بسم الله الرحمن الرحيم

PHYSICOCHEMICAL PROPERTIES AND QUALITY CHANGES OF GRAPEFRUIT (*Citrus paradise. Macf*) AND PUMMELO (*Citrus grandis (l.)*) CONCENTRATES DURING STORAGE

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Dedication

To *my great father & to my wonderful mother.*

To *my family.*

..... ***To*** *all whom I love.*

Acknowledgements

My *first thanks is to great Allah who gave me the patience and health to success this work.*

Secondly *I would like to express my sincere appreciation and thanks to professor* **Abdelazim Ahmed Mohamed Nour,** *for his supervision encouragement, precious care and insights.*

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ABSTRACT

Concentrated citrus juice had been made from two citrus species pummelo and grapefruit for the purpose of studying quality changes and shelf life in different storage conditions. The study intended to estimate the effect of adding citric acid and a high amount of sugar to concentrate juice.

Samples were purchased from central market of Khartoum and kept stored in polyethylene bags at room temperature until used and then washed and peeled using a sharp knife removing the outer peel.

Juice extraction had been done by hand squeezing after removing the seeds. Commercial sugar e.g. Kennana Sugar (1250 gm) had been added to raise the total soluble solids while powdered citric acid had been added to maintain the bitterness of the juice.

Juice had been packed in plastic bottles and stored for three months at refrigerator and room temperatures. Parameters for study included total soluble solids, pH, acidity and Vit. C content. The similar chemical structure of the two species didn't show a great difference in the results.

The results showed that shelf life of the concentrated juice was only one week at room temperature storage while refrigerator storage expired after two months.

The effect of different storage temperatures showed a loss in nutrient content such as degradation of Vit. C and deterioration of Total soluble solids and also color changing was observed. Citric acid played a good role in maintaining the bitter taste, but didn't affect the quality of juice, and also kept the acidity low for the first 15 days. Results didn't show a significant difference between the two species. Microbial activity was highly effected by the acidity and temperature.

خلاصة الأطروحة

استخدمت عينتين مختلفتين من صنف الموالح، القريب فروت والباملو لصناعة مركزات عصير من كل. تمت دراسة تأثير إضافة حمض ألستريك مع تركيز عالي من السكر في الحفاظ على جودة العصير وقياس صلاحية الاستهلاك بعد التخزين.

تم شراء العينات من السوق المركزي الخرطوم، غسلت العينات ثم تم تقشيرها باستخدام آلة حادة (سكين) لإزالة القشرة الخارجية فقط مع الحفاظ على بقاء القشرة الداخلية البيضاء، ثم تم عصر العينات عصر يدوي بعد إزالة البذرة من الثمار. تمت معاملة العصير معاملة حرارية بعد إضافة السكر التجاري 1250جم و1.5 جرام من حمض ألستريك بدون إضافة الماء أو أي لون أو نكهات صناعية

عبأ العصير على قوارير بلاستيك شفافة وخزن لمدة أقصاها ثلاثة أشهر على درجة حرارة الغرفة ودرجة حرارة الثلاجة.

تم قياس كل من نسبة المواد الصلبة الذائبة، الأس الهيدروجيني، نسبة الحموضة وحمض الاسكوربيك (Vit. C) كل 15 يوم من بدء التجربة. التشابه الكبير في التركيب الكيميائي للعينتين لم يظهر تباين واضح في القراءات.

أشارت النتائج إلي أن فترة صلاحية العصير لم تتجاوز الأسبوع عند التخزين في حرارة الغرفة بينما امتدت تلك الفترة إلي شهرين عند التخزين في درجة حرارة الثلاجة.

دراسة تأثير فترات التخزين مع تباين درجات الحرارة بين غرفة وثلاجة أوضحت فقدان في المحتويات الغذائية مثل حمض الاسكوربيك وتدهور في نسبة المواد الصلبة الذائبة كما لوحظ تغير واضح في اللون إلي اللون البني الغامق في التخزين في حرارة الغرفة مع درجة أقل من التغير عند درجة حرارة الثلاجة.

كان لحمض ألستريك أثر في الحفاظ على الطعم القابض للعصير ولم يكن له أي دور في الحفاظ على جودة العصير. كما كان له الأثر في ثبات نسبة التدهور في الحمضية بعد الـ15 يوم الأولي من التخزين إذ لم يكن هنالك تباين واضح في القراءات.

