Chapter II

LITERATURE REVIEW

Citrus fruits may be regarded as the most important fruit crop directly consumed as human food either in form of fresh fruit or preserved juice, so that many works concerned with preservation of citrus fruits and citrus juices have been done.

According to some experiments that have been done at the Department of Science, Ministry of Industry, it was found that single strength lime juice with 1000 ppm of potassium metabisulfite as preservative could be kept at $24^{o}C$ for one year without any change in qualities except the diminution of ascorbic acid content reaching zero at the end of second month and it is still acceptable. However, with cold storage condition ($5^{o}C$) the shelf life is very much longer (about 5-6 years). When 500 ppm sodium benzoate or sodium sorbate was used instead of potassium metabisulfite, the juices could be kept only for half a month and then its colour turned brown quickly.

Another result of the work concerning the effect of preservatives on maintaining qualities of lime juice which have been done at the Department of Food Science & Technology, Kasetsart University, have showned that lime juices treated with 200 ppm of potassium metabisulfite, 200 ppm potassium meta-bisulfite and 70 mg ascorbic acid per 100 ml, 300 ppm of potassium meta-bisulfite, 300 ppm of potassium meta-bisulfite and 70 mg ascorbic acid per 100 ml, could be kept for 3 months at 18-20°C. But lime juice treated with 0.1% sodium benzoate has colour and flavour that are unacceptable after one and a half month of storage at the same temperature.

During storage and processing of citrus juice, many physical and chemical changes as well as microbial deterioration such as loss of cloudy appearance, changes of colour and flavour, and fermentation which caused by micro-organisms always occur. Freshly-extracted citrus juices are generally opaque because of the presence of "a heterogeneous mixture of cellular materials and perhaps emulsoids held in suspension by pectin". Hydrolysis of pectin by enzyme pectinesterase will cause a phenomena of "cloud loss" and "gelation" occur in unheated citrus juices. To prevent the hydrolysis of pectins, the pectin enzymes should be destroyed by inactivating them through heating the juice for a short time to a minimum temperature of 85°C. But heat treatment and also high storage temperature will result in "cooked" taste which might be a result of loss and/or chemical alteration of the volatile substances, as well as through the formation of new odorous materials. Generally, all of these changes are undesirable and can be kept to a minimum. De-aeration, absence of excess essential oils, short-term processing, rapid cooling after pasteurization and low temperature storage condition are some of the precautions to be taken to avoid undesirable change.

Another serious problem in the production of citrus juice is the gradual development of bitterness in the juice after extraction from the fruit. When eaten fresh, these fruits are normally non bitter. Higby (1938) found that limonin caused bitterness in

Valencia and Washington Navel oranges. This phenomena is generally referred to as delayed bitterness. Two theories have been proposed for describing the delayed bitterness phenomena. One is the precursor theory which proposed by Higby (1938) and the other one is diffusion theory which proposed by Kefford (1959) and Joslyn et al. (1961).

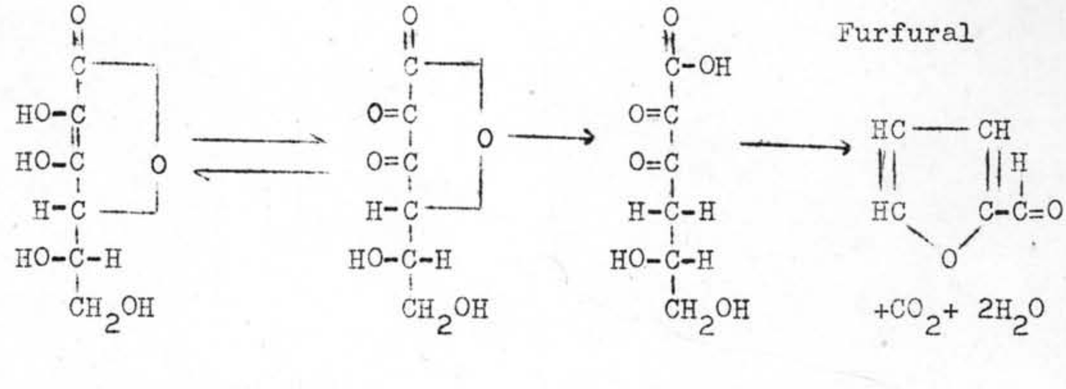
The precursor theory proposed that the fruit tissues contain limonoate A-ring lactone, a nonbitter limonoid which is located within the cell wall. When juice is extracted from citrus fruits, the acidic environment of the juice results in the eventual conversion of limonoate A-ring lactone (which enters the juice from the disrupted tissues) to limonin and the juice becomes bitter.

