared as described before and they were stored at refrigerator temperature and room temperature for 4 months. Changes of the lime juice qualities during storage were determined periodically.

3.5 Combined Effect of Potassium Meta-bisulfite and Potassium Sorbate

To study whether combination of potassium meta-bisulfite and potassium sorbate would have any effect that enhances the preserving qualities of lime juice which were stored at refrigerator and room temperature, the samples were prepared by adding various concentration of potassium meta-bisulfite and potassium sorbate as indicated in Table 3

Table 3

Treatment	ppm of potassium meta-bisulfite	ppm of potassium sorbate
III 1	200	300
III 2	250	250
III 3	300	200

All of the samples were prepared as described before and then were stored at refrigerator temperature and room temperature for 4 months. Changes of the lime juice qualities during storage were determined periodically.

3.6 Combined Effect of Potassium Meta-bisulfite, Potassium Sorbate, and Ascorbic Acid.

To study whether the combination of potassium meta-bisulfite, potassium sorbate and ascorbic acid would have any effect that enhances the preserving qualities of lime juice which were stored at refrigerator temperature and room temperature, samples were prepared by adding various concentration of potassium meta-bisulfite, potassium sorbate, and ascorbic acid as indicated in Table 4

Table 4

Treatment	ppm of potassium meta-bisulfite	ppm of potassium sorbate	mg ascorbic acid added per 100 ml of lime juice
IV 1	200	300	30
IV 2	250	250	30
IV 3	300	200	30

All of the samples were prepared as described before and then were stored at refrigerator temperature and room temperature for 4 months. Changes of the lime juice qualities during storage were determined periodically.

3.7 Combined Effect of Potassium Meta-bisulfite, Potassium Sorbate, Ascorbic Acid and Butylated Hydroxy Anisole (BHA)

To study whether combination of potassium meta-bisulfite, potassium sorbate, ascorbic acid and butylated hydroxy anisole would have any effect that enhances the preserving qualities of lime juice which were stored at refrigerator temperature and room temperature, samples were prepared by adding various concentration of potassium meta-bisulfite, potassium sorbate, ascorbic acid and BHA as indicated in Table 5

Table 5

Treatment	ppm of potassium meta-bisulfite	ppm of potassium sorbate	mg Ascorbic acid added per 100 ml juice	ppm of BHA
V 1	200	300	30	2
V 2	250	250	30	2
V 3	300	200	30	2

All of the samples were prepared as described before and then were stored at refrigerator temperature and room temperature for 4 months. Changes of the lime juice qualities during storage were determined periodically.

3.8 Effect of Air Corporating

To study whether corporated air during consumption would have any effect on continuing storage, after a certain storage time at room temperature (6,8,10 weeks), half of lime juice of the samples was poured out, and the storage of the remaining was continued at room temperature for one month. The qualities of lime juice were determined at the end of the storage.

3.9 Effect of Air Corporating and Temperature Changes

To study whether changes of temperature and corporated air during consumption would have any effect on continuing storage, after a certain storage time at refrigerator temperature (6,8,10 weeks), half of lime juice of the samples from experiment 5,6,7 was poured out, and then it was continued storing at room temperature for one month. The qualities of lime juice were determined at the end of the storage.