كان لارتفاع درجة الحرارة مع نسبة الحموضة أثر واضح في النشاط الميكروبي عند التخزين في درجة حرارة الغرفة.

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CHAPTER ONE

INTRODUCTION

Citrus is a common term and genus of flowering plants in the family Rutaceae, originally in tropical and subtropical Southeast Asia. The taxonomy of the genus is complex and the precise number of natural species is unclear as many of the named species are clonally-propagated hybrids and there is a genetic evidence that even the wild, true-breeding species are of hybrid origin. (Wikipedia, 2001).

The genus is commercially important as many species are cultivated for their fruit which is eaten fresh or pressed for juice, this is due to that citrus fruits are notable for their fragrance, which is partly due to presence of terpenes contained in the rind and most are juice laden. The juice contains a high amount of citric acid giving them their characteristics sharp taste and flavor; they are also a good source of vitamin C and flavonoids.

The popularity of citrus juice is certainly due to it's pleasant and refreshing flavor plus consumers know that they get the nutritional benefits from Vitamin C, folic acid and the dietary fiber in one serving, but the quick changes and degradation on the chemical and physical characteristics of the juice which is reflected on the taste, flavor and appearance need to be treated carefully with differ technological processes.(Johnson .M., 2006)

Recently food technologists applied new procedures for the commercial production of food industry with an advantage that makes the product offered in a suitable economical package size, which maintains foods nutritional benefits. These products are long shelf-life in suitable

storage conditions and low in microbial load and that is due to the technical sanitation procedures.

On general fruits are rich sources of vitamins and minerals and they can be used in an extensive way in food industry. They are ideal for use in recipes where the color, intense flavor and appeal of real fruit are needed, some of their application can be recognized in Bakery (pastry and cookies fillings, to add taste, color and natural flavor), dairy production (juices, drinks sorbets, ice cream, frozen novelties and desserts), sauce salad dressings, jams and jellies, confectionaries (gummies and hard candies) and beverage drinks, smoothies and juices.

A fruit juice is the natural sugar solution from a fruit that has been extracted/pressed and filtered from the skin pulp and seeds of that fruit and also the juice solution usually contain the majority of the acid and color content of the fruit.

A fruit juice concentrate is the single strength (S/S)sugar/acid/color solution depectinized, filtered and evaporated under vacuum to a specified brix level, usually folded 2 to7 times (x's) of the fruit's original S/S brix value. Concentration is done for case-of-use and economical storage and transport (University of Florida citrus department ,1998).

Generally the commercial process for citrus juice must be done by special mechanical techniques and several preparation steps which starts from fruit receiving from the market. For example the fruit is sampled during the process operation to determine maturity level and juice content. The sugar and acid level are also determined and often used as basis for fruit buying and for selective blending of loads to achieve specific quality parameters such as brix, a measure of sugar content and acid ratio. And they ultimately establish product quality standards

together with flavor and color, but today the taste is the concentration unit for the citrus industry.

Objective of the study:

1. To study the effect of the concentration procedure on juice quality.
2. To estimate the storage shelf life of the juice

CHAPTER TWO

LITERATURE REVIEW

2.1 Plant and fruit:

2.1.1 Scientific and common names:

The grapefruit is a subtropical citrus tree of the family Rutacae grown for its fruit, which is also known as grapefruit. Grapefruit got its name because it commonly grows in clusters on the tree just like grapes. The grapefruit was originally believed to be a spontaneous sport of the pummello. James Macfayden, in his Flora of Jamaica, in 1837, separated the grapefruit from the pummello, giving it the botanical name Citrus paradise *Citrus paradise. Macf.* And in about 1948, Citrus specialists began to suggest that the grapefruit was not a sport of the pummello but an accidental hybrid between the pummello and the orange (*Citrus maxima* x *Citrus sinensis*). The botanical name has been altered to reflect this view, and it is now generally accepted as Citrus x paradise (Morton, 1987).

The pummelo fruit scientifically named *Citrus maxima* (Merr. Burm. F.), also Citrus grandis (L.) Osbek is a citrus species which is considered to be one of three major, original citrus species

2.2 General description and origin:

Grapefruit is a medium to large size citrus fruit. It is larger than most oranges and the fruit may be flattened at both. The skin is mostly yellow but may include shades of green, white or pink. Skin color is not a sign of ripeness. But the pummelos are pear-shaped thick rind varieties of the same species as the grapefruit (Figures 1, 2, 3 and 4), grown mostly in the orient under the name of shaddock.(Winton. L.A., 1961).