The diffusion theory (Kefford, 1959; Joslyn, 1961) proposed that limonin itself is present in the fruit tissues but because of its low solubility it takes an appreciable time to diffuse from the tissue fragments of the juice into solution and to reach a concentration sufficient to impart a bitter taste.

Limonin, a limonoid bitter principle in citrus fruits, was first isolated from Washington Navel Orange juice by Higby in 1938. The amount of limonin required in a juice before bitterness becomes detectable varies with the sweetness and the acidity of the juice as well as the sensitivity of the taster. As a general rule a juice containing less than 6 ppm of limonin is unlikely to taste bitter, but a juice with more than 9 ppm will seem bitter to most tasters (Cartwright et al., 1975). Besides limonin, flavonoid naringin is also a bitter principle in citrus fruits (Braverman, 1949; Maier, 1969;

Kefford and Chandler, 1970). High concentration of these bitter substances in processed citrus products will affect consumer acceptance so that action should be taken to prevent their uptake in the juice. Fortunately the insolubility of both bitter substances aids in their removal. If the rag is removed rapidly from the juice phase there is insufficient time for the bitter principles to go into complete solution. Another convenient solution to the bitterness problem would be a debittering enzyme that could be added directly to juice or other fruit tissues. Preventing delayed bitterness by using an enzyme limonoate dehydrogenase from Artherobacter globiformis and Pseudomonas were studied by Hasegawa et al. (1973, 1974).

The colour of citrus juices is due to carotenoid pigments carried in the chromoplasts, which form part of the suspended cloud. The juices of some citrus fruits, such as lemon, grape fruit, lime and bergamot are yellowish green, while others, such as orange and tangerine, are orange to red in colour. The ratio of carotene and xanthophyll which are the main carotenoid present determines the colour of the juice. Unless citrus juices are preserved with sulfur dioxide, or unless some other antioxidants are added to them, they soon undergo a profound change in colour: they are darken and soon turn quite brown if stored at room temperature. (Braverman, 1949). This change is attributable to a process known as "non-enzymatic browning". Three main theories have been put forward to explain non-enzymic browning. (Clegg, 1964, Meyer, 1960). One pathway is the formation of sugar amine condensation product which, after undergoing Amadari rearrangement and a variety of secondary reactions, give rise ultimately to dark coloured 'melanoidin' compounds. The Amadori rearrangement requires a near neutral, or slightly alkaline medium for optimal efficiency of reaction; therefore, it is unlikely that this mechanism is the major contributor to the browning of a highly acid product such as lemon juice at pH 2.5. A second theory postulates that browning involves the decomposition of sugars and sugar acids to furfural-dehydes, or similar compounds, characterised by having an active carbonyl group. These products then condense with nitrogeneous compounds and/or polymerise to form brown resinous materials. The third theory is based on oxidation, yielding reactive products which similarly may polymerise or react with nitrogeneous constituents, but the precursors are specified as ascorbic acid or related compounds.



