STUDIES ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL ANALYSES OF COMMERCIALLY AND LABORATORY PREPARED ORANGE JUICES

BY

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(Msc/Sci/19209/99/2000)

AUGUST, 2005

DECLARATION

I hereby declare that this thesis is an original work carried out by me and no part of it has been presented elsewhere for a degree or diploma. The work of other investigators is acknowledged and referred to in this presentation.

OBASI, BLESSING CHIDI

AUGUST, 2005.

CERTIFICATION

This thesis titled "STUDIES ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL ANALYSES OF COMMERCIALLY AND LABORATORY PREPARED ORANGE JUICE" by Obasi, Blessing Chidi meets the regulation governing the award of the degree of Master of Science in Microbiology of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late beloved mother, MRS. ROSELINE OBASI, who laid the foundation of whatever I am today. Also my loving father MR SAMUEL OBASI whose support and encouragement has greatly contributed to the success of this work.

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ABSTRACT

Microbiological and physico-chemical analyses and sensory evaluation were carried out on commercially and freshly prepared orange juice (100%) in the laboratory and its shelf-life after the storage period of 90 days using various storage methods. At the end of the study, the result showed that the laboratory processed orange juice in terms of the nutritional composition when compared with the commercially processed orange juice had a better quality considering the parameters assessed. The results of the laboratory prepared orange juice showed that the chemically treated, pasteurized, concentrated and carbonated orange juice had total soluble solids (TSS) ranging from 26-33 ^{o}Brix. The vitamin C content for the untreated juice (fresh) and the commercially produced were 43 and 2.67 mg/100ml respectively. The experimental samples of chemically treated, pasteurized, concentrated and carbonated had vitamin C content ranging from 22-29, 27-32, 28-30 and 17-29 mg/100ml respectively. Potassium was the major mineral nutrient which ranged from 1.44 – 2.63%. Sodium and phosphorus were found in small amounts ranging from 0.01 -0.12 and 0.001-0.02% respectively in the orange juice. Among the various processing methods concentrated orange juice ranked first followed by chemically treated, pasteurized and carbonated in terms of the sensory evaluation as assessed by the panelists. The microbiological analysis showed the total plate counts ranged from bacteria $1.50x10^6$ to $3.75x10^6$ and the total yeast cell counts ranged from $1.7x10^6$ to $4.7x10^6$, colony forming units per ml (cfu/ml). The microorganisms isolated and tentatively identified included the following*; Escheriachia coli, Bacillus cereus, Pseudomonas aeruginosa,* yeasts and moulds which comprise *Saccharomyces cerevisiae*,

Saccharomyces rouxii, Sacharomyces telluris, Candida mensenterica, Rhodotorula mucilaginosa, Brettanomyces anomalus, Aspergillus, Mycelia, Helminthosporium, Gliocladium, Chrysosporium and *Blastomyces.* This study showed that the nutritional quality of orange juice can be maintained considering the physico-chemical analysis and that the level of microbial spoilage can be reduced to the acceptable limits if temperature fluctuations and oxygen present in the environment of storage are controlled.

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CHAPTER ONE

1.0 INTRODUCTION

Orange (*Citrus sinensis*) is one of the largest citrus grown fruit in Nigeria (Sumati, 1977) and is one of the main sources of vitamin C (ascorbic acid) in Nigerian diets (Sumati, 1977). Other vitamins that are of dietary significance are found in orange, folic acid and thiamine. Orange juice also contains niacin, riboflavin and pantothenic acid vitamins although in minute quantities of about 2 to 4% (Smoot and Nagy, 1980). Orange as one of the citrus fruits is non-climacteric; hence, they do not ripen or improve in quality after harvest (Davies, 1986b).

Various methods which can be used for the processing of orange juice include: the traditional thermal processing method (Pasteurization) which kills bacteria and extend the shelf-life of juices, but affects the taste of the product (Andres, *et al*., 2000). The pressurized carbon dioxide process, according to (Murat, 2000) showed that this process is as effective as heat pasteurization, but does not change the taste and preserves more of the vitamins found in fresh squeezed juices; pulse power submerged electrical arc technology is another method, which can give 5-to 7-log reduction of pathogenic bacteria in inoculated orange juice and the colour and flavour of the juice still remaining unaffected (Andres *et al*., 1998; 2001) and high pressure processing (HPP) a non – thermal technique which can minimize or eliminate thermal processing and or chemical preservatives while still ensuring safety (Stewart *et al*., 2001).

Production of orange juices and drinks involves washing and grading, juice extraction, clarification, pasteurization, evaporation (concentration), blending and

refrigeration storage. Products obtained from orange juice are frozen concentrated orange juice (FCOJ) in which about 85% of the water content is removed; freshly – squeezed or not from concentrate (NFC) also known as single strength orange juice (SSOJ) and powdered juice (Making Orange Juice Southern Garden Citrus) Florida (Anonymous, 2003).

Processed products are expected to be essentially uniform in composition and sensory characteristics from one batch to another. Infact, compositional limits for many processed foods such as orange juice are set out in official regulations and purchasing specifications. To obtain the required degree of uniform quality in these products, processors often have one option that is to use carefully selected raw materials (Board & Woods, 1983).

Iyang and Abah (1997) presented a comparison of the chemical composition of two juices showing that orange juice contained more sugars, titratable acidity and soluble solids but less ascorbic acid than cashew apple juice.

Commercially produced orange juice (pasteurized) can lose some of its flavour during storage because of reduced amounts of volatile flavour constituents, thus developing a stale flavour for juices packaged in plastic containers; major reason for this flavour loss is often caused by absorption of flavourings into the plastic container. (Moshonas and Shaw, 1998). Pasteurized orange juice not from concentrate is a premium product with a shelf-life of about nine weeks. (Moshonas and Shaw, 1998), with pasteurized orange juice packaged in laminated gable top cartons stored for the normal shelf-life of nine weeks at $2^{o}C$ and at $-18^{o}C$ (control sample) showed a gradual decrease during the first four weeks and a more rapid drop after 5-6 weeks of storage in flavouring

material present. Near the end of the storage life (7-9 weeks) a taste panel found a significant decrease in stored orange juice for seven weeks at 2^{o}C compared to that of the starting juice. However, the sensory panel has no preference for either juice. These results showed that despite a decrease in some flavourings, the product maintained acceptable quality during the recommended shelf-life period. Thus consumers of pasteurized orange juice are assured of a product with good flavour quality throughout its normal shelf-life.

Bezman *et al*. (2001) have shown that 2 methyl – 3 – furan – thiol and methional, at levels found in stored orange juice (21 days at 35^{o}C) contributed to stored orange juice off-flavour. Other volatile compounds which can affect the organoleptic properties during storage include P – Vinyl guaiacol (PVG) and 2, 5-dimethyl – 4-hydroxy furanone (DMHF).

A shelf-life study was carried on mango, orange and pineapple juices by Obeta and Ugwuanyi (1997) and these juices were inoculated with ascospores of *Neosartorya sp*. The result showed that only fruit juices at 4 -5^{o}C storage and those containing sodium benzoate stored at room temperature were protected from spoilage by these fungi for 64 days. Addition of sucrose at levels of 9% to mango juice, 30% to pineapple and 31.5% to orange juice significantly protected ascospores from inactivation during cold storage.

The aims of the present work are:

1. To find the most suitable method of processing orange juice so as to maintain its nutritional quality.
2. To study the effect of storage on the shelf-life of orange juices under different storage conditions such as freezers, refrigeration and room temperature.

3. To investigate the available parameters for assessing the quality of orange juice in order to ensure commercial sterility.
4. To determine the microbiological safety of orange juice.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 HISTORICAL BACKGROUND OF ORANGES

On a world wide basis there are five species of orange that are of economic significance namely; sweet orange, *Citrus sinensis* (Linn) Osbeck; the mandarins or tangerine orange, *C. reticulate* Blanco and C*itrus unshiu* Marc, grape Fruits, *C. paradisi* Macf, Lemons, *C. lemon* Burm. F. and limes, *C. aurantifolia,* L. Kumquats, *Fortunella species* and the shaddock or pummelo, *C. maxima* . are of economic importance in southern east Asia and China but not on a world wide basis. Others include citrons and sour or bitter oranges (Leon and Sinclair, 1961).

All the major five species of oranges are native to the tropical Asia and especially the Malay Archipelago. They have spread from there to practically all sections of the world that have climates suitable for orange production (Leon and Sinclair, 1961).

Limes (*Citrus aurantifolia* Swingle) apparently originated in the East Indian Archipelago. They were brought across the sea of Oman by Arabian sailors and transported subsequently to Egypt and Europe (Chapot, 1975; Barrett and Rhodes, 1976).

Lemons (*Citrus limon* Burmann) are of unknown origin, possibly a hybrid between citron and lime creating an intermediate species (Chapot, 1975; Barrett and Rhodes, 1976). Sweet oranges (*Citrus sinensis* (L) Osbeck) originated in southern China and possibly as far south as Indonesia (Weber *et al*, 1976).

The 'Washington' navel orange originated in Bahia, Brazil and is probably a mutation of 'seleta' sweet orange. It was introduced to Australia (1824), Florida (1835)

and California (1870) from Washington, DC, where it apparently received its name; Washington navel and cultivars arising from mutations of it have been distributed worldwide (Davies, 1986[a]).

The Shaddock or Pummelo (*Citrus grandis* (Linn) Osbeck) originated in the Malaysia and Indian Archipelagos and is widely distributed in the Figi Islands (Davies and Albrigo, 1994).

Grapefruits (*C. paradisi Macf.*), appear to have originated as a hybrid of pummelo and sweet orange (Scora, 1988) in the West Indies.

The area of origin for mandarins (Citrus *reticulata* Blanco) was probably Indo-China and South China with traders carrying selections to Eastern India. Traditional production areas of this species have been in Asia. Mandarins were transported from Asia to Europe much later than other citrus. (Davies and Albrigo, 1994).

2.2 CLASSIFICATION OF ORANGES

The oranges of commerce are made up of five species of citrus namely: Sweet oranges (*Citrus sinensis*), Grape fruits (*C. paradisi* Macf), Lemons and limes (*C. lemon* Burm), and *C. aurantifolia,* L. kumquats, *Fortunella* species and Shaddock or pummelo (*Citrus maxima* L). A brief description of three is given below in order of commercial importance.

2.2.1 Sweet oranges

The sweet orange species is by far the most important and includes the common deserts and juice oranges, represented by such varieties as the Washington Navel and

Valencia of California and Arizona, the Parson Brown, Hamlin and Pineapple of Florida, Shamouti of Israel, Cadenera of Spain, Pera of Brazil, Ovale and Tarcocco of Italy, and many other less important local varieties (Davies and Albrigo, 1994)

Sweet oranges may be separated into four groups based on fruit morphological characteristics:

i. the common or round oranges,

ii. the navel oranges

iii. the pigmented (blood oranges) and

iv. the acidless oranges

The common or round oranges include Hamlin, Pera and Valencia is those with normal fruit. They are the most important commercially and represent a major portion of sweet orange hectarage worldwide.

The navel oranges or those with abnormal fruits such as Washington Navel are by far the second most widely planted and commercially important navel orange cultivar (Davies, 1986a). Other examples are 'Atwood', 'fisher', Newhall, Navelina and Navelate, summer field, Baianinha (Davies, 1986a).

The pigmented oranges are of commercial importance in several Mediterranean countries including Italy, Spain, Morocco, Algeria and Tunisia, but are not widely grown outside this region. The most important cultivars of the "blood" oranges include the Ruby, Red, Maltaise Sanguine, Tarocco, Sanguinello, Sanguinelli and Moro varieties (Saunt, 1990). Acidless oranges are planted primarily for backyard use and are not of importance commercially.

Sweet oranges are also grouped based on season of maturity, viz early, mid or late season. In subtropical climates early season cultivars generally reach maturity within 6-9 months after full blown, midseason 9-12 months. These maturation rates, of course, depend on growing conditions and the factors chosen to define fruit maturity (Davies, 1986b).

Sweet oranges may also be classified based on seediness, cultivars range in seediness from commercially seedless (0-8 seeds) to moderately seedy (9-15 seeds), to very seedy (greater than 15 seeds). (Davies and Albrigo, 1994).

2.2.2 Mandarin oranges

There are two groups of mandarin or loose – skinned, oranges of commercial importance:

i. the Satsuma group composed of several varieties and of primary importance in Japan and

ii. the tangerine group, commercially grown in Florida. Fruits of the tangerine varieties such as Dancy and Clementine (Algerian) are of a deeper orange to reddish colour than the satsumas.

Tangerine oranges are a minor part of the Florida orange crop, constituting approximately 1.8% of the 1957-58 citrus crop, with slightly less than 1.2 million tree producing 2.1 million boxes. In Japan on the contrary, Satsuma mandarine is predominating (Davies and Albrigo, 1994).

2.2.3 Sour oranges

Sour oranges are a commercial crop in southern European countries, especially in Spain, where the Seville variety or Seville Bitter orange, comprises most of the crop of this species. These oranges are chiefly used to make orange marmalade, to which they impart a desirable bitterness.

The Bergamot variety of sour orange is grown in Italy for industrial use, primarily for essential oil Bergamot. The Bouquet variety is also grown for the same purpose. Certain varieties have ornamental value and are used for landscape purposes, some sour-orange juice was also used in the early centuries as a condiment on fish and meat dishes, as lemons and limes are used today (Davies and Albrigo, 1994).

2.3 WORLD PRODUCTION OF ORANGES

The world production of oranges is greater than that of any other fruit tree. Production has increased during the last 25 to 30 years. During the 5 year period from 1924 to 1925 average annual production was 126.2 million boxes and during the 5 year period beginning in 1950-51 it was 335.6 million boxes, an increase of 166%. Some of the leading orange-producing countries have had an increase of nearly 100% during this period of 23 years such countries are Mexico, the United States, Italy, Spain, Japan, Algeria, Morocco and the Union of South Africa. The approximate world production has increased 97% from 191,131 thousand boxes to an estimate of 377,220. The groups which increased orange production most rapidly during this period are the Union of South Africa (109%), North America (113%) and the Mediterranean area (121%).

In 1988, 46.7million tons of sweet oranges were produced. There exist about 1100 cultivars of sweet oranges which are divided into groups (Valencia, Shamouti), navel oranges (Washington, Thomson), blood oranges (Mediterranean region) (Sigmud and Gustave, 1991).

The second place in world production is taken by the mandarins, *C. reticulate Blanco* with 8.4 million tons in 1988. Approximately 500 types have been described and they are mostly classified in groups as botanical varieties e.g. vari deliciosa swingle tangerine, Clementine, var, unshiu swingle – Satsuma cultivars. The third place is taken by the lemons and their relations world production in 1988 was 6.0 million (Sigmud and Gustave, 1991).

Major citrus producing countries and their total production of various types of citrus as of 1989 – 1991 are shown in Appendix 1 with emphasis on oranges, (FAO, 1991). Brazil is now the largest producer of citrus worldwide having an industry oriented towards production of oranges for processing. Recently, China, a major area of origin, has significantly increased production to become the third largest producer of oranges worldwide and the fourth in overall production. This represents a tenfold increase from 1969 and shifts emphasis to oranges from mandarin type fruits which used to predominate (Wen-cai, 1981).

About four times more oranges than mandarins are produced in the Mediteranean region. Navels and Valencias are the predominant cultivars in Spain and Greece, but in Italy blood oranges such as “Tarocco”, “Moro” and “Sanguinello” constitute about 70% of the annual orange production of 1.18 million tones (Russo, 1981). ‘Biondo comune’ is

by far the most popular orange (0.4 million tones annually). Italy still produces some bergamots (sour orange hybrids) for perfume essence (Barone *et. al.,* 1988).