Fig. (1): Pummelo Whole fruit

Fig. (2): Pummelo after being cut

Fig. (3) Pummelo after being sectioned

Fig. (4) Flesh of the pummelo

2.2.1 Culture and climate:

In general, the agricultural practice on growing the trees of grapefruit is similar to that of orange, except wider spacing is necessary.

These fruits grow well in a warm subtropical climate. Temperature differences affect the length of time from flowering to fruit maturity. Humidity contributes to thinness of peel, while in arid climates the peel is thicker and rougher and the juice content is lower (Morton 1987).

2.2.2 Food uses:

These fruits are similarly the same in their uses but the grapefruit might be specialized in some uses. As a relatively new food, the grapefruit has made great advances. In 1970, consumption of grapefruit was temporarily lightened by a widely promoted "grapefruit diet" plan claimed to achieve a loss of 10 lbs (4.5 kg) in 10 days and continuous gradual loss until the achievement of normal body weight. In 1983, the Institute of Food and Agricultural Science reported that grapefruit has high vitamin C content and is therefore valuable to the immune system and it helps protect against colds flue and it has a very positive effect on obesity and also has diuretic properties, helping to remove excess water from the body.(Morton 1987)

It has an uplifting effect on the mood and helps with stress and depression. Grapefruit is customarily a breakfast fruit, chilled and cut into halves. The sections can be loosened from the peal and from each other by a curved knife and "half-shell". Some consumers sweeten it with sugar or honey and it can be used as an appetizer before dinner, grapefruit halves may be similarly sweetened, lightly boiled and served hot, often topped with a maraschino cheery. The sections are commonly used in fruit cups of fruit salads, in gelatins or pudding and tarts. They are

commercially canned in syrup. In Australia grapefruit is processed as marmalade. It may also be made into jelly. (Morton. 1987)

Grapefruit juice is marketed as a beverage fresh, canned or dehydrated as powder or concentrated and frozen. The juice can be made into excellent vinegar or carefully fermented as wine. Grapefruit peel is candied and is an important source of pectin for the preservation of other fruits. The peel oil, expressed or distilled is commonly employed in soft drink flavoring after removal of 50% of the monoterpens. The main ingredient in the outer peel oil is nookatone; the nookatone can be added to grapefruit juice powder to enhance the flavor of the reconstituted juice. Naringin, extracted from the inner peel (albedo) is used as a bitter taste in "tonic" beverages, and chocolates and ice cream. It is chemically converted into a sweetener about 1.5 times sweeter than sugar. After the extraction of naringine the albedo can be reprocessed to prepare pectin. The peel of the pummelo is also used in Chinese cooking or candied. In general citrus peel is often used in southern Chinese cuisine for flavoring, especially in sweet soup deserts. (Morton, 1987)

Grapefruit seed oil:

It is dark in color and bitter in taste. When refined it has a pale-yellow, bland much like olive oil in flavor, and can be used similarly. It is a saturated fat hence its production has greatly increased since 1966 (Morton, 1987).

2.3 Nutrition value and nutrition facts:

2.3.1 Nutritive values of fruit juices:

2.3.1.1. Carbohydrate:

Most fruit juices are high in sugars as they contain large amounts of dextrose and laevulose and in many cases sucrose as well. Grape juice is especially high in sugars. Concord juice oridinally contains 16 to 17% sugar; California grape juices are usually considerably higher than this. Passion fruit juice is also very high in sugar. Other juices relatively high in sugar are the apple (Lopez *et al*., 1958; Powrie and Asselbergs, 1956), appicot, orange, pineapples, and prune. Sugars are important foods, being easily digested and yielding energy.

2.3.1.2. Protein:

Most of the common fruits are low in protein. A considerable proportion of the protein content of fruits is insoluble and consequently remains in the pomace ; therefore most fruit juices are very low in protein.

2.3.1.3 Fat:

Nearly all of the fruit juices are very low in fat. Because of the small amount of fat found in fruits their juices are very low in fat.

2.3.1.4 Vitamins:

Most fruit juices are excellent sources of vitamin C, several are good sources of carotene and many contain moderate amounts of pyridoxine, inositol, folic acid and biotin.(Tressler, 1961). Composition of fruit juices in comparison of grapefruit juice are seen in table 2.