Ascorbic acid   Dehydro ascorbic acid

The third theory seems the most likely to apply to the conditions pertaining to an acidic product such as lemon juice; the concentration of ascorbic acid is relatively high and free amino acids are present to combine with the reactive products resulting from the oxidation of the ascorbic acid and lead to the formation of brown pigments. Study of nonenzymic browning of lemon juice have shown that browning of lemon juice and model systems was proportional to the level of ascorbic acid and the presence of atmospheric oxygen has been shown greatly to increase the development of brown pigments of non-enzymic origin (Clegg, 1964). Role of citric acid in the development of non-enzymic browning in the presence of ascorbic acid was studied by Clegg (1966).

The non-enzymic browning is enhanced by high storage temperture and high acidity so that it is advisable to store single strength, pasteurized lemon juice at low temperature preferably at or below 4°C (Braverman, 1949). Cold Storage greatly retards the browning process and also the decomposition of ascorbic acid. Adding proper amount of sulfur dioxide or sulfurous acid salts can prevent the browning reaction because of its activity toward carbonyl groups present in initial break down products (Roberts and McWeeny, 1972; Cartwright et al.,1975; Burton et al., 1963; Schroeter, 1966).

In view of spoilage of citrus juices by microorganisms, due to the range of pH value (1.5-4.5) and acidity of citrus juices, most organisms fail to multiply especially bacteria. Moulds and yeasts are however considerably more resistant to these high acidities and consequently are capable of growing. In citrus juices the major acid is citric acid which represents more than 95% of the total acidity. This acid not only has an inhibitory effect on bacteria through a lowering of pH but actually also has a powerful bactericidal action on bacteria which happen to find their way into the juice during processing. Moulds and yeasts are capable of utilizing the main fruit acids during their growth and are thus unaffected by any inhibitory action which the acids may have for other microorganisms. Most microorganisms, particularly the strongly aerobic types like moulds, grow best in the presence of oxygen. Inhibition of any mould growth cannot therefore be expected unless heating of the juice is followed by a canning or bottling procedure. Yeasts, however, are not as sensitive to reduced oxygen levels and can grow in canned or bottled juice.

On the whole, conditions in citrus juices favours yeasts more than other microorganisms, especially if it is handled in bulk in the absence of air. Yeasts that are capable of vigorous growth under anaerobic conditions are mostly species of Saccharomyces sps. Yeats Occurring in citrus products were studied by Recca and Mrak (1952), ninety-two yeasts were isotated from various sources in citrus processing plants. Species of Candida, Zygosaccharomyces, Hanseniaspora, Saccharomyces and Pichia were most commonly isolated. Combined effect of heat treatment and preservatives on yeasts in grape juice and orange juice were studied. (Djien et al., 1972; Djien et al., 1973; Djien et al., 1974). It was found that the pasteurizing effect of a mild heat treatment on yeast (*S. cerevisiae*) in grape juice is considerably enhanced by small concentrations of potassium sorbate and sodium benzoate. And also the pasteurizing effect of a mild heat treatment on yeasts (*S. cerevisiae*) in orange juice is considerably enhanced by small concentration of sodium benzoate.

The acidity of citrus juices excludes many bacteria but not the *acetic bacteria*. They, like moulds and yeasts, can tolerate the acidity but are not common agents of spoilage due to their requirement for an ample supply of oxygen. The most important spoilage bacteria are lactic acid bacteria, e.g. *Lactobacillus* and *Leuconostoc*, if the citrus juices are stored without refrigeration under carbon dioxide pressure.

The Role of Chemical Additives

According to chemical and microbial deterioration, many food additives were introduced into citrus juice for extending shelf-life.

Meta-bisulfite

Application of sulfiting to food products can prolong shelf life because of antimicrobial activity. Sulfurous acid inhibits yeasts, moulds and bacteria. Sulfur dioxide is very effective against molds because the oxygen present oxidizes some of the preservative and is thereby eliminated from the juice and the headspace. Besides antimicrobial activity, sulfiting can prevent enzymatic and non-enzymatic discoloration of food and helps in reducing losses of ascorbic acid. It is also used to prevent the oxidation of the essential oils and carotenoids and consequent development of off-flavour and loss of colour in citrus juices. It has no effects on the analysis for total acidity as citric acid (Vandercook and Guerrero, 1968) as well as no effect on pectic enzymes which are responsible for breakdown of tissue or which cause loss of cloud in citrus juices. However, it cannot be considered to have a permanent preservative effect in juices stored under conditions permitting reinfection. It reacts with some of the juice constituents (notably aldehydes, ketones, and sugars) to form compounds lacking in preserving power. Since high temperatures accelerate these reactions, it is advisable to store juices preserved with sulfur dioxide in cool places.