The major citrus – producing countries shown in Appendix 1 are also major fresh fruit exporters, with noticeable exceptions from Brazil (primarily Sao Paulo state) and to a lesser extent, the United States (Florida), where industries are more heavily based on citrus production for processing. The exceptionally large industries in these two locations are the result of their climatic and industrial capacity to produce high-quality processed, frozen concentrate orange juice (FCOJ). While only 33% of all citrus worldwide is processed, 83% of this is oranges with 82% of the orange processing occurring in Sao Paulo, Brazil and Florida, U.S.A. Florida produces juice primarily from the early, mid, and late season cultivars, Hamlin, Pineapple and Valencia (Florida Agricultural statistics Department, 1990), while in Brazil Hamlin, Pera, Valencia and Natal are the primary cultivars used (Amaro, 1984), 'Pera' accounts for over 50% and 'Natal' 25% of the trees in Sao Paulo.

The development of a large processing industry occurred because of the invention of frozen concentrated orange juice (FCOJ) in Florida in 1948, selection of orange cultivars with high yields, juice content and percentage soluble solids and the continued development of sophisticated industrial infrastructure to meet the world demand for this production (Albrigo and Behr, 1992). In the 1980s, many smaller producing countries in the Americas have begun to develop or expand their orange production with addition of processing facilities for frozen concentrated orange juice (FCOJ).

2.4 CULTIVATION OF ORANGES

Most Citrus species are propagated by grafting on a root stock which has been grown from seed. The stock often has a definite influence not only on the uptake of nutrients and the growing vigor, but also on disease resistance and fruit quality (Mendel, *et al*., 1989; Samson, 1970). The planting distances depend on cultivar and the stock (growing vigor), for oranges, 7.5-9 metres is needed, for mandarins 4.5-6 metres.

2.5 PHYSIOLOGY OF RIPENING

The fruit of an individual tree does not all mature at one time from a single bloom, but may do so over a period of three weeks or more. Fruit is usually harvested at the mature green stage (physiologically mature but unripe stage) and is allowed to ripen under ambient conditions (20-30^{o}C) (Davies 1986a). Fruits are not allowed to ripen on the tree for the following reasons: (Davies 1986a).

i. Majority of the fruits drop from the tree before they ripen for consumption,

ii. Tree – ripe fruits are inferior in taste and aroma to fruits that ripen after harvest and their shelf-life is drastically reduced;

iii. Unripe fruits reach the consumers in better physiological condition.

The physiology of ripening involves metabolic activities resulting in fruit of acceptable quality. These activities include changes in carbohydrates and acid (to give the desired sugar-to-acid ratio), vitamins, proteins, and amino acids and sometimes phenolic compounds, development of colour and flavour characteristics for consumer appeal. These biochemical changes take place within a period of 10-14 days at ambient temperature, depending on the variety (Subramanyam *et al*, 1975).

2.6 RESPIRATION

Most fruits fall into two distinct physiological classes with respect to their ripening behaviour (Arthey and Ashurst, 1998):

i. Climacteric fruits

ii. Non-climacteric fruits

The climacteric is a period in development of certain fruits, during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening.

The orange fruit belong to the non-climacteric type of fruit. These are fruits that do not undergo a rapid ripening phase. They mature slowly and their eating quality cannot improve after harvest. Non-climacteric fruits have relatively low respiration rates that decline slowly after harvest and they produce ethylene at low rates.

In citrus fruits, there is no conversion of an insoluble carbohydrate, such as starch, into glucose. The changes in pectic substances from the insoluble protopectin to the soluble forms (pectins, pectinic and pectic acids) are not as clear-cut in citrus as in other fruits which exhibit typical ripening and pass through the climacteric rise.

2.7 THE ROLE OF ETHYLENE (C_2H_4)

The ethylene-stimulated ripening of green oranges apparently occurs by the conversion of chlorophyll into colourless decomposition products it is also known that the physiological condition of the fruit affects the tendency to chlorophyll destruction. Immature, frost – affected or regreened fruit will not respond to ethylene ripening.

Varietal, cultural and climacteric conditions also affect chlorophyll destruction. Florida grown oranges, for example, are not as readily coloured as California, and fruit such as the avocado and the banana. Blanke (2002) studied respiration rate in apple, avocado ad orange fruit and discovered that the slowest respiration rate was shown by apple and the fastest by avocado.

2.8 BIOCHEMICAL CHANGES DURING GROWTH AND STORAGE

During growth, ripening and storage, many features, within the orange fruit change. These include organic acids, pigments, starch, sugars, pectin, vitamins, proteins and amino-acids.

2.8.1 Organic acids

The predominating organic acid in orange juice is citric acid representing from 80% of the total acidity in the juice from ripened oranges (Vandercook, 1977). Other organic acids have been reported to be present in the peels of citric fruits and these are malic, oxalic, malonic and quinic (Nagy and Attaway, 1980).

Using modern chromatographic techniques, L-quinic acid has been isolated from mature pineapple oranges in the amounts of 172 and 52 mg per 100grams of fresh weight of peel and pulp respectively (Ting and Deszyck, 1959). All these acids, when present in the juice, would have an additive influence on the titratable acidity. As orange fruits develop and ripen, the titratable acidity shows a marked decrease.

2.8.2 Pigments (colouring matters)

In the orange fruit, there are four groups of pigments which are classified based on their solubility in different solvents.

- Soluble in fat solvents
    i. The chlorophylls
    ii. The carotenoids
- Soluble in water
    i. The flavonoids
    ii. The anthocyanins

The skin colour of all unripe oranges is green, and in the tropics, the ripe fruit may retain a deep-green skin colour even though the flesh has developed the typical ripe-orange colour. The fruit normally losses its chlorophyll while still on the tree and develops an orange colour. If not picked it may revert to green as the chlorophyll is resynthesized. In practice, the chlorophyll can be destroyed and the orange colour of the flavedo developed in ripening rooms, by the use of ethylene. (Leon and Sinclair, 1961).

In the blood orange, the carotenoid may be masked or partially masked by the water soluble anthocyanin. The other colouring matters, the flavonoids especially the flavanone glycoside hesperidin, which occurs in the sweet orange, has negligible tinctorial power, particularly at the pH of the acid sap in which they occur.

Flavonoids comprise a large, fairly homogenous group of substances which are widely distributed in higher plants especially citrus plants. A number of types have been isolated from citrus plants/species. These are flavone (1), flavonols (2) flavonone (3) (Kramer,*et.al,* 1982).

These compounds are moderately soluble in water, largely because they occur as glycosides. The flavanones are relatively insoluble. The solubility is also affected by the number of hydroxyl groups and the extent to which they may be free or methylated (Whiteside *et al.,*1988).

The flavones and flavanones are ivory yellow to yellow in alkaline solution but are virtually colourless in acid solution (Mendt *et al*.,1984).

2.8.3 Starch and sugars

Carbohydrates account for 70-80% of the total soluble solids in the fruit. In the presence of oxygen, carbohydrates are completely oxidized to carbon dioxide and water, with the production of Adenosine Triphosphate (ATP), the form in which energy is stored within the cell. (Erickson, 1968).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2 + \text{Energy}$$

The major groups of carbohydrates in citrus fruits include monosaccharides (glucose, fructose), oligosaccharides (sucrose) and polysaccharides (cellulose, starch, hemicellulose, pectins). Sucrose is the primary non-reducing sugar and is the major tanslocatable carbohydrate. Erickson (1968) showed that the citrus peel contains about 1.99mg of sucrose, edible portion 4.41 and juice 4.73 grams. Fructose and glucose are major reducing sugars and are present at about one half to equal quantities of sucrose in most citrus juices. Small quantities of mannose and galactose have also been found in citrus juice.

Starch is present in small quantities particularly as the fruit matures and starch is converted to sucrose, fructose and glucose. (Erickson, 1968).

Total soluble solids levels increase as fruit size increases, becoming nearly constant or slightly increasing during stages of development (Albrigo, 1978).

The concentration of reducing sugars in mature orange juice amounts to approximately 50 percent of the total sugars, and the ratio remains fairly constant during the later development of the fruit (Ting and Attaway, 1971). This result compared favourably with the work of (George, 2001) which showed the amount of reducing sugars as percentages of the total sugars in Valencia oranges from different root stocks grown in two localities in California.

George (2001) has also determined the seasonal changes in reducing and non-reducing sugars of Valencia oranges grown in Florida. The soluble carbohydrates, ranged from 52.9% to 62.43%, while on the data of the last sample they range from 41.4 to 49.5%. Dugo, (1994) discovered that the ratios of reducing to non-reducing sugars decrease uniformly during maturity of the fruit, and found that the pH values of the juice increased. The rate of inversion of sucrose is highly correlated with the hydrogen ion concentration (pH value) of the juice and the length of time required for such conversion.

2.8.4 Vitamins

Nutritionally, the most important water-soluble vitamins in citrus fruits are ascorbic acid, folic acid and pyridoxine, while fat-soluble vitamins, although found in insignificant quantities include, vitamins A, and E. (Baig and Cerda, 1975).

2.8.4.1 Ascorbic acid (Vitamin C)

Ascorbic acid functions as co-enzyme and is an essential part of the human diet (Nagy and Attaway, 1980). Levels of ascorbic acid, however, are quite variable among citrus fruits and tend to decrease seasonally. Ascorbic acid levels are expressed as mg $(100ml\ juice)^{-1}$ and ranges from 18 to 20 in some tangelos to over 70 for 'pineapple' sweet oranges. Erickson, (1968) noted that ascorbic acid levels are generally high in the peel than extracted juice and found that the ascorbic acid levels in 'valencia' oranges in the peel was 0.29, while in the juice it was 1.02 gram (100g-1). Nearly, three quarter of all vitamin C in an orange and five-sixths in grapefruit are found in the peel (Basak and Ramaswamy, 1996).

2.8.4.2 Folic acid

Citrus juice both in frozen or fresh form is a rich and stable source of folate. The presence of vitamin C in orange protects it from oxidation, and unlike other nutritional sources of folate, the folate in orange juice is not subjected to destruction caused by cooking of foodstuff (Streiff, 1971, and Streiff *et al*, 1978). Orange juice contains more folate than any other fruit juice (Hill *et al.,* 1971).

Research studies have shown that in addition to being rich in folate that citrus juice contains N-5 methyltetrafolate as the most predominant chemical form, which is metabolically active and most readily available. Its natural occurrence is unique in citrus fruits (Streiff, 1971; Streiff *et al.,* 1978).

2.8.4.3 Vitamin A

Vitamin A is present in appreciable quantity as carotenoid provitamin A in citrus (Ting *et al*., 1977). The carotenes (both the alpha and beta forms) and the cryptoxanthins in citrus are considered as the main precursors. The carotenes are only a minor component of the carotenoids of oranges ranging from about 5 to 10 percent (Ting *et al.,* 1961). Stewart (1977) analyzed the carotenoids of several varieties of oranges and mandarins, and found that beta-cryptoxanthin is the main provitamin A in citrus. The mandarin type citrus fruit is a good source of provitamin A, whereas oranges are significantly poorer.

2.8.4.4 Vitamin B6

Analysis of reconstituted frozen concentrated orange juice has shown that it contains an average of 55 microgram per 100ml (Ting, *et al*., 1974), reported that cow's milk supplies an average of 54 microgram of vitamin B6 per 100ml and orange juice 56 microgram in equal volume. Vitamin B6 is a group of three related compounds, namely pyridoxine, pyridoxamine and pyridoxal. It is essential for many of the enzymes in amino acid metabolism.

2.8.4.5 Niacin, Riboflavin and Pantothenic Acid

These vitamins are present in all citrus juices but the amount of each present in orange juice is only between 2 to 4 percent. U.S. recommended daily allowance is 177ml serving (Ting *et al*., 1974).

2.8.4.6 Stability of Vitamin C in Citrus fruits and juices during storage

Considerable variations in vitamin C content can be found in different products due to such factors as variety, maturity, fresh fruit handling, packaging and cultural practices of the fruit, from which the products originate and to the processing practices and storage conditions of these products before they reach the consumer. Aerobic and anaerobic mechanisms are mainly responsible for the destruction of vitamin C in processed products (Smoot and Nagy, 1980).

Vitamin C content of the early and mid-season are higher than that of late season fruit. These facts indicate that high quality does not correlate with higher nutritional quality. The more mature fruit and those of the late season variety are generally regarded as of better quality (Kilm and Nagy, 1988).

Vitamin C in citrus juice is remarkably stable during the short period it is generally kept after being extracted from the fruit. Freshly extracted orange and grape fruit juices retained about 98% of their original vitamin C at 21.1^{o}C for three days. At 4.4^{o}C orange and grape fruit juices retained 96 and 99 percent respectively, of the original amount after one-week's storage (Cameron *et al*., 1998). Orange juice that has been heated to boiling for 15 minutes still retained about 96 percent of vitamin C (Lopez *et al*, 1967). Atmospheric oxygen is an essential element in the loss of the vitamin C. In sealed containers, there is rapid loss of the vitamin followed by a slow decrease and becoming relatively constant. (Smoot and Nagy, 1980), found that when stored at high temperature the loss of vitamin C continues even after the headspace oxygen is exhausted and that the di-ketogulonic acid fraction increased noticeably.

The loss of Vitamin C in intact oranges during their marketing period are not expected to be more than 10 percent of the original (Harding, *et al*., 1966).

2.8.4.7 Pectins – cell wall constituent

It is generally accepted that softening and textural changes occurring during the ripening process are associated with pectic changes. Pectins consist of long chains of poly galacturonic acids in which the carboxylic groups are partially esterified (Nagy and Attaway, 1980).

Citrus fruits, especially certain of their component parts constitute one of the richest sources of pectin on a dry weight basis, as much as 30% of orange fruit albedo may contain pectin (Leon and Sinclair, 1961). The rag, comprising the fruit core and segment membranes after juice extraction is also a rich source. Since pectin is a cell wall component, it follows that little would be present in juice expressed from fruit. For example concentrations ranging from 0.01 to 0.13% in orange juice have been reported (Rouse *et al*., 1977). Much of these would be present at cell wall fragments and particulate material in juice pulp and cloud. Even at this levels, pectin influences juice quality, both positively and negatively, by its contribution to viscosity, gelation and cloud stability (Rouse *et al*., 1977).

The principal enzymes associated with the solubilization of protopectin are the pectolytic enzymes, pectinesterase and polygalacturonase. Pectinesterase de-esterifies the methyl esters, freeing carbonyl groups, while polygalacturonase splits the poly galacturonides into smaller units and finally to d-galacturonic acid residues. The insoluble pectins (known as proto-pectin) which are formed by reaction of carboxylic

groups of pectin with calcium are commonly found in unriped fruits. As ripening progresses, partial enzymatic hydrolysis results and the proto-pectin decreases with corresponding increase in soluble pectin, (Salunkhe *et al*., 1974). This results in the softening of fruit. The uronic acid and carbonyl contents of the water-soluble fraction of pectin increases to a maximum when the fruit is ripe and subsequently decreases when the fruit is over-ripe.

2.8.4.8 Proteins and amino acids

The actual protein values obtained by (Clement, 1970) from orange juice were about 20% of the acetone powder. Nearly 30 percent of the alcohol-insoluble solids of juice and about 20 percent of that of vesicular pulp were found to be protein as determined by the Kjeldahl procedure (Attaway, *et al*., 1972). These values are the actual protein that was precipitated by alcohol and are only a fraction of the total protein values usually reported for orange juice (Watt and Merrill, 1963). The main source of proteins in citrus juice is probably in the form of enzymes and the plastids. At least 47 different enzymes have been reported to occur in citrus fruits (Vandercook *et al*., 1977). Citrus fruits also contain several phenolic amines (Stewart and Wheaton, 1964), some of which such as synepherine may have physiological importance (Stewart, *et al*., 1964).

Among the various free amino acids reported in citrus juices (Attaway, *et al*., 1972), arginine is the only semi-indispensable amino acid that occurs in moderate amounts.

Evidence was presented for the presence of small amounts of histidine, leucine, isoleucine, tyrosine and methionine in orange juice (Vandercook and Price, 1972).

Vandercook and Price, (1974); Vandercook *et al*., (1975) detected arginine and γ-aminobutyric acid in orange juices and they suggested that the value and ratios of these amino-acids with other constituents such as phenolics and sugars would be helpful in determining adulteration in orange juice.