Table 1: Nutritive value of grapefruit juice unsweetened

Nutrient values/100g.

Protein	0.4g./ 100g.
Fat	0.2g. /100g.
Fiber	trace-0g /100g.
Vit . C (ascorbic acid)	34.0mg /100g
Moisture	89.2-90.4g. /100g.
Carbohydrate	9g. / 100g.
Sugar total	9g. /100g.
Lactose	0g. /100g.
Fructose	3.8g. /100g.

National Public health Institute of Finland (2003-2006)

2.3.1.5 Ascorbic acid (vitamin C)

All fruits contain more or less of the vitamin C. Most of them are good sources and several others are very rich in the vitamin. Fruits especially high in vitamin C, includes gooseberries, grapefruit, lemons, citrus fruit, red and black currants, guavas and strawberries. This vitamin acts as a scavenger to harmful elements in the body. It is one of the most powerful antioxidants, vitamin C neutralizes free radicals (harmful elements naturally occurring in the body and environment factors) It helps the cells and tissue against damage that could lead to disease, including cancer and heart diseases. Vitamin C also helps the body to fight infections (Block, 1991).

2.3.2 Nutrition facts about grapefruit and grapefruit juice:

Consuming citrus fruit e.g. grapefruit and grapefruit juice can help boost the absorption of non-heme iron (the iron found in plant not meat products). This means that if you drink a glass of grapefruit juice before eating a meal rich in iron for example a spinach salad the body absorbs two or four times as such iron (Rampersaud, 2002).

One half of a piece of grapefruit has more dietary fiber than many other popular fruits including, bananas, apples and strawberries. In recent

years, low fat diets high in fruits and vegetables containing dietary fiber have been shown to a variety of health benefits, especially in the reduction of risk of cardiovascular disease and cancer (Rampersaud, 2002).

One grapefruit can provide more than 188 mg potassium which meat the daily requirement, and also provide the necessary daily requirement for vitamin C, which is important to support a healthy immune system. The high level of pectin and fiber found in citrus fruits like grapefruit may help to maintain health cholesterol levels (Rampersaud, 2002).

2.4 Juice production:

Many fruits are eaten fresh, but high demand for fruit juice has increased (Galaszewiski *et al*., 1998). The suitability of fruits for juice production is greatly affected by cultivars, maturity and growing conditions (Robert, 1974).

The word juice can be defined as a fluid naturally contained in animal or plant tissue for example Grapefruit juice is the liquid extract of the fruit of grapefruit. Juice may be supplied in a concentrate form, where consumer adds water to reconstitute the liquid back to approximately the original state.

A concentrate is a form of substance where the majority of its base component in a liquid state is removed typically this will be the removal of water from fruit juice. The importance of producing a concentrate is the reduction of weight, and volume. The concentrate can be reconstituted at the time of usage by the addition of the water.

Table (2) Composition of fruit juices:*

Name	Constituents of 100 grams proximate composition							Minerals					Vitamin				
	Caloris	Water Gm.	Protein Gm	Fat Gm.	Ash Gm	Total carbo-hydrates Gm	Crude fiber Gm	Calciu m mg	Phosph orus mg	Iron mg	Sodium mg	Potassi um mg	A.I.U	B_1 mg	B_2 mg	Nicotin ic acid mg	C, mg
Apple juice, frozen canned	50	85.9	0.1		0.3	13.8			10	0.5	4.0	100	40	0.02	0.03	Tr.	
Apricot nectar	52	86.1	0.3	0.1	0.5	12.4	0.2		13	0.3	2.9	98	1090	Tr.	0.01	Tr.	
Grape juice, cad, sw.	67	81.0	0.4		0.4	18.2		10	10	0.3	1.0	120		0.04	0.05	0.2	Tr.
Grapefruit juice, canned, sweet	52	85.3	0.5	0.1	0.4	13.7	0.1		13	0.3	0.4	150	Tr.	0.03	0.02	0.2	35
Lemon juice, end.	24	91.4	0.4	0.2	0.3	7.7		14	11	0.1	1.1			0.04	Tr..	0.1	42
Lime juice, fresh	24	91.0	0.4		0.3	8.3		14	11	0.1		176		0.04	Tr.	0.1	28
Orange juice, fresh	44	87.5	0.8	0.2	0.4	11.0	0.1	19	16	0.2	3.6	182	190	0.08	0.03	0.2	40
Orange juice, end.	44	87.5	0.8	0.2	0.4	11.1	0.1	10	18	0.3	0.5	190	100	0.08	0.02	0.2	42
Orange and grapefruit juice, end, sw.	52	85.1	0.5	0.1	0.4	13.9	0.1		15	0.3	0.4	170	40	0.07	0.02	0.2	38
Pineapple juice, end	49	86.2	0.3	0.1	0.4	13.0	0.1	15		0.5	0.5	140	80	0.05	0.02	0.2	
Prune juice, end	71	80.0	0.4		0.3	19.3		25	40	1.8	2.0	260		0.03	0.08	0.4	
Tangerine juice, end.	39	89.2	0.9	0.23	0.4	9.2		19	16	0.2	0.6	170	420	0.06	0.03	0.2	26
Tomato juice, end.	21	93.5	1.0	0.2	1.0	4.3	0.2		15	0.4	230	230	1050	0.06	0.03	0.8	16