The amount of sulfur dioxide required depends on the pH of the juice and the amount of suspended pulp. The use of sulfites is limited by the fact that at residual levels above 500 ppm, the taste begins to be noticeable. However, up to 1000 ppm of benzoic acid and sorbic acid can be added for improving antimicrobial activity. The sulfite salts and sulfur dioxide are generally recognized as safe for use in foods by the U.S. Food and Drug Administration. Potassium meta-bisulfite ($K_2S_2O_5$) is white crystal or powder having odor of sulfur dioxide that is freely soluble in water. Under humid conditions, the meta-bisulfites are more stable than the sulfites. Finally, sulfur dioxide is the only chemical preservative which can be removed from the juice by applying heat or vacuum.

Sorbic acid

Sorbic acid and its salts have broad spectrum activity against yeast and moulds, but are less active against bacteria. Their range of optimum effectiveness extends up to pH 6.5-considerably above that of the propionates or sodium benzoate. As with other weak acid microbial inhibitors, activity of sorbate increases as the pH of the medium declines. Sorbic acid and potassium sorbate are generally recognized as safe (GRAS) for use in foods under regulation of the U.S. Food and Drug Administration. Application to fruit juice and derivatives, potassium sorbate can be used at 0.025 to 0.10%, or they can be used together with other preservatives, each at a lower level.

Ascorbic acid

The concentration of ascorbic acid, an important nutritional factor, in citrus juices varies from 10 to 80 mg/100 ml according to variety. During storage of citrus juice, in the presence of oxygen and a suitable catalyst, ascorbic acid is oxidized to dehydroascorbic acid, which is fairly stable below pH 4.0. Dehydro-ascorbic acid still has probably 75-80% of the Vitamin activity of ascorbic acid. Addition of some ascorbic acid to extracted lime juice may increase the nutritional value.

Many works concerning retention of ascorbic acid in citrus juices have been done. It was reported that the retention of ascorbic acid in Valencia orange juice from Florida over the 6 month period at storage temperature of -17.8^{o}C was 68.1%. Analysis at the end of each month of storage showed a steady loss in ascorbic acid (Tingleff &

Milier, 1960). Ascorbic acid studies on chilled, fresh and fermented orange juice have revealed that dehydroascorbic acid and diketogulonic acid were found in samples of chilled orange juice tested. Where reduced ascorbic acid was present in low amounts compared to fresh juice, the amounts of dehydroascorbic acid and diketogulonic acid were relatively high. The ascorbic acid content of freshly squeezed orange juice refrigerated at 4-5°C decreased 25% during four weeks and did not increase substantially in oxidized forms until two weeks later, (Lamden et al., 1960). Another investigator has reported that during storage at 5°C for 3 weeks, more than 40% of Vitamin C were still remained in citrus juices such as lemon and grapefruit juices. The amount of ascorbic acid that present in lemon juice after the second week has a lower value than that present in the week later, this result indicates the variability of Vitamin C retention during the storage, but it still has a tendency of decreasing (Saito et al., 1974).

Butylated hydroxy-anisole (BHA)and Butylated hydroxy-toluene(BHT)

Butylated hydroxy-anisole is generally recognized as safe (GRAS) for use in food to prevent oxidation. Application to beverages, BHA and BHT can be used at 2 ppm level. Besides preventing oxidation BHA also have a profound antimicrobial effect against *Aspergillus parasiticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* (Chang & Branen, 1975).