2.8.4.9 Phenolic compounds

Phenols are present in many fruits in the form of 'tannins'. These polymeric phenolic substances are associated with the sensation of astringency.

The phenolics of citrus represent a varied and widely studied class of compounds. They range from the lipid-soluble methoxylated coumarins and psoralens to the water-soluble glycosides of the flavanones and flavones. They include the intensely bitter naringin and the highly insoluble hesperidin (Petrus and Vandercook, 1980). Rouse *et al.,* 1977 found more flavonones in water extracts of orange pulp than orange juices collected from the same processors at the same time.

2.9 STORAGE CONDITIONS

Citrus fruits may be stored to provide a source of fruit at times other than during the normal harvesting season, to hold fruit until market is available or while fruit is in transit to a distant market (Brown, 1980).

Nevertheless, storage conditions are cultivar dependent and fruit quality changes occur during prolonged storage. Various methods have been used to extend shelf life of the fruits during storage. These methods include both physical and chemical methods. These methods do not only ensure prolonged shelf-life but also maintain the organoleptic

qualities of the fruits. Unlike deciduous fruits, citrus fruits do not undergo rapid chemical or physical changes after harvest. Citrus fruits are non-climacteric; hence, they do not ripen or improve in quality after harvest. The only exception may be lemon, which is packed hard and green, and which increases in juice and becomes yellow during normal storage periods. If citrus fruits are stored under proper temperatures, humidity, and atmospheres, and are marketed after a proper duration of storage, the quality of the fruit does not differ greatly from that which existed at harvest (Brown, 1980)

2.9.1 Low temperature (cold storage)

One of the methods commonly used to extend storage life of fresh fruit is to employ refrigeration. Storage of fruit at lowered temperatures reduces fruit respiration, or the rate of metabolic processes resulting from post-harvest changes in respiration and ripening. It also reduces rate of growth of decay pathogens with a resulting proportionate increase in fruit storage life (Ting and Attaway, 1971).

Some of the changes characteristics of maturation, notably decreasing acidity, continue after storage. The fruit begins to lose weight as soon as it leaves the tree and the general loss of soluble solids and ascorbic acid, on the basis of the original fresh weights of the fruit. If the weight loss is excessive, these constituents may actually increase in concentration. (Kefford and Chandler, 1970). However, some quite significant losses in ascorbic acid have been reported, particularly with lemons and tangerines. (Bratley, 1976).

Quality of Valencia oranges stored for 12 and 18 weeks at 15 and 5^{o}C, respectively, was compared to similar fruit left on the tree (EL-Zeftawi, 1976). Storage

fruit was definitely of better quality. Color was better, since regreening which occurred in fruit on the tree did not occur in storage. Granulation and stem – end browning also developed in fruit held on the tree but not in storage fruit. Cool-stored fruit was also higher in Brix, Brix / acid ratio, and ascorbic acid. Loss of flavour in storage after 24 weeks at 15^{o}C was associated with a rapid decline in Brix / acid ratio, ascorbic acid, polyphenols, and flavonoids of the juice. Valencia oranges stored at 3.3^{o}C for 6 months decreased in acidity, increased in total soluble solids, and varied in ascorbic acid content (Isshak *et al.*, 1974). Citric acid was the main acid in juice, malonic and malic acids predominated in the rind of shamouti oranges stored 3 months at 17^{o}C (Sasson and Monselise, 1977). With aging, malonic, succinic and adipic acids increase in rind and juice, (Sasson *et al* 1977) but citric acid and malic acid decreased in the juice and suggested that malonic acid could serve as an indicator of fruit tissue senescence in storage, because of the accumulation of the acid within the rind begins at fruit maturity and increased three-fold during three months of post-harvest storage. Davis (1971) also suggested that ethanol may be an indicator of fruit conditions since it showed greater changes during storage and subsequent holding than did acetaldehyde, total soluble solids, titratable acid, or pH. In oranges, ethanol increased more at 10^{o}C than at 4.5 or 1.0^{o}C. Ethanol content in juice of Hamlin, Pine apple and Valencia oranges and Marsh grapefruit increased with decreasing oxygen (O_2) concentration in controlled atmosphere storage and with wax applications (Davis, 1970). Under these various storage conditions, the ethanol content was apparently insufficient to affect flavour of the juices significantly. The condition of fruit during cold storage (Caro *et al.*, 1977) was estimated by continuously monitoring the carbon dioxide produced hourly and 1kg of fruit and

calculating the relative increase in the respiratory intensity and increases in respiration were due to infected fruit plus the effect of ethylene emanation on metabolism of healthy fruit.

2.9.2 Pre-cooling

Pre-cooling, is a technique used to remove field heat from citrus fruits, fruit is often delivered from the field at relatively high temperature. Processes within the packing house such as degreening, colour-adding, and drying contribute further heat to the fruit. Pre-cooling can be accomplished by air-cooling or hydro-cooling. Forced air at $2^{o}C$ for 6 to 24 hours prior to ventilated shipment of Shamouti, Valencia and Navel oranges and Marsh grape fruit reduced weight loss during stimulated shipment and storage (Chalutz, *et al*., 1974). Pre-cooling of fruit to temperatures below $0^{o}C$ is not advised because of possible effects on quality. Pre-cooling of florida oranges (Grierson and Hayward, 1960) initially reduced decay levels but then decay levels increased during stimulated shelf life. Water damage from hydro-cooling caused increased decay of trangerines and grape fruit (Hayward, 1962).

2.9.3 Controlled atmosphere storage

Use of controlled atmosphere (CA), means an atmosphere containing more carbon dioxide (CO_2), less oxygen (O_2) than air and high nitrogen, to maintain the quality of citrus storage. These combinations lower the rate of respiration during storage of fruits. Various results have been obtained and indicated that factors other than oxygen and CO_2

concentrations, such as variety, maturity and humidity may be involved (Chace, *et al*. 1970).

Valencia oranges held in 15% O_2 to 0% CO_2 for 12 weeks at 1^oC plus 1 week at 21^oC had higher flavour ratings than similar fruit held in other controlled atmospheres or in air, (Chace, 1968). Addition of either 2.5 or 5% CO_2 controlled aging. No marked differences were found in total soluble solids, total acid, pH, ascorbic acid or hesperidin between Valencia oranges from CA storage and those from air (Chace, *et al.*1967). Less decay developed following storage of Valencia oranges at 15 or 10% O_2 with 0% CO_2 than when stored in air (Smoot, 1977). Storage of navel oranges at 15^oC at high O_2 levels (40-80%) improved the orange color of the endocarp and of extracted juice (Houck *et al*., 1978).

2.9.4 Anti microbial treatment

Various anti-microbial agents have been used to reduce losses during storage and ripening of citrus fruits. Solutions of soap or detergents are used for cleaning purposes, elimination of total mesophilic microorganisms such as *Salmonella typhimurium, Listeria monocytogenes*, and as a mold retardant for decay control (Saper, 2001).

In Australia, Saper (2001) conducted an extensive study of the effects of chemicals on rottening and on rind blemishes. He found that soap, chlorous acid (268mg/l), sodium hypochlorite (200mg/l) and sodium acetylsulphonate reduced rotting but increased blemishes. Saper (2001) reported that chlorine is the most widely used sanitizing agent for fresh produce surfaces because published data showed population reductions in the range of 90-99 percent. Other workers (Anonymous, 2002) tested

various fungicides, a 4 to 8 percent solution of Borax and found reduction in *Penicillium* rots but little effect on stem-end decay.

2.10 PROCESSING OF ORANGE JUICE

There is much that can be said in favour of the consumption of fresh fruits and vegetables in the daily diet. The consumption of non-carbonated fruit juice drinks has become increasingly important worldwide. Both canned and pasteurized ready to serve drinks and concentrates, frozen or pasteurized are of great importance. In most part of the world citrus is consumed primarily as the fresh fruit. The main staple of processed citrus juices for example in the U.S. is frozen concentrated orange juice (FCOJ).

Processed orange products accounted for the usage of about 81% of the domestic orange crop between the years 1973 and 1978. About 94% of the Florida orange crop went into the production of orange juice products during the 6 year period, 1973 – 1978, and frozen concentrated orange juice accounted for approximately 81% of that usage (Florida crop and Livestock reporting service (Anonymous, 1978).

Various techniques which can be used for the processing of orange juices include: the traditional thermal processing method (pasteurization) also called high temperature short time (HTST), evaporation (concentrated juice), sterile filteration, pressurized carbon dioxide process (carbondioxide saturation), high pressure processing (Hpp), pulse power submerged electrical arc technology method and spray drying (Andres, *et al*., 1998; 2000 and 2001).

2.10.1 Orange juice extraction

The fruits are graded to remove unwholesome damaged fruits. They are washed and the skin oil is removed by passing the fruit over an oil extraction machine which inflicts hundreds of small cuts on the skin. The oil sacs in the surface of the skin are raptured and the oil is removed by washing. The oil and wash water are centrifuged so that the emulsion is removed from the water, which is returned to the machine (Rutledge, 1998).

Citrus juice is extracted by either reaming action or crushing action, using the FMC In-line Extractors. (FMC is a trade name). The reaming operation involves cutting the fruit in half followed by the automatic presentation of each half to the remears to remove the contents. The FMC systems use a cup-shaped set of fingers top and bottom mesh. The fruits are crushed between the cups, a small piece of the skin is cut from the centre of the bottom cup and the contents are squeezed through the cut hole into a tube, which is perforated, and partial screening process is carried out at this stage Juice cells and excess pulp that remain in the juice are easily removed during screening operation. Centrifugation is sometimes carried out to remove pulp, which can then be used as by-products (Rutledge, 1998).

2.10.2 Clarification

In the clarification of juice the initial stage involves the removal of excess pulp and seeds which can be carried out by centrifugation in a decanting centrifuge or by finishers with fine screens. Juice is clarified by removing pectin, starch, gums proteins,

polyphenolics, metal cations and lipids which may cause hazes before or after presentation (Anon, 1985).

At this stage also enzymes can be used to remove the pectins (depectinization). The action of enzymes depends on their ability to hydrolyze pectin and thereby reduce the viscosity of the juice, making it easily filtered. The most concentrated enzyme preparations are in liquid forms while the more dilute types are in dry powder from 0.2% of "pectinol O" is recommended (Mans, 1988).

The traditional method of fining is to heat the juice to the required temperature and stir in the enzyme or enzyme mixture, wait until the enzyme has acted and the agents that will bring down tannins and other unwanted materials (Heather Bell, 1976 a, b). These agents are gelatin, bentonite, and silica-sot and flocculation usually occurs rapidly after their addition. This is carried out at temperatures near ambient to get good flocculation. However, (Grampp, 1977) describes a system for hot clarification that is faster than the traditional method. The juice is decanted or racked from the lees and filtered using a filter press or diatomaceous earth filter such as a rotary vacuum filter.

The problem with enzyme treated juice is the formation of clear juice during storage, which is undesirable. To overcome this, starch-free and liquid pectin are added to the juice after the treatment with enzymes (Mans, 1988).

Control of the traditional flocculation occurring after filtration, however, membrane filter systems have become a practical reality since the cross flow system started to be used industrially in the 1970's (Pepper, 1987) and these have eliminated many of the haze problems.

Ultra-filtrations of fruit juice has become the industry standard because of the system. The juice is enzymed to reduce clogging of the filter and then passed across the filter pressure. The pores in the filter are small enough to hold back tannins and other compounds that cause haze formation in the clarified juice. Yields of juice from ultra-filtration are typically in the 95 to 97% range, compared with 90 to 93% from the traditional method (Rutledge, 1998).

2.10.3 Blending

The finished or clarified juice is conveyed to the blending tank where the acidity and soluble level are determined. Blending is carried out to achieve the best possible physical characteristics of the juice that is colour, cloud, sweetness, and acidity. After blending the juice is deaerated, deoiled, pasteurized and packaged. Deaeration is utilized to remove oxygen from the juice, thereby retarding the oxidation of ascorbic acid and flavour changes that may result form a combination of oxidative browining and oxidative changes in the flavouring oils (Casimir, *et al*., 1981).

2.10.4 Evaporation

Technical developments in high vacuum evaporation techniques have been responsible for the rapid growth of the domestic citrus industry. According to Cook (1970), the first commercial orange concentrate was produced in Florida in 1938 on a low temperature (20-25^{o}C) evaporator with 13 stages that operated under high vacuum. But prior to 1947, most of the evaporators used, utilized high temperatures and long residence times. These operators operated somewhere between 48.9 and 82.2^{o}C. Fruit solids

remained in these evaporators for a minimum of 30 minutes hence the products produced on such evaporators were of poor quality and exhibited a strong heat processed flavour.

The first commercial frozen concentrated orange juice was produced in a falling – film type evaporator at low temperature and high vacuum. This evaporator employed a large steam jet pumping system to remove water vapour at high enough vacuum to maintain an operating temperature of about 18.3^{o}C. The juice stayed in the evaporator for a long period of time but the concentrate produced was far superior to that produced in high-temperature evaporators (Varsel, 1973)

Presently, better evaporator units have been developed and they are employed in the domestic citrus industry. These units are known as TASTE (Termally – accelerated short time evaporation) evaporators. The water removal capacity is normally 18,000 to 23,000 liters per hour, but the new units being constructed have water removal capacities of nearly 41,000 liters/hour (Cook, 1970).

2.10.5 Ion-exchange processing

This involves the removal of acid from citrus juice and it was first reported (Kilburn and Drager, 1965). They employed electrodialysis to remove citrate ion from citrus juice. Later, (Berry and Wagner, 1973) used calcium hydroxide for precipitation of citrate in citrus juices.

A more recent process, that is, acid reduction by anionic ion exchange has been developed (Johnson and Chandler, 1985). Removal of citrate ion by an anionic ion-exchange process is accomplished by exchange with hydroxyl ion and the subsequent

formation of water, which is a component of juice and which can be removed by evaporation. The ion-exchange process is illustrated in the following equation;

$$3R^{+}.\ OH^{-} + C_6H_5O_7^{\ -3} \rightleftharpoons (R^{+})_3.\ C_6H_5O_7^{\ -3} + 3OH^{-}$$

Where R^{+} equates to a positively charged unit of the resin structure.

The ion-exchange resin employed in the acid reduction process is weakly basic and because the anionic resin is weakly basic, the retention of stronger acids is favoured. As a result, when processing juice, the retention of citric acid is favoured with respect to the weaker organic acids, ascorbic and folic, which are well recognized nutrients in orange juice (Johnson and Chandler, 1985).

The process permits the treatment of either bulk concentrate or freshly extracted juice. Freshly extracted juice is first stabilized at (79.4^{o} – 82.2^{o}C) then centrifuged in a high speed centrifuge to effect a pulp reduction to 2% to inhibit development of excessive back pressures in the column due to plugging. The pulp can be re-added to the juice stream following acid reduction in the ion – exchange column. Bulk concentrate is first diluted to about 15^{o} Brix after which pulp reduction is accomplished by centrifugation (Casimir, 1983).

Acid reduction of orange juice is effected by downflow passage through the resin. Juice is passed through the column until the eluate (reduced acid juice) drops to a pH below 4.6. This method assures minimal loss of ascorbic acid (Berry and Veldhuis, 1977).

The column eluate is discharged into an evaporator feed tank where its pH is adjusted to a maximum of 4.6 through the addition of freshly extracted, but untreated, juice or concentrated orange juice. This adjustment of pH ensures that no growth of pathogenic organisms can occur, and studies from researches have confirmed this (Nagy, *et al*, 1993).

2.10.6 Pasteurization

Pasteurization or thermal processing is a term given to heat processes in the range of 60 – 99^{o}C for a fraction of 40 seconds; this is also known as high temperature short time (HTST) process.

The main goal of thermal processing is to eliminate specific pathogens and a large proportion of potential spoilage microorganisms thus producing a safe product with enhanced shelf-life. This method is also useful in juice processing because it makes the juice to possess a natural cloudiness which some manufacturers desire for their product. This treatment helps in stabilizing the cloud suspension by destroying those enzyme systems which can act upon the suspended particles themselves or upon the natural pectin present which helps to support them (Robbins, 1967).