*From the Henz Handbook of Nutrition Burton, Ediotor 1995 Black Div. McGraw. Hill book Co.

Ashuirst *et al*. (1998) reported that juice processing technology in general could be in the following steps: fruits are collected, sorted and washed and them subjected to a mechanical compression that is relevant to type of concerned. And in some fruits addition of degrading enzymes to breakdown the cellular structure and obtain best yields from certain fruits. If juice is not to be sold as (not-from concentrate), it is usually screened and pasteurized immediately after pressing, on operation with two main objectives; first is to control inborn microbial spoilage (mostly yeast and molds). The second is to destroy the pectolytic enzymes that occur naturally in fruit, which could breakdown the cloudy nature of the juice.

2.4.1 Citrus juices:

Citrus juice are rich sources of vitamins and minerals especially vitamin. C they also contain citric acid, which gives them their characteristic sharp taste. Other chemical components, which play an important role in that physical and chemical characteristic and this can be reflected noticeably in the degradation and spoilage which occurs during their storage period or the time between production and consumption. Fortunately, relatively little ascorbic acid is lost during preparation and canning of citrus juices.

(Wanger *et al*. 1954) made a study of grapefruit canning operations In 12 canneries of Rio Grande Valley of Texas and found that the retention of this vitamin during the canning operation ranged from 92.2 to 99.6% with an average of 97.6% for all plants. (Moore *et al*.1944) and (Ross. 1944) found similar loss of ascorbic acid occurs during canning Florida grapefruit juice noting an average retention of 97%.(Krehl and Cowgill. 1950) noted an average drop from 55. 8 to 53.9 mg of ascorbic

acid per 100ml. Of Florida Valencia juice during canning, a loss of only 3.4%.

The reason for relative stability of the ascorbic acid in citrus juices has been the object of extensive researches by (Inagaki 1943, 1944 and 1947). This investigator has found many substances in natural fruit juices which retard oxidation of ascorbic acid, including the following: meta phosphoric acids, saturated fatty acids some aromatic carboxylic acids, mineral acids, certain poly basic acids, certain monobasic acid, hydroxyl acids and chlorine substituted acids, hesperdin, naringin, pectin, hespertin, harningenin, thiamin, vitamin B_6 and beta-carotene.

Neither canning (heat processing) nor freezing of citrus juice cause the destruction of appreciable amount of biotin, folic acid, pyridoxine and inositol.

On the other hand, canned fruit juices lose considerable amounts of ascorbic acid and thiamin during long storage. The higher the storage temperature the more rapid the loss. Moore (1949) reported that canned orange juice stored for 18 months at 40^{o}F, retained 93.2% and bottled juice 89.2% of the original ascorbic acid content, but when this juice was stored for this period at an average temperature of 76^{o}F, the canned juice retained only 59.8% and the bottled product only 50.9% of this vitamin. Similar results were obtained in a storage test of grapefruit juice.

2.4.2 Citrus juice concentrates:

DuBois and Kew (1951), Huggart *et al*. (1954) and McColloch *et al*. (1957) noted only very small losses of ascorbic acid during the storage of frozen concentrates. Anderson and Fagerson (1952) and Anderson *et al*. (1953) determined the ascorbic acid content of a large number of reconstituted samples of orange juice. The lowest sample contained 28.7

mg and the highest 51.5 mg ascorbic acid per 100ml reconstituted juice. However, 154 of the 20 brands examined average over 40 mg ascorbic acid per 100 ml. The average ascorbic acid value of 48 cans of frozen grapefruit after constitution was 25.1 mg/100ml. Reconstitution frozen tangerine juice average 25.9 mg ascorbic acid per 100ml.

