CHAPTER V

DISCUSSION

The 4 methods of determination of limonin from preserved lime juice were considered feasible. These are the methods developed by Chandler and Kefford, Maier and Grant, Kruger and Colter, Wilson and Crutchfield. Chloroform was found to be better solvent in limonin extraction than dichloromethane.

The first method of detecting limonin attempted was the method by Kruger and Colter (1972) using gas chromatography. Although this method seems to be the most practical, the gas chromatograph was not suitable for this experiment since it requires high temperature. The second method proposed by Wilson and Crutchfield (1968) based on the formation of the colored feric complex of the hydroxamic acid derivative of limonin, whose concentration was spectrophotometrically determined. Ferric perchlorate, one of the reagents used in this method is expensive and was not available at the time, therefore this method has not been attempted. The third method attempted was the method by Maier and Grant (1970), using thin-layer chromatography. The concentration of limonin was estimated by visual comparison with standard therefore it is inconvenient for routine analysis when several samples have to be determined at the same time. The fourth method also used thin-layer chromatography proposed by Chandler and Kefford, (1966) which based on the colorimetric analysis of the eluted limonin 2,4-dinitrophenylhydrazone spot. This method was the simplest and most satisfactory, although the method is time consuming but it is still possible for routine analysis. Therefore this method was selected as a method of analysis. The results were reproducible and reliable (Appendix III). The limonin content of lime juice was determined in triplicate analysis.

A linear relationship ($y = bx + a$) was established between the absorbance (y) of the limonin dinitrophenylhydrazone solution and the amount (x μg) of limonin which was calculated from 0.723 multiplied by the amount of limonin dinitrophenylhydrazone (Appendix II). One mole of limonin was converted into one mole limonin 2,4-dinitrophenylhydrazone. The regression coefficient (b) which must be determined independently for each set of laboratory conditions was found from 65 determinations to be 1.327. The equation is $y = 1.327x + 0.40$. The value 0.4 could be omitted due to the experimental error. The equation became $y = 1.327x$ for the determination of limonin content. Thin-layer chromatography gave Rf value of limonin dinitrophenylhydrazone of 0.33. The limonin content of the juice was given by;

$$\text{ppm limonin} = \frac{\mu g \text{ limonin from standard curve} \times \mu l \text{(solvent)dilution}}{\mu l \text{ sample spotted} \times g \text{ weight of juice}}$$

The experiment on the effect of potassium metabisulfite on limonin content showed that during the first 6 weeks the additive had a slight effect on limonin content in lime juice. As it can be seen that untreated sample had slightly more limonin. (1-2 ppm) than the treated samples. After 6 weeks concentration of limonin varied irregularly with a tendency of constant value. Due to the limonoate

A-ring lactone, non bitter limonoid precursor of limonin, which was located within the cell wall, seed, rag in contact with the acidic juice was converted to limonin. It appeared that most of limonoate A-ring lactone had been already converted to limonin during pasteurization. The limonin contents in freshly extracted juice was observed to range between 4.55-6.0 ppm and increased to the range of 14-15 ppm just after pasteurization, then gradually increased until the end of the sixth week. These findings agreed with the work of Tariq et al., (1974) that most limonin content of Valencia orange had been formed from its non-bitter precursor during pasteurization. Samples at room temperature had slightly higher limonin contents than at refrigerator temperature, since high temperature enhanced the rate of conversion.

Potassium sorbate had no effect on limonin content at both temperatures. After pasteurization, all limonoate A-ring lactone in all samples were converted gradually to limonin until the end of the sixth weeks, then the limonin remained nearly constant. The bitterness was incorporated into the juice from rag, peel and seeds of the fruits in form of limonoate A-ring lactone. The extent of limonin content depended upon the variety (Scott, 1970), rootstock (Wilson and Crutchfield, 1968), methods of extractions and stage of maturity (Kefford, 1959). The qualities of full bottle lime juice treated with potassium metabisulfite is found to be better than the others. The experiment showed that potassium metabisulfite can prevent browning development better than potassium sorbate. Our experiment showed that the 300 ppm of potassium metabisulfite was the best in vitamin C retention and appearance.

There is slight different vitamin C content between the early and late season lime. The latter initially contain more vitamin C, other chemical properties ie pH, ^{o}Brix, % acidity are not different. Because of the difference, our experiments should be limited to the same seasonal period and early season limes are employed.

In studying the effect of single additive, the incorporated air during preparation and incorporated air introduced afterward (when the juice was partially removed from the bottle on utilization), on preservation of lime juice, it was found that the most important quality was its general appearance especially color and cloudy appearance. Besides these the amount of vitamin C which to of nitritious value and the flavor must also be taken into consideration by comparing with the qualities of fresh lime juice.

The experiment showed that potassium metabisulfite can prevent browning development better than potassium sorbate because of its activity toward carbonyl group present in the initial break drown products resulted from the degrad ation of ascorbic acid (Schroeter, 1966).

It was found that potassium metabisulfite is effective in browning prevention in full-bottle-lime juice however in half-bottle it becomes useless. This is because sulfur dioxide gradually volatilises from potassium metabisulfite in the juice to fill the head space during storage. Hence the efficiency in browning prevention is reduced. Therefore potassium metabisulfite which is very effective against molds and loss of ascorbic acid, can prevent browning development only when there is no headspace. On the other hand potassium sorbate is only effective against yeast and molds, consequently either potassium metabisulfite or sorbate cannot prevent browning development in half-bottle-juice. Stannous chloride, another preservative was considered to replace both additives. The preliminarly study used the maximum allowable amount of stannous chloride which is 200 ppm. The results are very satisfactory in preventing browning. The loss of vitamin C is due to air oxidation therefore lime juice in the full bottle can retain more vitamin C than in the half-bottle. From our experiments stannous chloride is the most effective antioxidant whereby it can prevent not only browning but vitamin C loss as well.