Orange juice is heat treated as soon as possible after extraction to inactivate pectinases (enzymes) naturally occurring in the juice and also to destroy spoilage organisms. The pectin in the juice is responsible for holding the 'cloud' or suspended solids in the juice. If the pectin is degraded by the pectin esterase, then the attractive cloud in the juice will fall leaving a serum (clear liquid) and a deposit on the bottom of the container. The required heat treatment is 95^{o}C for 30 seconds which is more severe

than that required for microbial stability (Hatcher, *et al*., 2001). Heating to temperatures of only 65.5^{o}C will destroy most spoilage organisms but some heat resistant molds may require pasteurization temperatures as high as 98.9^{o}C for control.

Studies have shown that processing of the orange juice at temperatures of 76.6 to 99^{o}C for a fraction of a second to 40 seconds will destroy the pectinesterase activity in citrus juices and pectinmethylesterase (PME), an enzyme causing the breakdown of citrus juice cloud is usually inactivated by pasteurization at 90^{o}C for 1 minute for orange juice (Cameron *et al*., 1998).

The orange juice is pasteurized by the HTST that is high temperature short time, process in which the juice is heated to a high temperature in the order of 79.4^{o} – 82.2^{o}C for a very short time of about 0.5 second. It is then cooled and filled into cartons at about 0^{o} – 4.4^{o}C. Such a process is not damaging to the flavour and texture of the juice and the resulting product has a very acceptable flavour and aroma (Kelly and Russel, 2001).

2.10.7 Concentrated juices

As the fruit industry grew, the financial advantages of storing juices in a concentrated form became increasingly obvious. Unfortunately, despite the availability of several types of evaporator, none of these could produce a fruit juice concentrate which, when reconstituted, approached the quality of the original straight juice. The reason is because most of the compounds which give a fruit juice its flavour and aroma are volatile, and lost in the usual evaporation process.

Orange juices, concentrates and the more recently developed comminuted ‘bases’, (Charley, 1963) are normally pasteurized and preserved with benzoic acid or sulphur

dioxide. They are usually quite stable and can be stored satisfactorily in their original containers at ambient temperatures.

Established evaporation methods for concentrating fruit juices cease to be effective when the natural pectins in the juice become dominant and form a gel. Recently, a team of scientists at the Hafia Techion, Israel have developed a method of breaking down, the pectins in orange juice using ultra – sonic techniques, Zimmerans (1967). This has resulted in an eight-fold concentrate to be produced twice as concentrated as any other known orange concentrate. It is claimed that this juice does not require the addition of any preservatives for the state of the juice is such that it deters the growth of bacteria.

2.10.8 Pressurized carbon dioxide process (carbon dioxide saturation)

This involves the use of a high pressure pump for impregnating fruit juice with carbon dioxide. The optimum concentration of the gas to be used is about 1.5kg / 100ml of juice, requiring a pressure of about seven atmospheres at $15^{o}C$..

2.10.9 High pressure processing (HPP)

This method involves the use of pressure treatment for the processing of orange juice. High pressure processing provides a way for solving the problem encountered in the use of the traditional thermal processing methods which causes detrimental changes in flavour, texture, colour and nutrient value of foods for example orange juice (Uwe and Thomas, 2001).

High pressure processing (HPP) a non-thermal technique provides a means for addressing this conflict as it can minimize or eliminate thermal processing and or

chemical preservatives while still ensuring safety. (Stewart *et. al.*, 2001). In 2001, a multidisciplinary project was initiated by food science Australia to study the sensory, safety quality, storage and cost issues associated with processing Australian orange juice products using HPP. The effect of HPP on the refrigerated storage life of orange juices was compared with untreated and heat pasteurized juices. Pressure treatment for 1 minute at 600MPa extended the refrigerated shelf-life to at least 2 months even when the product was temperature abused. Sensory characteristics were comparable to untreated juice throughout the storage time.

Consequently, it can be said that fruit juice processing methods have been developed primarily to achieve products which will remain stable between one fruit season and the next, even though these methods may have caused initial changes in flavour.

2.11 Effect of processing on chemical, nutritional and flavour quality of orange juice

Like most fruit juices, orange juice undergoes changes in chemical, nutritional and flavour characteristics during processing. Processing requires the input of heat to effect pasteurization, enzyme stabilization and concentration.

Wolfrom *et al*.(1974) studied the non-enzymes browning of dehydrated orange juice and concluded that 4-aminobutyric acid was of particular significance in the formation of coloured products. The initial phase of the browning reaction led to a loss of D-glucose and 4-amino butyric acid. Kampen (1976) stored freeze – dried orange juice crystals and a synthetic mixture for 40 days at 50^{o}C and monitored losses of total amino

acids (73%), ascorbic acid (100%), citric acid (5.1%), and sucrose (4.4%). The orange juice crystals were discoloured from Maillard browning, and several carbonyl compounds and furfural derivatives were identified as products of the reactions. Berry *et al.,* 1971 studied foam at dried instant orange juice stored at 21^{o}C and at 29^{o}C and reported that the stability of the product was improved by the use of more acidic juices, but adding acid, or removing sugar. They reported an inverse relationship between stability of the instant orange juice and the pH of the orange concentrate dried.

2.12 Storage of orange juice

The processing of fruit juices such as orange juice presents the problem of storing an unstable product for periods of 12 months or more. These juices are susceptible to three types of deterioration; microbial spoilage, colour changes and off – flavours due to enzymic reactions (Robins, 1967). Several techniques of preserving fruit juices exist. This includes carbon dioxide saturation, sterile filtration, pasteurization, evaporation and spray drying.

A major advance in the storage of fruit juices was discovered by Bohl in 1912, a Swiss chemist, that a high degree of stability could be achieved if the juices were saturated with carbon dioxide. The optimum concentration of the gas used was 1.5kg/100ml of juice, requiring a pressure of about seven atmospheres at 15^{o}C. This method prevents the development of yeasts, probably by retarding the conversion of pyruvic acid into acetaldehyde and carbon dioxide. Moulds and bacteria are less affected and could cause spoilage with time.

Concentrated juices produced through the evaporation method by the use of evaporator are another method of storing juices, but these when reconstituted could not approach the quality of the original. The reason is because most of the compounds which give a fruit juice its flavour and aroma are volatile, and lost in the usual evaporation process (Moshonas and Shaw, 1998).

Orange juice that has been extracted and pasteurized for few seconds to inactivate yeasts and moulds will have a shelf life of up to 21 days when stored at 4^{o}C or less. A shelf-life of up to three months at chill temperatures (4.4-7.2^{o}C) can be attained with orange juice treated with chemical preservatives. Preservatives used are typically sulphur dioxide at 100 ppm and a combination of sorbic and benzoic acids at up to 400ppm (Townsend, 1976). The antioxidant properties of sulphur dioxide being partially helpful.

2.13 SOURCES AND TYPES OF MICROORGANISMS IN FRUIT JUICES

2.13.1 Sources of microorganisms

Presence of microorganisms in any fruit/beverage drink arises from faulty processing technology or post – handling (Bowen and Beech, 1964, Nwashille and Ogbonna 1985, Deboye and Akinpelu, 1986). The processing of these juices relies on the ingredients, containers and processing equipment that carry only small amount of micro-organisms; however, some industrially processed fresh juice contain a variety of micro-organisms including yeasts, moulds, and bacteria (Lott and Carr, 1964; Kolfschoten and Yarrow, 1970) Sand and Van Grinsven, 1976; Ofuya and Kine, 1983 Goverd *et al*., 1979).

Yeasts from skin of ripe fruits often get into processed fruit juices and their presence could either be beneficial or detrimental (Bowen and Beech, 1964). Internal tissue of undamaged fruit generally contains no yeasts. Yeasts such as *Saccharomyces, Cryptococcus* and molds such as *Aspergillus, Penicillium, Mucor, Rhizopus, Botrytis, Fusarium* and *Alternaria* have been reported to be predominant organisms in frozen fruits, although small number of soil bacteria for example species of *Bacillus, Pseudomonas, Archromobacter* survive freezing. Yeasts are most likely to grow during slow thawing (Bowen and Beech, 1964).

The numbers of microorganisms in frozen fruit juices depend upon the condition of the fruit, the washing process, the method of filtration and the opportunities for contamination and growth before freezing. There may be from a few hundred to over one million organisms per milliliter present in the juice at the time of freezing. The inclusion of rotten parts of the fruits increases the numbers of organisms during storage in the frozen condition. The kinds of organisms are mainly those of soil, water and rots, together with the natural surface flora of the fruits. Prominent usually are coliforms, enterococcci, Lactis e.g. *Leuconostoc* and *Lactobacillus* species, *Alcaligenes* and yeasts.(Deboye and Akinpelu, 1986).

Coliform bacteria, mostly of the *Enterobacter aerogenes* type, form part of the natural flora of fruits, they are present in both fresh and frozen fruit juices. The use of decayed fruit for the juice increases the number of coliforms, but these organisms decrease during storage (Deboye and Akinpelu, 1986).

Microorganisms also enter the processing plant from soil, surface water, dusty air and from cross-contamination with decomposed fruits (Tresler, 1971). Presence of viable

pathogens in fruit juices are thought to come from animal manure used to fertilize the orchards and gained access to the juice because of failure to clean and sterilize the equipment (Anon, 1975). The degree of contamination of fruit juices depend on handling methods and shelf-life (Anon, 1975).

2.13.2 Types of microorganisms isolated from fruit juices

In Nigeria, as in most developing parts of the world, very little or no microbological research on fruit juices has been reported. However, there are few reports on locally processed fruit juices. Ofuya and Kine (1983) reported the presence of *Lactobacillus, Pseudomonas* and Coliform*s* in orange and pineapple squash. Spores of *Bacillus* species were isolated from various Egyptian canned juices and vegetables (Gabriel and Abdul, 1973). Industrially produced fruit juices contain a variety of microorganisms including yeasts, moulds and bacteria (Lott and Carr 1964; Govered, *et al*, 1979).

Studies carried out by Egamana (1988) showed that apple juice had a microbial load of between $2.4x10^1 – 5.0x\ 10^6$ cfu/ml, while Brahama orange juice had $3.0x\ 10^4$ cfu/ml. Ten strains of yeast were also isolated form apple juice. These were identified as *Canndida etchelsil* (2), *Brettanomyces* spp(4), *Candida versatalis* (3) and a strain of *Candida* spp. Three species of *Aspergillus* were identified from each of the lolly orange juice. These include *Aspergillus flavus, A.niger, A. Ochraceous* and *A. fumigatus* Egamana (1988) and Splittstoesser (1978) reported that manifestations of yeast growth are the formation of turbidity and sediments, changes in colour and development of off-flavours.

2.13.3 Potential hazards of microorganisms in fruit juices

Despite, the high water activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and moulds especially molds. The degree of specificity shown by many species of molds, active in the spoilage of harvested fruits, reflects their possible role as pathogens, thus *Penicillium italicum* and *P. digitatum* show considerable specificity for citrus fruits, being the blue mold and green mold respectively of oranges, lemons and other citrus fruits (Anon, 1975).

The pH range of juice from apple is below 4.0 which is believed to inhibit the growth of food poisoning organisms including *Salmonella* (Anon, 1975). In 1974, however, an outbreak of gastroenteritis caused by *Salmonella typhimurium* in chilled non-sterile apple juice occurred in New Jersey U.S.A. Samples of the juice recovered during the outbreak had low pH ranging from 3.4 – 3.9 but they contained viable organisms (Anon, 1975). So far there are no known reported cases of infections or intoxications which are traceable to consumption of fruit juices such as orange juices in Nigeria. The presence of bacteria, particularly the coliforms as reported by Ofuya and Kine (1983) in Port-Harcourt from orange and pineapple squash shows the potential dangers consumers of such fruit juices face if enteric pathogens are consumed.

2.13.4 Shelf-life of fruit juices

The period between manufacture and retail of food products during which the product is of satisfactory quality is referred to as its shelf-life (Woodroof and Philips, 1974).

Practically, fruit juices/beverages deteriorate with age. Apart, from environmental and physical factors, microbiological factors such as microbiological load play significant role in determining the shelf life of beverages (Korab, 1976).

2.14 QUALITY CONTROL OF FRUIT JUICES

Fruit juices such as orange juice may be sources of food-borne diseases. Presence of microorganisms serves as an indication of quality of the products since they (microorganisms) play significant role in reducing its shelf-life. The fact that several microorganisms including coliforms have been isolated from soft drinks and fruit juices (Logan, *et al*., 1981, Deboye and Akinpelu, *et al*. 1986) makes these products potentially hazardous form the view point of public health. There is a need for quality assurance to safeguard health of consumers and to reduce the risk of economic loss incurred by manufacturers. Among the several agencies established to safeguard and to ensure the maintenance of quality of goods is the joint Food and Agricultural Organization (FAO) / World Health Organisation (WHO), Codex Alimentarius Commission which works out International Standards for food (Townsend, 1976). Under this commission, there is codex co-ordinating committee for Africa that was set up to help the various members in setting up national food and drug control authorities.

The 1974 Food and Drug Acts established for National Agency for Food and Drug Administration Control (NAFDAC) makes provision for the prevention of deceit and fraud in manufacture and sale of foods, drugs, cosmetics and medical devices and establishing standards for importation, manufacture, sales and advertisement of food and drugs. NAFDAC, in addition to its inspectorate and laboratory services also carried

regulatory services where detailed specifications are spelt out about food processing, food standards, hygiene practices, packaging and labeling, food additives and advertising.

Nigerian processors of fruit/juices employ techniques similar to or adapted from those of their foreign partners. The processes were designed among other things to maximize the shelf life of the products by controlling microbial contamination and growth. The Standard Organization of Nigeria (SON) (1976) has documented the permissible concentrations of additives and preservatives applicable to fruits juices and this information is disseminated to local factories.

The standards set by SON, (1976) and NAFDAC on permissible concentrations of additives, preservatives and microbiological limits of fruit juices (orange juice) and soft drinks for Nigerian processors are given as follows: Orange juice should be prepared with

- sound orange, fruit or fruits, potable water, sugar, dextrose or liquid glucose with or without citric acid, tartaric acid or lactic acid and food colours and preservatives such as sodium benzoate, sodium metals; sulphite (sulphur dioxide), benzoic acid at permitted levels of 350ppm (0.035%) respectively.
- They should contain not less than 25 percent v/v of orange juice and should not contain any flavouring substance other than those naturally present in fruit or fruits of orange. Microbiological limits /guideline by NAFDAC is as stated below.

- Total aerobic mesophilic bacteria plate count, the minimum acceptable level in a sample unit is $2x10^2$ colony forming unit per mililitre (cfu/ml) and the maximum acceptable level is 10^2cfu/ml.
- Total coliform, minimum acceptable level is less than 3 cfu/ml, maximum acceptable level is 10 cfu/ml
- *E. coli* is 0 cfu/ml for both minimum and maximum acceptable level.

Therefore any of these organisms when it exceeds the maximum acceptable limits in any fruit juices (orange juice) and soft drinks in Nigerian market is termed unsafe for human consumption.

Also FAO/WHO Codex Alimentarius Commission (2002), provided microbiological criteria for citrus juices/fruits and they are as given below:

- Processed orange juice either by thermal pasteurization that is high temperature short time (HTST) or high pressure processing (HPP) should achieve a minimum of 5-log reduction (1 million fold) of the target pathogen(s) in citric juices are permitted.
- "Untreated fruit juice or vegetable juice or citric juice" must contain no detectable *E. coli* / ml and *Salmonella sp*., should be absent in 25g when the sample is analyzed by the prescribed method. Untreated fruit juice or vegetable juice or citric juice means the juice which, in its production, after cleaning and culling of fruit and at a single facility, has not undergone a process capable of producing a minimum of a 5 – log reduction in the most treatment resistant organism of public health significance that is likely to occur in the juice.