2.4.3 Degradation of Ascorbic acid (vit. C) in citrus juice concentrates during storage:

Ascorbic acid (Vit. C) is an important component of our nutrition and used as an additive in many foods because of its antioxidant capacity. Thus it increases quality and technological properties of food as well as nutritional value (Larisch *et al*., 1998 and Solmon *et al*., 1995). However, vitamin C is an unstable compound and under less desirable conditions it decomposes easily (Fennema, 1997 and Lee and Coates, 1999).

The loss of some nutrients such as Ascorbic Acid (Vit. C) might be a critical factor for the shelf-life of some products such as citrus juice concentrates (Laing, Schlueter and Labuza, 1978) since vitamin C content of citrus juices undergoes destruction during storage. (Johnson, *et al.* 1995; Lee and Negy, 1998a and Solomo, *et al*. 1995).

Degradation of Ascorbic acid proceeds both aerobic and anaerobic pathways (Huelin, 1953 and Johnson *et al*., 1995) and depends on many factors such as oxygen, heat, light (Roberton and Samaniego, 1986), storage temperature and storage time (Fellers, 1988 and Gordon *et al* 1990). Oxidation of ascorbic acid occurs mainly during the processing of citrus juices (Helin, 1953), whereas anaerobic degradation of Ascorbic acid mainly appears during storage (Johnson, *et al*., 1995; Lee and Nagy, 1988 and Solomon *et al*., 1995), which is especially observed in thermally preserved citrus juice. It was reported that several decomposition relative occur via the degradation of vitamin C (Eskin, 1990 and Huelin, Coggiola, *et al.*, 1971) and these compounds may

combine with amino acids, thus result in formation of brown pigments (Clegg, 1964 and Larisch *et al*., 1998). Hydroxy methyl furfural (HMF) is one of he decomposition products of Ascorbic acid (Eskin, 1990 and Solomon *et al*., 1995) and suggested that a precursor of brown pigments.

It is used to evaluate severity of heating applied to fruit juices during processing and taken into account for quality control (Lee and Nagy, 1988b). Other pathways of HMF accumulation are known as degradation of reducing sugars (Ibarz, *et al*, 1999 and Lee and Nagy, 1988b) and Millaird reaction .

Table (3): Vitamin C degradation in citrus juice concentrates during storage (mg/100g).

Variety	Temp.	Storage week								
		0	1	2	3	4	5	6	7	8
Orange	28	232.9	224.1	226.4	214.0	214.0	210.0	207.0	189.9	194.9
	37	232.9	208.0	196.6	138.9	121.5	106.2	91.3	69.0	52.4
	45	323.9	198.8	153.4	95.2	72.9	56.3	39.3	38.4	39.3
Lemon	28	225.0	198.8	188.8	173.5	166.9	163.8	148.1	148.1	122.8
	37	225.0	188.3	153.4	118.8	80.4	73.4	101.8	101.8	54.6
	45	225.0	191.4	152.9	109.2	112.7	80.4	65.9	65.9	45.0
Grapefruit	28	205.8	194.0	184.4	164	160.0	159.1	159.1	155.0	144.0
	37	205.8	180.0	136.8	119.9	108.3	95.7	82.1	82.1	55.5
	45	205.8	152.0	115.8	90.9	71.2	44.1	41.9	41.9	31.4
Tangerine	28	97.9	95.3	80.4	80.9	81.5	73.0	77.0	77.0	65.0
	37	97.9	88.7	68.6	60.3	55.0	34.1	38.5	38.5	23.1
	45	97.9	68.6	51.5	40.6	30.1	24.0	18.0	13.9	14.8

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2.4.4 Browning in grapefruit juice:

Browning reactions in food are widespread phenomena that take place during processing and storage (Eskin, 1990), these reactions involve carmalization, ascorbic acid degradation and milliard reaction (Gazzani *et al*., 1987), it may be desirable in baked fried or roasted foods (Ashoor and Zent, 1984) as in the manufacture of coffee, tea, bear, bread and cake (Martin *et al.*, 2001). However, non-enzymatic browning reactions are often responsible for important quality changes that occur during the storage of foods, limiting their shelf-life (Burea *et al*., 1987).