- Samples of apple juice (cider in the U.S.) may contain 1 *Escherichia coli*/ml. if these *E. coli* were a pathogenic strain, then a reduction to fewer than 1 cell per 100ml serving would be the minimum necessary to provide consumer protection. However, if a 100-fold safety margin is applied then a 5-10 log reduction to 10^{-4} *E. coli* would be required. This would be equivalent to one viable *E. coli* in 10 litres of juice.

2.15 Physico-chemical analyses

2.15.1 pH

The pH of orange juice is an important parameter or attribute to be considered in heat processing orange juice is classified as an alkaline. This is because the organic acids are destroyed by the process of digestion, and the final reaction of the juice in the human body is determined by its mineral content, the main constituents of which are the basic elements (Nelson and Tressler, 1980).

At pH values greater than 4.5 severe heat treatments is required to kill spores of *Clostridium pasteurianum*, yeasts and moulds. Such treatment would cause heat damage to the flavour of orange juice. By adjusting the pH to below 4.5 much less severe heating is required and flavour changes are minimized. The pH of the juice is normally determined and acidified sufficiently to render the pH below 4.5. 0.01 percent citric acid is normally added to adjust the pH below 4.5. (A.O.A.C, 1980).

2.15.2 Titratable acidity

The acidity of orange juice is usually calculated as citric acid that is the predominant organic acid in orange juice is citric acid. The acidity in orange juice decreases during growth and maturity of the fruits while the concentration of sugar increases (Davies and Albrigo, 1994).

2.15.3 Brix: Acid Ratio (maturity ratio)

The test of maturity for oranges is based on the ratio of total solids to acid present in the juice. Since the major part of the total solids is sugar, this is often termed the sugar: acid ratio for oranges and the minimum ratio is 8:1. the flavour of orange juice is related to the ratio of soluble solids to the total acidity called the "maturity ratio" which increases as fruit ripens and is sometimes used for assessing the quality of the juice (that is ratio determination is required for grades and for fruits maturity test). The ratio is determined by dividing the degrees Brix of a sample by the percentage of titratable acidity of a sample (A.O.A.C., 1980).

2.15.4 Total soluble solids:

The soluble solids of oranges are composed mainly of soluble sugars and organic acids which are stable compounds. About 15% of the soluble constituents of orange juices are other than sugars and organic acids. This fraction consists of inorganic compounds, amino acids, ascorbic acid and small amounts of pectins, essential oils, esters, glucosides and other organic compounds. These compounds are commercially important in that the occurrence of off-flavours in either natural or processed orange juice

is in no small measure due to oxidation and decomposition of these substances (A.O.A.C., 1980).

2.15.5 Vitamin C (ascorbic acid) content

The standard ascorbic acid is used to titrate against sodium 2.6-dichlorophenol dye using metaphosphoric acid as an extracting solution following the methods of analysis of the Association of Official Agricultural Chemists (A.O.A.C., 1980).

2.15.6 Total solids

This can be described as the amount of insoluble matter such as peels, cellulose pulp, vesicles present in fruit juices, jams, fresh and canned fruits, jellies, syrups, marmalades and preserve. Total solids are determined to ascertain the amount of these insoluble matters since their presence can bring about off-flavour and cloudiness in the fruit juice. Degree of concentration is usually assessed from refractive index or specific gravity (A.O.A.C, 1980)

2.15.7 Moisture contents

Food products contain water in different forms. The most common form is free or absorbed water. Moisture content in foods can be determined by direct or indirect methods. Moisture is normally removed from the material by oven drying, desiccation, distillation, freezing – drying, extraction and other physico-chemical techniques. Methods based on these principles which are usually employed in the laboratory, have a high

degree of accuracy and in most cases with proper precautions in sampling can be made to yield absolute values. (Karmas, 1980).

2.15.8 Ash content

The ash content is the inorganic matter or constituents contained in fruit juices. It is the soft grey powder that remains after the dried solids from orange juice has been ignited at 550^{o}C in a muffle furnace for several hours. Ash content serves as a measure of the inorganic salts that were present the original material.

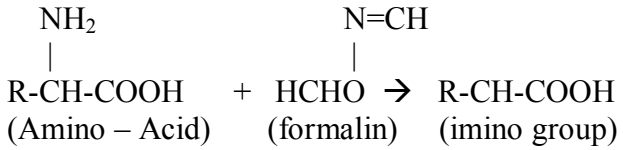
2.15.9 Minerals (macro elements)

Orange juice has become over the years one of the most widely accepted natural source beverages. This is due to its invigorating flavour, its source of quick energy in the form of natural sugars, its nutrient qualities, especially ascorbic acid (Vitamin C), and its high potassium content relative to other inorganic metals, particularly sodium.

The elemental composition of orange juice can be expressed in terms of macro (those elements above 1%), major trace (elements from 10ppm to 1%) and minor trace (elements less than 10ppm) (Karmas, 1980).

2.15.10 Estimation of amino acids

The formol titration method is used for the estimation of amino – acids. When formalin is added to neutralize aqueous solution containing protein, the amino-group reacts to form methylene-imino group. – N = CH_2 with the release of a proton which may be titrated.



The formol titration involves mixing a mixture of 1:2 or 40% formaldehyde solution and distilled water. The mixture is neutralized (titrated with 0.1m NaOH) using phenolphthalin as the indicator.

2.16 SENSORY EVALUATION

Sensory evaluations are made by the senses of taste, smell, visual and touch. The combined sensation or the interaction of these senses measures food quality in the mind of the taster. Sensory panels (taste panels) may be used to measure general appearance factors such as colour, odour, viscosity and textural factor such as "mouth feel".

Different methods of sensory evaluation exist: (Odumosu, 1983).

1. triangular test
2. product rating
3. consumer preference panel
4. storage stability

2.16.1 Difference testing (triangular test)

This makes use of three samples which are presented to the taste panel members. Two of the samples are the same and one is different. The panel is then asked to detect the odd sample. This method is used in quality control to detect, whether there is batch to batch variation.

2.16.2 Product rating

This involves product rating in which quality scores are given to different quality parameters by a small group usually about 5 of highly trained panelists. The quality parameters may include: colour, odour, taste or mouth feel and viscosity.

2.16.3 Consumer preference panel

This method is used in product developments or marketing exercises. Usually, a group of about fifty is asked to 'score' or 'rank' products in terms of their own preference.

2.16.4 Storage stability

This test is carried out in order to maintain quality in storage. Sensory tests for storage stability involve:

i. Difference tests to establish that stored lot is not different from the control. If no difference is found, product stability is good.

ii. Descriptive and scalar tests to describe the changes that may have occurred or to grade the product as acceptable or not acceptable.

A group of about 3 to 10 trained panelist and 1-18 experimental samples are used to determine if difference exist in the samples quality at storage with that of the control.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Mature ripe sweet oranges (*Citrus sinensis*) were used and they were purchased from Zaria market, Kaduna state.

3.2 PREPARATION OF JUICE

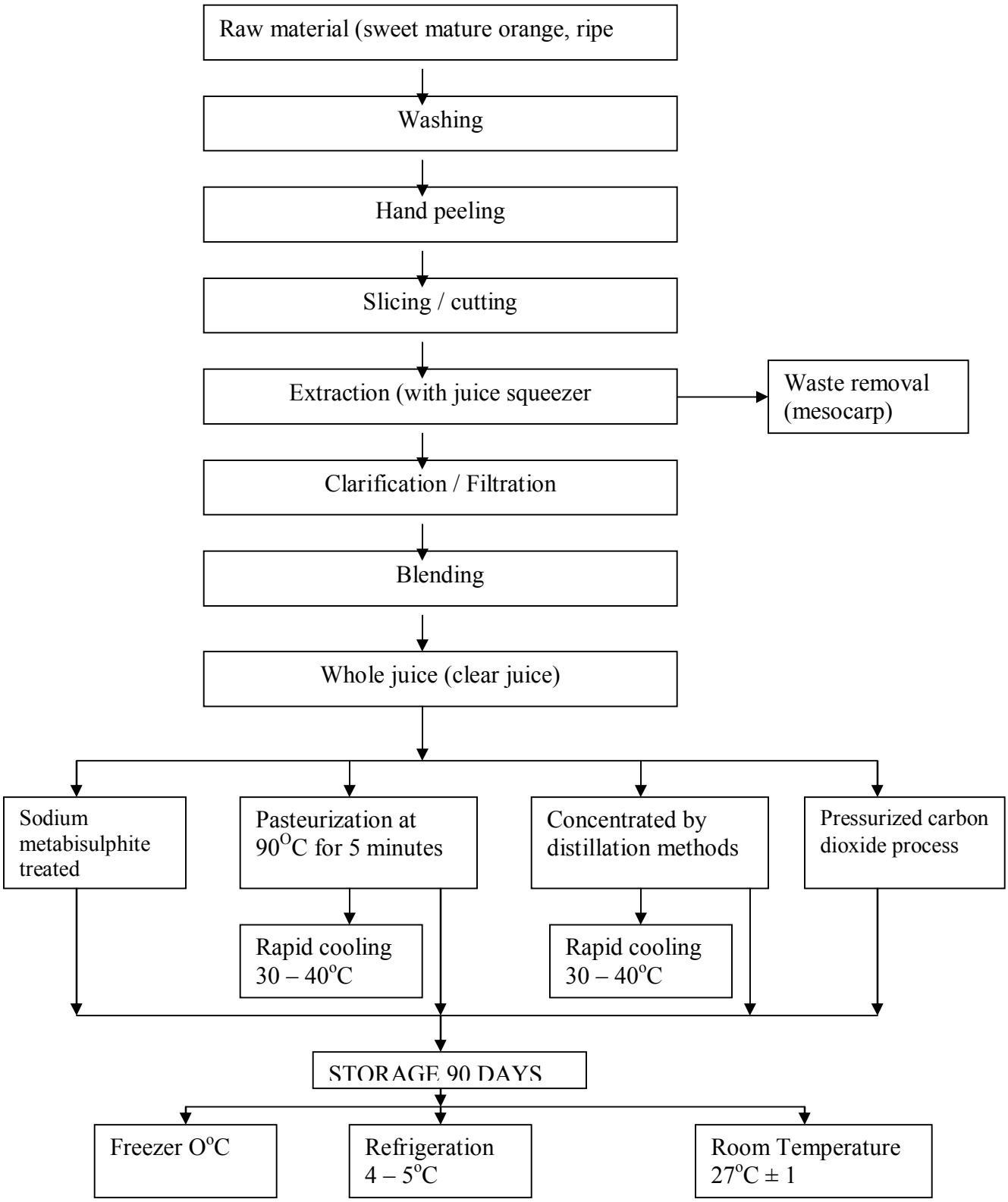
Six hundred medium sized ripe fruits of sweet oranges were washed, peeled and sliced/cut into two halves using a sharp stainless steel knife. The cut orange (mesocarp) was pressed with a juice squeezer to extract the juice. The juice and pulp obtained were homogenized in a Monilex blender. The homogenate was filtered using a cheese cloth to obtain a whole juice. This was divided into five batches of 400ml each in a plastic container and given the following different treatments:

- Fresh orange juice was treated with sulphur dioxide, i.e. 0.035gm of sodium meta-bisulphite was added into each 100mls of the fresh juice sample (for 400ml 0.14 gm was added).
- Orange juice was pasteurized at 90^{o}C for 5 minutes.
- Orange juice was concentrated by heating to boiling and 10mls of distillate was collected from every 100mls of orange juice using distillation apparatus.

- Pressurized carbon dioxide (carbon dioxide saturation) was introduced into the juice at the Coca-cola bottling plant in Kaduna. The concentration of gas used was 1.0kg/100ml of orange juice with a pressure of about three atmosphere at $10^{o}C$
- The freshly laboratory prepared orange juice without any treatment serving as control
- and a second control sample, that is a commercially prepared orange juice, a product of Prestige ventures, Mushin, Lagos was purchased from Zaria market and assessed along with the treated samples for their physico-chemical, sensory evaluation and microbiological qualities. The pasteurized and the concentrated samples were allowed to cool to 30-$40^{o}C$. All the samples with different processing techniques were stored for 90 days under freezer, refrigeration and room temperature respectively.

The foregoing protocol above was adopted from (Akpapunam, *et al*, 1993) with some modifications and the flow chart is shown in figure 1.0 below:

FIGURE 1: Flow chart for the preparation of orange juice



Source: Akpapunam *et al,* (1993) with some modifications

3.3 PREPARATION OF MEDIA

All the media that were used were prepared according to manufacturers' instruction. These media include:

- MacConkey Agar (Oxoid) for isolation of coliforms
- Potato Dextrose Agar (Oxoid) for the isolation and enumeration of yeasts and moulds.
- Standard plate count Agar (SPA) of (PCA) was used for the determination of total aerobic plate counts.
- Yeast extract powder (Oxoid) was used for sugar fermentation.

3.4 ISOLATION AND ENUMERATION OF MICRO-ORGANISMS

Procedures similar to those described by (Gilliland *et al*., 1976) was used to enumerate microorganisms from fresh orange juice, pasteurized orange juice, pressurized carbon dioxide (carbonated) orange juice and control sample of already processed orange juice which included the following:

3.4.1 Enumeration of Total Viable Count

Each of the various samples of orange juice both treated and control samples were analysed. The stock culture were prepared by taking 10mls of the orange juice sample, mixed with 90mls of sterile peptone water. Each sample was serially diluted (10 fold dilution of 1:10, 1:100, 1:1000, 1:10,000) in sterile peptone water and 0.1ml amounts of appropriate dilutions was spread on various agar media; standard plate count agar,

MacConkey Agar and Potato Dextrose Agar using a sterile bent glass rod. All samples were plated in duplicates.

Standard plate count agar (PCA) (Oxoid) was used to determine the total aerobic plate counts, while coliforms and other enterics were enumerated on MacConkey Agar (Oxoid) (Rafai, 1979). 1.75gm of PCA, 5.2gm, of MacConkey Agar/100ml of the distilled water each sterilized by autoclaving for 15 minutes, poured into plates and inoculated with the samples. All samples were incubated at 37^{o}C for 24-48 hours.

Potato Dextrose Agar (PDA) (Oxoid) of 3.5g/100ml of distilled water, sterilized by autoclaving for 15 minutes and streptomycin 0.1gm dissolved in 1ml sterile distilled water was added into the medium for isolation and enumeration of yeasts and moulds. The streptomycin serves as a bacteriostat to the sterile molten potato dextrose agar (PDA). Enumeration of yeasts was done after three to four days when isolates were picked, and isolates of moulds were picked after four to five days. All enumerations were expressed as colony forming units per millilitre (cfu/ml) of samples plated.

3.4.2 Identification of microorganisms

Isolate representative of the growth on each medium used for enumeration of microorganisms was picked and purified by subculturig several times on the same medium and the purified isolates were stored at 5-10^{o}C on slants of the same medium of isolation.

The purified bacterial isolates were gram stained and examined for gram reaction and morphology.

Yeasts and moulds were identified based on their morphological features. The method used by (Okafor, 1971); Smith (1969) and Gilman, (1957) were used and they are as described below.

The yeast isolates, observations were made on the appearance and pigmentation of the colonies of each isolate. To achieve these, the organisms were purified by subculturing on a fresh Potato Dextrose Agar (PDA), at room temperature (27 ± 1^{o}C). Microscopy of slide stained with lactophenol cotton blue was carried out.

The moulds were subcultured on freshly prepared Czapek Dox Agar from old culture on PDA before identification after an incubation period of four to five days. The purified isolates were stained using lactophenol cotton blue for morphological examination and observation.

3.5 BIOCHEMICAL TESTS

Various biochemical tests were carried out to test for the physiological characterization and the confirmation of the isolated organisms as described by (Gilbert *et al.,* 1981). The descriptions of the tests are given below:

3.5.1 Coagulase test (slide method)

A drop of physiological saline was placed on a sterile slide and a colony of the test organism from a nutrient agar culture slant was collected with a sterile wire loop and emulsified in the drop of the saline. A loop full of citrated human plasma was added to the bacterial suspension in the normal saline and tilted back and forth for 1 minute. The presence of slots or clumps of cells is indicative of positive coagulase test.