For instance, browning is the most common quality problem of many concentrated fruit juices (Toribo and Lozano, 1984) and cause loss of nutrients and the formation of intermediate undesirable compounds, like furfural and 5-hydroxymethyl furfural (Duedoet *et al*., 2001). In citrus juices non-enzymatic browning is due to the reactions of sugars, amino acids and ascorbic acid (Johnson *et al*., 1995). However, the decomposition of ascorbic acid is reported to the major deteriorative reaction during the storage of orange juice (Solomon *et al*., 1995). Lee and Nagy (1988) also reported a high correlation between the percentage loss of ascorbic acid and increase in browning in grapefruit juices. On the other hand, sugar-amino acid reactions of the classical milliard type are of minor importance in citrus juice browning because of the high acidity (pH 2.0 – 4.0) (Clegg, 1964).

Russell, *et al*. (1989) mentioned that under non-refrigerated storage conditions processed grapefruit juice darkens with increasing storage time due to the formation of browning pigments. This non-enzymatic browning has been a visual defect of processed grapefruit juice for many years and is one of the factors determining shelf-life. Browning in grapefruit juices is accompanied by an increase in off-flavours, which also limits shelf-life.

2.5 Grapefruit chemical composition:

The processing of grapefruit to yield grapefruit juice is complicated by several factors, which affect the bitterness of final product (Burdick, 1961; Hagen *et al*., 1966 and Maier, 1969). It would be advantageous for the processor to have the capability of controlling the final product bitterness without waste or adverse effect on other desirable properties of the juice. The control of excessive bitterness in grapefruit products has

been reviewed by many researchers (Chatidler and Nicol, 1975; Danlop *et al*., 1962; Goldstein *et al*., 1971; Ono *et al*., 1977 and Maier *et al*., 1977).

Naringin is the major flavonoid glycoside in grapefruit and gives grapefruit juice its bitter taste. It is metabolized to the flavonone naringining in humans. Both naringin and hesperetin, which are the aglyconens of naringin and hesperiting, occur in citrus fruits. Naringin is present in some juices in the rang of 700 – 800 ppm, amounts definitely affected to be responsible for making the juice bitter (Wikipedia, 2001).

2.5.1 Naringin activity:

Naringin exerts a variety of pharmacological effects such as antioxidant activity, blood lipid, lowering, anti-carcinogenic activity and inhibition of selected cytochrome P450 enzymes including CYP3A4 and CY1A2, which may result in several drug interactions *in vitro* (Alferdd, *et al* (1979).

2.5.2 Grapefruit interactions with drugs:

Grapefruit can have a number of interactions with drugs, often increasing the effective potency of compounds. Grapefruit contains naringin and berga mottin, which the cytochrome P450 isoform CYP3A4 in the lier. It is via inhibition of this enzyume that grapefruit increases the effects of buspirone (Buspar) caffeine, simvastation, terfenadine, felodiine, verapamil ostradiol, midazolam, tacrolimus, dextromethorphen (significant only at recreational doses). This effect was only discovered after being responsible for a number of death due to overdosing in medication. (Wikipedia, 2001).

2.6 Limoniods and flavonoids in grapefruit juices:

Limoniods is a naturally occurring highly oxygenated compound in plants, including citrus. These compounds participate in the improvement of human health and nutrition. (Journal of Nutrition 2002),

Flavonoids are also naturally occurring compounds found in fruits and vegetables Citrus are a rich source of such as hesperidin, naringin, tangeretin narirutirin nobietin, many flavonoid component are known by antioxidant activity and many protect against a variation of health problems such as cancers, cardiovascular degenerative eye disease and damage cause by aging. (Journal of Nutrition 2002).

Oroblanco and melogold are hybrids obtained from pummello and grapefruit. Hsu *et al*. (1998) mentioned that limonoids and flavonoids in juices of melogold and oroblanco grapefruit hybrids were analyzed. The juices contained low concentrations of limonoid glucosides, an average of 99 and 59 ppm, respectively.

However, they contained relative high concentrations of bitter limonoid aglycones, limonin and nomilin, at levels about the limonoid bitterness threshold. For comparison limonoid glucosides in juices of grapefruit, another pummello hybrid, were also analyzed. Limonin glucoside was the major limonoid glucosides in all juices analyzed. Nomilin glucoside and nomilinic acid glucosides were also present. Oroblanco and melogold juices contained bitter flavonoids normally found in grapefruit and pummello including naringin, neohesperidin and poncerinin in total amount of 440 ppm in oroblanco juice and 495 ppm in melogold juice. They also contained several other non-bitter flavonoids found in grapefruit.