3.5.2 Catalase test

A drop of 6% hydrogen peroxide (H_2O_2) was placed on a glass slide. A portion of the test bacterial growth from a nutrient agar slant culture was collected and placed on a drop of the hydrogen peroxide. The release of bubbles and froth is indicative of a positive result.

3.5.3. Citrate utilization test

A simmon citrate agar (oxoid) slant was inoculated with the test organism and incubated at 37^{o}C for 24-48 hours and observed. The development of a deep blue colour showed an indication of a positive reaction. That is the utilization of citrate where the appearance of an alkaline colour of bromothymol blue from pale green to deep blue after 48 hours of incubation.

3.5.4 Tripple sugar Iron Agar test

Slants of triple sugar iron Agar was prepared. To this, the test organism was inoculated into it from peptone water. The surface of the slant was streaked and a stab was made into the slants. It was then covered with a cotton wool and incubated at 37^{o}C for 24 hours. After 24 hours, all change of colour, presence or absence of gas and black ring were observed and recorded.

3.5.5 Methyl Red-Voges-Proskauer test (MR-VP)

Aliquots of 0.5ml of sterile glucose phosphate broth was inoculated with the test organism and then incubated at 37^{o}C for 24 hours. After 24 hours incubation 2-3 drops of methyl red solution was added. The reaction was observed and recorded. The development of bright red colour is an indication of a positive result, while a yellow orange colour indicates a negative result.

For the Voges – Proskauer (V-P) test 2ml of sterile glucose phosphate peptone water was inoculated with the test organism. It was incubated at 37^{o}C for 48 hours. At the end of 48 hours, 3ml of sodium hydroxide of 40% was added and shaken properly, the bottle cap was removed and left for 1 hour. The reaction was observed and recorded. The development of pink red colour indicates that the culture produced acetoin and which shows a positive result, while the non development of pink colour indicates that acetoin is not produced and thus a negative result.

3.5.6 Nitrate reduction test

Aliquots of 0.5ml of sterile nitrate broth was inoculated with a heavy growth of the test organism and then incubated at 37^{o}C for 4 hours. After 4 hours, one drop of sulphanilic acid reagent and one drop of α-napthylamine reagent was added and observed for any colour change. A red, pick or maroon colour gives a positive test i.e. nitrate is reduced while a test without colour change is an indication of a negative result (i.e. nitrate not reduced).

3.5.7 Starch hydrolysis

One gram of potato starch was suspended in 10mls of cold distilled water, mixed with 100mls of nutrient agar, autoclaved, cooled to 45^{o}C and thoroughly shaken to mix before dispensing into sterile Petri-dishes. The plates was then inoculated with loopful cultures of the test organism and incubated at 37^{o}C for 48 hours. After 48hours, the plates were then flooded with iodine solution. A zone of clearance underneath the growth indicates starch hydrolysis i.e. a positive result.

3.5.8 Sugar fermentation

Test for sugar fermentation was conducted in solution of the sugars in tubes with inverted durham tubes. The medium contained 0.25% Yeast Extract powder (Oxoid), 0.75% peptone water (Oxoid) and 0.01% bromothymol blue as indicator. This was distributed in 4.0ml aliquots in 150 x 15mm tubes which were cotton-plugged and sterilized by autoclaving at 121^{o}C for 15 minutes. The carbon sources that were used included membrane filter-sterilized 6.0% (w/v) solutions of glucose, galactose, maltose, sucrose, melibiose and 12.0% solution of raffinose. Two millitres of each sugar solution was aspectically added to each tube and inoculated with 0.1ml of the test organism in sterile normal saline of 18-24 hours old culture of the yeast strain. The tubes were observed at two, three and seven day intervals.

A basal medium without any of the sugars was also inoculated with a strain of the yeast to serve as control. A positive fermentation result is indicated by a gas-filled durham tube.

3.5.9 Urea hydrolysis

Slants of sterile urea agar base tubes were inoculated with a heavy growth of the test organism from a potato dextrose agar culture. The tubes were incubated at 37^{o}C and examined half hourly for colour change to purple over a four hour period. Appearance of purple colouration within the test period was considered to be indicative of hydrolysis of urea.

3.6 PHYSICO-CHEMICAL ANALYSIS

Chemical analysis was carried out using the methods of Association of Official Analytical Chemists (A.O.A.C.) (1980), on fresh samples of orange juice (FRH), concentrated orange juice (COND), carbonated orange juice (CAB), chemically treated (CHEM) and the commercially processed orange juice (COS)

3.6.1 pH

The pH meter was first standardized in buffer solution of 4, 14, and 7. Aliquot of 5ml each of the orange juice sample was used to determine the pH using pH meter at 20^{o}C.

3.6.2 Titratable acidity

Aliquot of 5ml of each sample was titrated with 0.1M of sodium hydroxide (NaOH) using phenolphthalein as the indicator. The percentage acidity as citric acid was determined as described in the Association of Official Analytical Chemists (A.O.A.C., 1980). The percentage titratable acidity was calculated using the relation below:

Total titratable (% citric acid)

$$= \frac{\text{Titre x 0.1m NaOH x 100}}{\text{Volume of juice used}}$$

3.6.3 Brix: Acid Ratio (maturity ratio)

Citric acid of (8.0%) was added to 5ml of each sample, to obtain the degree brix which is determined by ratio. For oranges the minimum ratio is 8:1. The ratio was determined by dividing the degrees brix of the samples by the percentage of titratable acidity of each of the various samples and given by this relation;

$$\text{Brix: acid ratio} = \frac{^{o}\text{Brix}}{\text{titratable acid}}$$

3.6.4 Total soluble solids

The total soluble solids content (^{o}brix) was determined using the Abbe 60 refractometer at 20^{o}C based on the percentages by weight of anhydrous citric acid that was added to the refractometer.

Citric acid of (7.0%) was added to 5 ml of the orange juice sample to obtain the degree brix which was determined by using the correction table of the refractive indices (n) of sucrose.

3.6.5 Vitamin C (ascorbic acid) content

Aliquots of 10ml of each sample was taken and transferred to 125ml Erlenmeyer flask. 10ml of 3% of metaphosphoric acid solution was added to each of these samples.

Then each example was titrated immediately with the standard dye solution of sodium 2, 6-dichlorobenzone indophenol to a faint pink end point which persisted for 15 seconds. The concentration of ascorbic acid was then calculated as follows.

Dye titer x titration x 100 = Mg Ascorbic Acid per 100 ml juice.

$$= \frac{\text{Dye titer x titration x 100}}{10 \times 100}$$

3.6.6 Total solids

Ten milliliters of each of sample was weighed into a crucible and dried at 70^{o}C for 2 hours in a vacuum oven. The calculation of the total solids was done as follows:

$$\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where

W_1 = weight of crucible

W_2 = weight of sample + crucible before drying

W_3 = weight of sample + crucible after drying

3.6.7 Moisture content

Aliquot of 10 ml of each sample was added into a silica dish. This was immersed in a water bath until the water was completely evaporated. The dried solids in the silica dish is then removed from the water bath and dried at 70^{o}C in a vacuum oven for 3 hours,

and was then placed in the desiccator for 10 minutes for cooling. The content of the dish was weighed and the moisture content calculated as follows.

$$\% \text{ moisture content} = \frac{\text{weight of sample after drying}}{\text{weight of sample before drying}} \times 100$$

3.6.8 Ash content

Aliquots of 10ml of each sample juices were weighed into a silica dish for the determination of the ash content. The samples were evaporated in the water bath and charred on the Bunsen burner. The charred samples were then ignited at $55^{o}C$ in the muffle furnace for 6 hours. At the end of ashing (the inorganic matter) it was weighed and the percentage of ash calculated.

$$\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where

W_1 = weight of crucible

W_2 = weight of sample + crucible before drying

W_3 = weight of sample + crucible after drying

3.6.9 Minerals (macro-elements)

The procedure used was the selected method of soil and plant analysis from International Institution For Tropical Agriculture (IITA) Ibadan and soil science department, Ahmadu Bello University, Zaria.

Five milliliters of each of the samples was added in a digestion beaker for the determination of phosphorus, potassium and sodium. Ternary acid of 15ml was also added to each sample and digested until white fumes were produced. Then the digested

materials was transferred into 25ml volume flask and made up to mark with distilled water.

Phosphorus was further developed by adding 2mls of ammonium metavanadet and the reading was taken using spectrophometer 20 at 440 – 460nm for each of the samples of orange juice.

Potassium and sodium were determined by using flame photometer Beckman model.

The calculations were carried out for each nutrient as follows:

For phosphorus:

$$\frac{PPM}{A} \times EX.\ Vol. \times D \times \frac{F}{V} = Phosphorus$$

Where PPM = Perse per million

Ex. Vol = Extract volume

D x F = Dilution factor

V = Volume of sample

A = Absorbance

Potassium and sodium

$$PPM\ (GRD - GRD\ of\ blank) \times D \times \frac{V}{W} = Potassium/Sodium$$

Where PPM = Parts per million

GRD = Graph reading

GRD of blank = graph reading of blank

D = Dilution Factor

V = Volume of sample

W = weight of sample used

3.6.10 Estimation of amino acids

Five milliliters of each sample measured and 10ml of formaldehyde solution 40% concentration with distilled water was titrated against 0.1M NaOH. The volume of sodium hydroxide used in all the samples was noted. The titration was carried out in duplicate for all samples and the amount of amino – acid was estimated as follows:

$$\text{Amino-acid content} = \frac{\text{titre x 0.1M NaOH}}{\text{Volume of juice used}}$$

3.7 SENSORY EVALUATION: (PRODUCT RATING METHOD)

Taste panel tests were carried out on the laboratory processed orange juice for fresh, pasteurized, concentrated, carbon-dioxide saturation (pressurized), chemically treated and the commercially processed orange juice which served as control sample, using the product rating method. The samples were presented to the panelists which consisted of five members; one undergraduate student, two research students, two members of staff. The panelists were requested to assess the samples and give scores ranging from 1-5. The quality parameters that were used in assessing the product include colour, viscosity, odour and flavour.

The panelists were also requested to assess the samples by personal preference. Five samples were presented to each of the panelists at the same time. The panelists rinsed their mouths with warm water after assessing the first sample. The taste panel

score sheet that were used in assessing the samples using the products rating method is given in Appendix 3.

3.8 STATISTICAL ANALYSIS

Randomized block design using factorial concept was used in analyzing the results of the laboratory and commercially processes orange juices. This involved the use of analysis of variance (ANOVA), the post Hoc test using multiple Duncan range procedure (DMRT) in order to determine the levels of significance between the different methods and the control.

CHAPTER FOUR

4.0 RESULTS

The results of microbiological, physico-chemical and sensory evaluation of the commercially and laboratory prepared orange juice are presented in tables 1, 2, 3 and 4. Table I shows number of bacterial and yeast cells / ml from the different juices stored under different storage conditions; freezer, refrigeration and ambient temperature. The total aerobic plate counts (TAPC) per ml (colony forming unit/ml (cfu/ml) for all the samples tested ranged from 1.50 x 10^6 to 4.1x10^6 for bacteria. The bacteria counts for the carbonated and pasteurized sample stored in the freezer had the highest counts of 8.1x10^5 and 9.1x10^5 cfu/ml respectively. The chemically treated, carbornated, concentrated and pasteurized samples stored under the refrigerator had counts of 3.75x10^6, 3.11x10^6, 4.1x10^6 and 3.4x10^6 cfu/ml respectively. The samples stored under the ambient temperature had the counts of 1.50x10^6, 1.73 x 10^6, 2.7 x 10^6 and 3.4 x 10^6 cfu/ml for the chemically treated, carbonated, concentrated and pasteurized samples. The fresh and commercial samples had 1.6x10^5 and 4.40x10^5 respectively.

The total plate counts for yeast cells ranged from 1.7x10^6 to 4.75x10^6. The samples stored in the freezer had the counts of 5.95 x 10^5 , 1.99 x 10^6, 6.4 x 10^5 , 5.75 x 10^5 cfu/ml for pasteurized, carbonated, concentrated and chemically treated. The refrigerated samples had the counts of 1.7x10^4, 1.77x10^6, 1.7x10^6 and 4.75x10^6 cfu/ml for concentrated, carbonated, chemically treated and pasteurized respectively, while the counts for the samples stored under ambient temperature had counts of 8.0x10^5 , 9.2x10^4, 2.58x10^6 and 3.4 x 10^6 cfu/ml for carbonated, chemically treated, pasteurized and

concentrated respectively. The fresh and commercial samples had counts of 8.5 x 10^4 and commercial 5.5x10^4 cfu/ml respectively.

Table 2 shows the physico-chemical properties of the laboratory prepared orange juice fresh, pasteurized, pressurized carbon dioxide (Carbonated), chemically treated, concentrated and commercially processed orange juice. For all the samples the pH ranged from 1.6 to 4.5 and the pH values for the commercially processed sample showed the lowest value compared with the laboratory prepared orange juice with different treatments. The titratable acidity values ranged from 0.03 to 0.24, the Brix acid ratio from 1.16 to 4.73, the total soluble solids (TSS) 26-33^o Brix, the vitamin C content from 2.79 – 43.78% (mg/100ml juice), total solids from 3.46-7.43%, moisture content 2.0 – 10.59%, estimation of amino – acids from 0.08-0.25 molarity, ash content 0.29 to 1.29%, minerals (macro-nutrients): potassium from 0.51 to 1.73%, sodium 0.01 to 0.12% and phosphorus 0.01 to 0.02%.

A correlation analysis of the different variables of the physico-chemical properties of the laboratory prepared orange juice is shown in Table 3. The result showed that there was a significant correlation between pH and the percent Brix:acid ratio ($r = 0.58$, $P < 0.05$), total soluble solid ($r = 0.52$, $P < 0.05$), potassium ($r = 0.7$, $P < 0.05$), and phosphorus ($r = 0.63$, $P < 0.05$). There was a significant correlation between percent Brix:acid ratio and total solid ($r = 0.59$, $P < 0.05$), and level of amino acid ($r = 0.52$, $P < 0.05$). Also there was correlation between level of amino acid and sodium ($r = 0.05$, $P < 0.05$), percent Ash and potassium ($r = 0.57$, $P < 0.05$), total soluble solid and potassium ($r = 0.64$, $P < 0.05$), potassium and phosphorus ($r = 0.72$, $P < 0.05$). There was no significant correlation between other variables.

Table 4, shows the microscopic and colonial morphological characters and identities of all the moulds isolated from commercially processed orange juice and the laboratory processed orange juice, they were tentatively identified to the genus level *Mycelia* sp was the predominant genus having accounted for five out of 11 isolates. Other moulds isolated included *Helminthosporium sp.,Blastomyces sp, Chrysosporium sp, Aspergillus sp* and *Trichoderma sp.*

Table 5 shows the sensory evaluation of the laboratory prepared orange juice. The table shows the mean values of the results of test methods and treatments. Means with different superscripts within columns were significantly different at ($P<0.05$).

The biochemical characteristics of the isolates of bacteria are shown in tables 6. The isolates of bacteria species had the Bacillus as the predominant genus and these are *Bacillus megaterium, Bacillus cereus, Bacillus pantothenticus;* others included *Pseudomonas aeruginosa, E. coli and Shigella.*

Table 7 shows the yeast isolates identity. The species of the yeast isolated were *Saccharomyces cerevisae, Saccharomyces telluris, Saccharomyces rouxii,* Rhodoto*rula mucilaginosa, Brettanomyces anomalus* and *Candida mesenterica and* appendix II shows the biochemical characteristics of yeast isolates from orange juice (laboratory produced)

All these isolates were obtained from commercially, fresh, pasteurized, carbonated, concentrated and chemically treated laboratory prepared orange juice using the various standard comparism tables for identification of different microorganisms.

Table 1: Microbial load of the various types of orange juices

S/No	TYPE OF CONTAINER	SAMPLE CODE	TYPE OF TREATMENT	TOTAL AEROBIC PLATE COUNT (cfu/ml) OF BACTERIAL CELLS /ml	TOTAL PLATE COUNT OF YEAST CELLS / ml (cfu/ml)
1	Transparent plastic container	FRH	No treatment (freshly processed)	1.6×10^5	8.4×10^4
2	" "	COS	Factory treatment (pasteurization)	4.40×10^5	5.5×10^4
3	" "	CHM_1	Chemical preservative (sodium metabisulphite)	1.68×10^6	5.75×10^5
4	" "	CHM_2	" "	3.75×10^6	1.7×10^6
5	" "	CHM_3	" "	1.50×10^6	9.2×10^4
6	" "	CAB_1	Carbonated	8.1×10^5	1.99×10^6
7	" "	CAB_2	" "	3.11×10^6	1.77×10^6
8	" "	CAB_3	" "	1.73×10^6	8.0×10^5
9	" "	$COND_1$	Concentrated	3.75×10^6	6.4×10^5
10	" "	$COND_2$	" "	4.1×10^6	1.7×10^4
11	" "	$COND_3$	" "	2.7×10^6	3.4×10^6
12	" "	$PASD_1$	Pasteurized	9.1×10^5	5.95×10^5
13	" "	$PASD_2$	" "	3.4×10^6	4.75×10^6
14	" "	$PASD_3$	" "	3.4×10^6	2.58×10^6

KEY

FRH → Fresh laboratory (lab) produced orange juice
COS → Commercially processed orange juice
CHM_1 → Chemically treated lab produce orange juice stored in the freezer
CHM_2 → Chemically treated lab produce orange juice stored in the refrigerator
CHM_3 → Chemically treated lab produce orange juice stored in an ambient temperature
CAB_1 → Carbonated lab produced orange juice stored in the freezer
CAB_2 → Carbonated lab produced orange juice stored in the refrigerator
CAB_3 → Carbonated lab produced orange juice stored in an ambient temperature
$COND_1$ →Concentrated lab produced orange juice stored in the freezer
$COND_2$ → Concentrated lab produced orange juice stored in the refrigerator
$COND_3$ → Concentrated lab produced orange juice stored in an ambient temperature
$PASD_1$ → Pasteurized lab produced orange juice stored in the freezer
$PASD_2$ → Pasteurized lab produced orange juice stored in the refrigerator
$PASD_3$ → Pasteurized lab produced orange juice stored in an ambient temperature

Table 2: Physico-chemical properties of various types of orange juices

S/N	Sample code	pH value	Titra table Acidity (%)	Brix: Acid Ratio (maturity ratio)	Total soluble solid (^{o}Brix)	Vitamin C content mg/100ml	Total Solids (%)	Estimation of amino acids (%)	Moisture content (%)	Ash content (%)	K (%)	Na (%)	P (%)
1	FRH	3.5	0.1	3.39	33..88	43.78	4.81	0.12	7.53	0.63	1.73	0.04	0.02
2	COS	1.6	0.03	8.55	26.67	2.79	3.46	0.08	10.97	0.29	0.51	0.02	0.001
3	CHM_1	3.4	0.20	1.50	30.05	22.89	5.61	0.13	10.59	0.55	1.67	0.04	0.01
4	CHM_2	3.3	0.22	1.54	33.88	29.65	5.09	0.22	4.46	0.45	1.47	0.12	0.02
5	CHM_3	3.5	0.07	3.81	26.67	25.97	4.86	0.23	4.38	0.48	1.44	0.11	0.01
6	CAB_1	3.4	0.20	1.59	31.70	17.01	5.33	0.08	10.79	0.45	1.83	0.01	0.01
7	CAB_2	3.2	0.31	0.92	28.37	29.45	5.39	0.23	4.79	0.62	1.65	0.12	0.01
8	CAB_3	3.0	0.06	4.45	26.67	29.75	5.34	0.17	5.12	0.62	1.62	0.01	0.01
9	$COND_1$	3.1	0.07	1.28	30.60	10.75	6.74	0.18	12.40	0.65	2.27	0.01	0.02
10	$COND_2$	4.5	0.12	2.78	33.34	21.99	3.25	0.12	2.81	0.72	2.30	0.02	0.02
11	$COND_3$	3.4	0.24	4.73	28.37	8.96	4.18	0.13	3.56	0.99	2.63	0.01	0.02
12	$PASD_1$	3.4	0.12	4.61	32.25	24.88	5.65	0.09	11.06	1.29	1.74	0.01	0.01
13	$PASD_2$	3.4	0.06	1.16	27.80	31.44	7.43	0.25	7.26	0.62	1.77	0.01	0.01
14	$PASD_3$	3.2	0.12	2.36	28.37	32.84	6.99	0.20	4.99	0.84	1.93	0.01	0.01

KEY

FRH → Fresh laboratory (lab) produced orange juice
COS → Commercially processed orange juice
CHM_1 → Chemically treated lab produce orange juice stored in the freezer
CHM_2 → Chemically treated lab produce orange juice stored in the refrigerator
CHM_3 → Chemically treated lab produce orange juice stored in an ambient temperature
CAB_1 → Carbonated lab produced orange juice stored in the freezer
CAB_2 → Carbonated lab produced orange juice stored in the refrigerator
CAB_3 → Carbonated lab produced orange juice stored in an ambient temperature
$COND_1$ →Concentrated lab produced orange juice stored in the freezer
$COND_2$ → Concentrated lab produced orange juice stored in the refrigerator
$COND_3$ → Concentrated lab produced orange juice stored in an ambient temperature
$PASD_1$ → Pasteurized lab produced orange juice stored in the freezer
$PASD_2$ → Pasteurized lab produced orange juice stored in the refrigerator
$PASD_3$ → Pasteurized lab produced orange juice stored in an ambient temperature

K → Potassium
Na → Sodium
P → Phosphorus

Table 3: Correlation test results of the physico-chemical properties of the commercially and laboratory processed orange juices

CORRELATION	pH	ACID	BRAT	TSS	Vit.C	TOSOL	MC	EMACID	ASH	K	NA	P
pH	1	0.2723	0.5818	0.5232	0.088	0.0184	0.4435	0.1382	0.3703	0.7161*	0.0681	0.6334*
ACID	0.2723	1	0.4499	0.2514	0.2237	0.0877	0.2651	0.0709	0.1265	0.3122	0.4445	0.2497
BRAT	0.5818	0.4499	1	0.3402	0.0378	0.5931	0.0871	0.5211	0.0193	0.4777	0.2486	0.4001
TSS	0.5232	0.2514	0.3402	1	0.0704	0.1444	0.0952	0.3178	0.1812	0.294	0.0626	0.642*
Vit.C	0.088	0.2237	0.0378	0.0704	1	0.1013	0.1914	0.2355	0.1854	0.4979	0.2724	0.208
TOSOL	0.0184	0.0877	0.5931	0.1444	0.1013	1	0.2657	0.5303	0.179	0.1852	0.1412	0.0805
MC	0.4435	0.2651	0.0871	0.0952	0.1914	0.2657	1	0.4729	0.0696	0.2763	0.3757	0.3345
EMACID	0.1382	0.0709	0.5211	0.3178	0.2355	0.5303	0.4729	1	0.157	0.055	0.5246	0.0973
ASH	0.3703	0.1265	0.0193	0.1812	0.1854	0.179	0.0696	0.157	1	0.5744	0.3217	0.2498
K	0.7161*	0.3122	0.4777	0.294	0.4979	0.1852	0.2763	0.055	0.5744	1	0.3065	0.7233*
NA	0.0681	0.4445	0.2486	0.0626	0.2724	0.1412	0.3757	0.5246	0.3217	0.3065	1	0.0625
P	0.6334*	0.2497	0.4001	0.642*	0.208	0.0805	0.3345	0.0973	0.2498	0.7233*	0.0625	1

NOTE: N OF CASES = 14, 1-Tailed Significant : * 01, ** 001
"" "" is printed if a coeficient cannot be computed

Key:
pH → hydrogen ion concentration.
Acid → titratable acidity
Brat→ brix: acid ratio
Tss → total soluble solid
Vit . c → vitamin c
Tosol→ total solid
Mc → moisture content
Emacid → estimation of amino acid
K → potassium
Na → sodium
P → phosphorus

Table 4: Microscopic and colonial morphology of mould isolates from commercially and laboratory processed orange juices

SAMPLE CODE	ISOLATE CODE	MICROSCOPIC OBSERVATION	COLONIAL OBSERVATION	IDENTITY OF MOULD ISOLATES
COS CAB_2 CHM_3 $COND_3$ $PASD_1$	A	Irregular groups of cells or hard masses of hyphae without spores	Colonies are widely spread, white and pink mycelium cell masses. Reverse is colourless	*Mycelia sp*
$COND_3$	B	Multiseptate conidia with smooth walled conidiophores	Colony growth is rapidly spreading, slight pink, and woolly with black reverse	*Helmintho-sporium sp*
CAB_1	C	Spherical conidia borne on short hyphae branches	Colonies are dimorphic in shape. The entire surface is covered with a white woolly mat.	*Blastomyces sp*
$PASD_1$	D	Simple branched conidiophores in chains	The surface is rough, woolly dark green. The reverse is pale to colourless	*Gliocladium sp*
$PASD_1$	E	Irregular branched conidiophores. Some appear singly and some in short chains	Colony growth appear waxy to velvty and look pink in colour. The reverse is colourless	*Chrysosporium sp*
$PASD_1$	F	It has a swollen vesicle at the tip of the conidiophores	Colonies are fine velvty woolly or cottony acrial hyphae with abundant mycelium. Reverse is colourless. Conidia heads are blackish	*Aspergillus sp*
CAB_3	H	Branched conidiophores some appear singly and some in groups	Colonies shows white hyphal growth with compact woolly tufts, looking green.	*Trichoderma sp*

Key

FRH → No growth
COS → Commercially processed orange juice
CAB_1 → Carbonated laboratory (lab.) produced orange juice stored in the freezer
CAB_2 → Carbonated lab. produced orange juice stored in the refrigerator
CAB_3 → Carbonated lab. produced orange juice stored in an ambient temperature
$COND_3$ → Concentrated lab. produced orange juice stored in an ambient temperature
CHM_3 → Chemically treated lab. produced orange juice stored in an ambient temperature
$PASD_1$ → Pasteurized lab. produced orange juice stored in the freezer

Table 5: Sensory evaluation of the orange juice s (commercially and laboratory processed) sample results of test methods (x [± SD]) (a)

CODE	COLOUR	VISCOSITY	ODOUR	FLAVOUR	ACCEPTABILITY
PASD	3.8667[b]	3.1333[a]	3.6667[a]	2.6667[b]	3.2667[b]
CHM	4.0667[a]	3.1333[a]	3.4667[a]	2.1333[b]	2.7333[b]
CAN	4.1429[a]	3.3571[a]	3.8571[a]	1.9286[c]	2.7143[b]
COND	3.2667[b]	2.9333[b]	3.7333[a]	2.5333[b]	4.0000[a]
COS	3.2000[b]	2.2000[b]	2.8000[b]	3.4000[a]	3.8000[b]
FRH	3.6000[b]	3.6000[a]	2.2000[b]	3.0000[a]	4.0000[a]
TOTAL	3.7681	3.1014	3.5072	2.4493	3.2899

Sample Code Results of Test Treatments Methods (X [± SD]) (B)

CODE	COLOUR	VISCOSITY	ODOUR	FLAVOUR	ACCEPTABILITY
$PASD_1$	4.0000[b]	3.2000[a]	3.2000[b]	3.4000[a]	3.8000[a]
$PASD_2$	3.800[b]	3.2000[a]	4.2000[a]	3.0000[a]	3.4000[a]
$PASD_3$	3.800[b]	3.0000[a]	3.6000[b]	1.6000[c]	2.6000[b]
TOTAL	3.8667	3.1333	3.6607	2.6667	3.2667
CHM	4.2000[a]	2.6000[b]	2.6000[c]	3.2000[a]	3.6000[a]
CHM_2	4.2000[a]	3.4000[a]	4.6000[b]	1.8000[b]	2.000[b]
CHM_3	3.8000[b]	3.4000[a]	3.2000[b]	1.4000[b]	2.6000[b]
	4.0667	3.1333	3.4667	2.1333	2.7333

CODE	COLOUR	VISCOSITY	ODOUR	FLAVOUR	ACCEPTABILITY
CAD_1	4.4000[a]	3.4000[a]	3.0000[a]	3.0000[a]	4.0000[a]
CAD_2	3.8333[b]	3.6667[a]	4.1667[a]	1.3333[b]	1.8333[c]
CAD_3	4.3333[a]	2.6667[b]	4.6667[a]	1.3333[b]	2.3333[b]
TOTAL	4.11429	3.3571	3.8571	1.9286	1.6838
$COND_1$	3.2000[a]	3.0000[a]	3.6000[b]	2.8000[a]	3.8000[b]
$COND_2$	3.8000[a]	2.8000[b]	4.000[a]	2.4000[a]	4.2000[a]
$COND_3$	2.8000[b]	3.0000[a]	3.6000[b]	2.4000[a]	4.000[a]
TOTAL	3.2667	2.9333	3.7333	2.5333	4.0000

NOTE: a, b, Means with the same alphabet are not significantly different at 0.05 level of significant
* All values represent means of duplicate reading
* Values are means and standard deviation of the mean

Table 6: Biochemical characteristics of isolates of bacteria from commercially and laboratory prepared orange juices

test results of isolates of bacteria

ISOLATES CODE	CATALASE	CITRATE UTILIZATION	METHYL RED	VOGES PROSKAUER	NITRATE REDUCTION	STARCH HYDROLYSIS	HYDROGEN SULPHIDE PRODUCTION(TSI)	ACID PRODUCTION	INFERENCE (ORGANISMS)
FRH, CHM_1 PASD, CAB_1	+	+	+	-	-	+	-	A	*Bacillus megaterium*
FRH, $PASD_2$, CHM_2, CAB_1	+	+	-	+	+	+	-	A	*Bacillus cereus*
FRH, CHM_1,$PASD_3$, CAB_3	+	-	+	-	-	+	-	A	*Bacillus pantothenticus*
CHM_3,COS, CAB_2	+	-	+	-	-	-	-	A	*Bacillus aeruginosa*
COS, CHM_3	+	-	-	-	-	-	-	A	*Escherichia coli*

KEYS

FRH → Fresh laboratory (lab) produced orange juice
COS → Commercially processed orange juice
CHM_1 → Chemically treated lab produce orange juice stored in the freezer
CHM_2 → Chemically treated lab produce orange juice stored in the refrigerator
CHM_3 → Chemically treated lab produce orange juice stored in an ambient temperature
CAB_1 → Carbonated lab produced orange juice stored in the freezer
CAB_2 → Carbonated lab produced orange juice stored in the refrigerator
CAB_3 → Carbonated lab produced orange juice stored in an ambient temperature
$PASD_1$ → Pasteurized lab produced orange juice stored in the freezer
$PASD_2$ → Pasteurized lab produced orange juice stored in the refrigerator
$PASD_3$ → Pasteurized lab produced orange juice stored in an ambient temperature

TSI → Tripple Sugar Iron agar

\+ → Positive
\- → Negative

Table 7: Yeast isolates identity

SAMPLE CODE	FERMENTATION OF SUGARS							IDENTITY OF YEAST
	Glucose	Galactose	Maltose	Sucrose	Lactose	Melibiose	Raffinose	
FRHW, CAB_2W, CHM_2, $COND_2W$	+	-	+	-	±	-	-	*Saccharomyces, cerevisiae* *Saccharomyces rouxii*
$PASD_1P$, CHM_1P, FRHP	+	-	-	-	±	-	-	*Saccharomyces telluris*
CAB_3, FRHW	-	-	-	-	±	-	-	*Rhodotorula mucilaginosa*
FRHP, $PASD_1P$ CHM_3, $COND_1$	+	-	-	+	±	-	-	*Brettanomyces anomalus*
$PASP_3$, $COND_3$, CAB_1P, FRHP	+	-	-	-	±	-	-	*Candida mensenteric*

Key:

+ = Positive result

- = Negative result

± = Doubtful result

FRH = Fresh laboratory produced orange juice

CHM_1 = Chemically treated laboratory (Lab) produced orange juice stored in the freezer

CHM_3 = Chemically treated Lab produced orange juice stored in an ambient temperature

CAB_1 = Carbonated Lab produced orange juice stored in the freezer

$PASD_1$ = Pasteurized Lab (Lab) produced orange juice stored in the refrigerator.

$COND_1$ = Concentrated Lab produced orange juice stored in the freezer

$COND_3$ = Concentrated Lab produced orange juice stored in an ambient temperature

$PASD_3$ = Pasteurized Lab (Lab) produced orange juice stored in an ambient temperature

CAB_2 = Carbonated Lab produced orange juice stored in the refrigerator.

CHAPTER FIVE

DISCUSSION

The laboratory freshly prepared orange juice, the differently treated orange juice samples and the commercially processed sample were analysed for their microbial content. The microbial population consisted of *Bacillus megaterium, Bacillus cereus, Bacillus pantothenticus, Pseudomonas aeruginosa and E. coli*. Growth and metabolic activities of these organisms contributed to the spoilage of the orange juice in storage. Fruit juices and concentrates are known to contain adequate nutrients which can support microbial growth (Goverd *et al*, 1979). Use of these nutrients by the micro-organisms could result in production of substances such as diacetyl and acetyl methyl carbinol (Logan *et al*, 1981). These substances will influence the pH of the juice and cause spoilage. Ofuya and Kine (1983) had earlier suggested that pH could be used as indicator of spoilage of fruit juices.

All the orange juices tested had a microbial count which was relatively high ranging from $1.50x10^6$ to $4.1x10^6$ for bacteria and 1.7×10^6 to 4.75×10^6 for yeast. cfu/ml of the orange juice under different processing methods and storage conditions. The control samples freshly prepared and commercially processed had also high count of 1.6×10^5 cfu/ml and $4.40x10^5$ respectively. The high microbial counts (presence of bacteria, yeasts and moulds) in the samples exceed the acceptable limit of 2×10^2 cfu/ml (Rafai, 1979 and NAFDAC) and therefore could constitute a health hazard to consumers. The presence of bacteria, yeasts and moulds could be attributed to the fact that fruits mainly are contaminated with various microorganisms at the harvest time mostly from soil and

dust. Also a high viable count often indicates unsatisfactory sanitation, unsuitable time or temperature conditions during production or storage (Parish and Haggins, 1989). Moreover, studies have shown that moulds and yeasts tolerate high osmotic and low pH and can grow at refrigeration temperatures and can therefore cause spoilage in processed product (Covadonga, *et al*, 2003). Citrus juices are acidic beverages (pH 3.0 - 4.0) with high sugar content (15^{o} Brix), under these conditions bacteria, yeasts and moulds comprise the typical microbiota present in citrus juice.

Reports by (Ofuya and Kine, 1983; Foster and Hall, 1990, and Parish *et al.*, 1997) showed that bacteria species of *Pseudomonas* and *E .coli* can grow in orange juice and pineapple squash. In 1997, Food Safety and Hygiene discussed the survival of Entero haemorrhagic *E. coli* (EHEC) and *Salmonella* species in fruit juices (Pasteurized orange). They concluded that EHEC and Salmonellae can survive in fruit juices and some other acid foods with a pH below 4.5 for many days especially at refrigeration temperatures (Anomymous, 1997). These reports confirms the result of this work where *Pseudomonas* and *E. coli* was among the bacterial strains being isolated from the orange juice sample pasteurized and stored in the refrigerator. Spores of *Bacillus* species were isolated from various Egyptian canned juices and vegetables (Gabriel and Abdul, 1973). This report thus confirms the possible isolation of the species of *Bacillus* being isolated from the orange juice samples analysed especially, the fresh sample and those stored in the freezer, refrigeration and ambient temperatures, Reports by (Beech and Davenport 1970), showed that species of *Bacillus* and *Pseudomonas* can survive freezing temperature.

The high acid and sugar content of fruits often permits yeasts and moulds to predominate in fruit juices. Of all the samples tested the genus *Mycelia sp* were

predominant mould contaminants. The yeasts and mould species isolated from the test samples were mainly from the sample stored in the freezer and this shows that fungi are more likely to grow during slow thawing especially yeasts. The detection of *Candida sp, Saccharomyces sp* and *Brettanomyces sp* as yeast contaminants of fruit juice especially orange juice agrees with the report of (Put and Sand, 1974; Sand & Van Grinsvan, 1976; Covadonga, *et al*., 2003). *Saccharomyces sp* have been known to be responsible for large scale spoilage, while slow spoilage has been caused by members of the genera *Brettanomyces* and *Candida* as reported by (Put & Sand, 1974; Sand & Van Grinsvan, 1976) in fruit juices and soft drinks. Statistically, there were no significant differences in numbers of bacteria and yeasts obtained in the test samples and in the control ($P>0.05$).

The results in Table 2 indicate a consistence in the pH value of the freshly prepared orange juice which was affected by the different treatments used in processing and storage in this study. From the result in Table 2 it shows that the pH in the commercially processed orange juice was very low when compared with the experimental sample. With the commercial sample, the drastic decline in pH shows the effect of heating on orange juice during pasteurization. This is because the temperature necessary to stabilize the juice is pH dependent. However, the experimental samples showed a progressive decline in pH value in chemically treated, carbonated and pasteurized but with an increase in pH value in the concentrated juice. The decline in pH was in good agreement with previous work of (Akpapunam *et al*., 1993). The increase in hydrogen concentration could be explained by the dissociation of component acids by heat. The increase in soluble solids contents and the reduction in total solids show the effect of a decrease in acidity. The reduction in acidity during ripening plays an important

role in the Brix: acid ratio and consequently in influencing the taste and flavour of the juice. The increased soluble solids contents obtained compares with the result of Braddock and Marcy, (1985). The increase in soluble solids is explained by the evaporation of water during pasteurization and concentration of the juice (Akpapunam *et al*. 1993).

The vitamin C of the orange juice studied under different processing conditions compared favourably with freshly prepared juice. More of the vitamin C content was conserved in the pasteurized, chemically treated, and carbonated.

The results showed that the chemically treated and carbonated orange juice is as effective as pasteurized because it does not change the taste and it preserves more of its vitamins found in fresh-squeezed orange juice. Thus this result agrees with the work of (Murat, 2000). The loss in vitamin C which was observed drastically in the commercially processed sample as stated by Nagy and Smoot (1999) could be attributed to the oxidation by residual air layer within the container during processing. According to Nagy and Smoot, (1999) temperature and storage affects the presence of vitamin C content in orange juice and orange fruit. This also accounts for the low level of vitamin C observed in the juice stored at the freezer and ambient temperature for carbonated and concentrated juice.

Considering other parameters like, amino-acids, ash content and mineral elements, potassium was a major macro mineral in orange juice, followed closely by phosphorus, and the result compared favourably with the work of Parichart *et al*., (1999). The result of amino acid showed that the treatments did not show any significant

difference in the nutrient compositions during the period of storage and it was consistent with the data obtained from (Mears and Shenton, 1973).

Statistically, a correlation test shown in table 3 was applied to assess the nutritional components in order to determine possible significant relationships between the parameters analyzed. In the test, pH value was significantly related to total soluble solids, Brix : acid ratio, potassium and phosphorus ($P < 0.05$). No significant relationship was found between pH and the other parameters. Brix: acid ratio was negatively correlated with pH, total solids and estimation of amino acids ($P<0.05$). Total soluble solids were significantly related to pH and phosphorus. There was no significant relationship between Vit. C, acidity, moisture and ash among the other selected parameter ($P >0.05$).

The result of the sensory evaluation in Table 5 revealed that for the parameters used in the study to assess the quality of the orange juice, that there was no significant differences between colour and viscosity ($P>0.05$) but their was significant difference between odour and flavour ($P \leq 0.05$) of the samples in the present study when compared with their rating with the control samples for general acceptability. The result of this present study showed an off-flavour and aroma in the orange juice in storage that is the laboratory prepared juice. Moshonas and Shaw, (1998) reported that orange juice can lose some of its fresh flavour during storage because of decreased levels of volatile flavour constituents, thus developing a stale flavour. They also reported that for juices packaged in plastic containers, a major contributor to this flavour lose is often caused by absorption of flavour constituents by the plastic containers.

Also the differences in aroma and flavour could also be attributed to the presence of some off-taste compounds known to be formed in orange juice, such as, P-vinyl guaiacol (PVG) and 2, 5-dimethyl-4-hydroxyfuranone (DMHF) Eggstad (2003), Bezman *et al*. (2001) suggested in their studies that 2-methyl-3-furanthiol and methional found in stored orange juice (21 days at 35^{o}C) contributed to stored orange juice off-flavour and aroma. There was no significant differences between the various methods as regards aroma and other parameters assessed.

At the end of storage for 90 days, the panelist had preference for concentrated juice stored under the refrigeration temperature and the room temperature, pasteurized and carbonated both were stored under the freezer and room temperature. The assessment by the panelist revealed that despite a decrease in some flavourings, the products still maintain acceptable quality during the recommended shelf-life when compared with the study of Shaw and Moshonas (2000). Thus consumers of these products especially pasteurized and carbonated orange juice are assured of a product with good flavour quality throughout its normal shelf-life of nine weeks (Shaw and Moshonas, 2000).

The deterioration of orange juice during storage comprises changes in organoletic quality or sensory evaluation, nutritional value and appearance. The main parameters that influence the deterioration of orange juice is the temperature and the presence of oxygen. From this study, it was observed that the predominant microorganism causing spoilage in the orange juice depends on either inherent abilities of the organisms to survive in the environment of study or that these organisms were either inherent in the fruits because most of the fruits are contaminated from the harvest time.

In terms of mould growth sources of contamination could be from the spores of mould in the air in the environment or from the additives used since it is known that some of these additives deteriorate with time if they were not properly sealed hence growth of moulds. This is evidently seen in the present study where the level of sodium metabisulphite of 350ppm (0.035%) source of sulphur dioxide were ineffective in inhibiting yeast and mould growth in the orange juice in storage at pH values of 3.0 – 3.5 and even 4.5. Although Beuchat (1976) in his investigation using several antimycotic agents found sulphur dioxide (SO_2) to be inhibitory against *Byssochlamys niver* ascospores amongst the antimycotic agents tested. Therefore, it would not be a good preservative for fruit juices especially orange juice and soft drinks in Nigerian markets since many species of yeast and moulds were isolated from the orange juice samples tested in storage.

5.1 CONCLUSION

Nigerian processors of fruit juices employ techniques similar to the ones that was adopted in this study. These techniques are pasteurization, carbonation, concentration and use of chemical preservatives and their products being stored under similar methods applied in this work; freezer, refrigeration and ambient temperature. The processes were designed among other things to prolong shelf-life of the products by controlling microbial contamination and growth, maintain nutritional value and the chemical composition. From this study, it was observed that the different methods employed, showed that orange juice can store in any of these methods when suitable conditions are being applied in terms of satisfactory cleaning/sanitary conditions of handling and processing methods

and electricity supply based on the result obtained, it can be said that the product under study can still be acceptable and fit for human consumption since the result of the nutritional values that is the chemical composition when compared with other workers like Parichart *et al*. (1999) showed a consistence in keeping with the standards stipulated by the Standard Organization of Nigerian (SON) (1976) and the International Standards Recommended by Codex Alimentarius Commission for juices of orange, grape fruits, lemon and apple.

Therefore, orange juice under proper quality control and application of good manufacturing practices will still maintain its nutritional values, organoleptic qualities, not exceeding its microbiological limits as provided by Nigerian Agency for Food and Drug Administration Committee (NAFDAC) still serves as a most invigorating fruit drink.

5.2 RECOMMENDATIONS

On the basis of the observations and results of this study, the following recommendations are made:

A combination of sodium metabisulphite (100ppm) and sodium benzoate (350ppm) (food preservatives) should be used as an antimicrobial agent, a flavouring agent and as adjuvant in the processing of orange juices in Nigerian markets

A safe microbial load in orange juices can be achieved by combination of various methods of processing, thus, chemical preservatives should be combined with pasteurization, concentrated and carbonated under controlled microbiological environments.

The Standard Organization of Nigeria and NAFDAC should define more specifically the quality control standards of locally manufactured commercial orange juices, stating clearly the minimum / tolerable numbers and types of microorganisms.

Good agricultural manufacturing practices and application of hot water and chemicals sanitizers such as chlorine dioxide, ozone and peracetic acid should be used on surfaces of orange fruits prior to juice extraction in order to avoid and reduce microbial contaminants of fruit products.

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Appendix I: World Citrus – production (1989-1991)

*All values are expressed in 1000 x tonnes

Location	Total	Oranges	Mandarins	Lemons/ Limes	Grape fruits
World	69,967.9	48,568.4	8,580.9	6,404.7	4,413.9
Northern hemisphere	47,155.5	30,835.1	7,510.1	4,916.5	3,893.9
United States	9,987.6	7,163.0	262.7	638.1	1,923.9
Mediterranean region	17,297.7	10,807.6	3,301.8	2,476.7	711.6
Greece	1,186.8	875.7	74.9	229.3	-
Italy	3,233.3	2,025.0	509.0	691.0	8.3
Spain	4,862.8	2,613.4	1,519.9	708.0	21.5
Israel	1,279.5	720.7	125.8	39.0	394.1
Algeria	298.6	185.0	108.0	3.1	2.6
Morocco	1,251.8	944.8	285.1	15.0	7.0
Tunisia	276.7	172.6	36.6	15.6	52.0
Cyprus	313.0	144.6	-	55.5	103.0
Egypt	1,887.7	1,498.7	175.0	212.0	-
Lebanon	447.8	279.1	-	-	-
Turkey	1,492.5	804.1	328.5	320.0	39.5
Former USSR	178.0	178.0	-	-	-
Japan	2,423.0	239.5	2,181.5	-	-
Cuba	1,020.0	520.0	30.0	70.0	400.0
Mexico	3,303.5	2300.0	183.5	711.0	109.0
China	5,511.4	4,711.9	385.0	149.5	265.0
Southern Hemisphere	20,128.8	17,733.3	1,070.8	1,488.2	520.1
Argentina	1,585.0	750.0	245.0	405.0	185.0
Brazil	14,300.0	13,050.0	581.0	644.0	25.0
Uruguay	193.8	83.5	49.5	53.0	-
Venezuela	427.0	427.0	-	-	-
Australia	629.5	519.5	-	35.0	31.0
South Africa	874.0	691.0	-	60.0	123.0
Chile	-	-	-	75.0	-

Source: FAO (1991)

Appendix II: Biochemical characteristics of yeast isolates from orange juice (laboratory produced)

Test results of isolates of yeast (Codes)

Fermentation of Sugars	FRHW	FRHP	CAB_1P	CAB_2W	CAB_3W	$COND_1W$	$COND_2W$	CHM_1P	CHM_2W	$PASD_1P$	$PASD_2W$	CHM_3W
Glucose	+	-	-	+	+	+	+	-	+	-	+	+
Galactose	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	-	-	-	+	+	-	-	+	-	+	+
Sucrose	-	-	-	-	-	+	-	-	-	-	+	+
Lactose	±	±	±	±	±	±	±	±	±	±	±	±
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis of Urea	-	+	+	-	-	-	-	+	-	+	-	-

Key:

+ = Positive result
- = Negative result
± = Doubtful result

Appendix III: Taste panel score sheet, orange juice

Date: ……. Sample code ……..

Please score by putting a tick ( ) in the appropriate box, the impression which you think best describes the sample.

Quality parameters	Descriptive terms for different score				
	1	2	3	4	5
Colour: orange	Dark orange	Light orange	Pale orange	Orange	Orange/yellow
Appearance: Viscosity	Nil	Slight	Moderate	Thick	Very thick
Odour: Orange odour	Nil	Slight	Moderate	Strong	Very strong
Flavour: General	Nil	Slightly sweet	Moderately sweet	Very sweet	Extremely sweet
Personal preference (Acceptability)	Bad	Poor	Average	Good	Excellent

Comment on sample

_____ _____

Name of panelist (please print) signature