2.7 Aroma composition:

Lin *et al*. (2002) studied the aroma composition and changes of grapefruit juice produced from thermal concentration, they found differences in aroma components and total volatiles between a single unpasteurized grapefruit juice and its 65 Brix concentrate reconstituted to 10 Brix. Results showed that total volatiles in the reconstituted concentrate were reduced to less than 5% of initial values, but 75% of total aroma remained. Forty-one aroma-active compounds were observed in unpasteurized single strength juice, whereas 27 components were found in the unflavored reconstituted concentrate. Aroma-active compounds were classified into grapefruit/sulfurgy, sweet/fruity, fresh/citrusy, green/fatty/ metallic and cooked/meaty groups. Five of six components in the sweet/fruity and 14 of 18 green/fatty/metallic components served thermal concentration. However only 4-mercapto-4 methyl 1-2 pentanne in the grapefruit-sulfury group, and linalool and nootkatone from the fresh/citrusy group, were found in the reconstituted concentrate. Methional was the only aroma compound in the cooked/meaty category found in both juice types. Beta-Damascenone and 1-p-methen-s-thiol were found only in the reconstituted concentrate. 4-Marcapto-4-methyl 1-2-pentanol was found for the first time in grapefruit juice.

2.7.1 Odor-active volatiles:

Buettner A.& Schieberie P. (1999) reported that thirty-seven odor active compounds were detected from an extract prepared from fresh grapefruit juice and subsequently identified .Among them the highest odor activities were determined for ethyl butanoate, p-1-menthene-S-thiol, Z-3 hexal,4-5-eoxy-(E)-2decenal,4mercapato-4-methylpentane-2-one,1-heptane-3-one and wineacetone. Beside the 5 last mentioned

compounds a total of 13 further odorants were identified for the first time flavor constituents of grapefruit.

2.8 Juice shelf-life:

The shelf-life of fruit juices and concentrates is limited primarily by microbial, enzymatic and chemical reactions that adversely affect the nutritional quality, color and flavor. Pasteurization requirements of freshly extracted citrus juices are based on thermal inactivation enzymes. Whereas requirements for reconstituted juices are based on destruction of microbial populations capable of causing spoilage. Aseptic processing produces a higher quality juice than hot filling; however, differences in quality may disappear during storage at ambient temperatures (Bockelman *et al.,* 1977).

Oxygen dissolved in the product, in the container headspace or permeating through the container, accelerates the rate of ascorbic acid destruction and nonenzymatic browning and hence reduces shelf-life. The most important factor in determining the shelf-life of aseptic juice and concentrate is storage temperature .

Beal (1998) identified many factors that can affect the shelf-life of beverages (juices, milk, etc). Some of the more important factors that affect shelf-life are: raw materials, product formulation, processing, hygiene, packaging, storage and handling.

Preservation by chilling is normally limited to durations ranging from2 days at (3–5^{o}C) to 7 days at (0 to 3^{o}C). Most commercial reconstituted fruit juices are pasteurized and refrigerated at around 4^{o}C for 30 days. Pasteurization increases the product preservatability (Ciobnu *et al*., 1976).

2.9 Microbiology of juices:

Microorganisms associated with the soft drinks industry are usually confined to some yeast, acid-tolerant bacteria, a few non-acid-tolerant bacteria and a selection of molds. Of these the yeasts are the most important Dockelmann and Bockelmann (1998) reported that the main spoilage organisms in juices are yeast and molds, primarily lactobacillus and streptococcus. These can multiply at low pH values i.e. below 4.6 and hence able to affect the product. Yeast is the predominant organism in spoilage of acid foods because of their high acid tolerance and the ability to grow aerobically. Molds and lactic acid bacteria also play role in spoilage of fruit juices. Juices as fruit products or as ingredients have to be pasteurized or must receive an equivalent process that insures the production of juice free from pathogenic microorganisms. Previous washing of citrus fruits with chlorinated water is recommended to remove the soil and born organisms. If washing is done properly microbial population can be lowered.

Most fruit juices have high acidity and accordingly low pH values in the range of (5.0 - 2.5) being the most important factor in determining the types of microorganisms that can spoil fruit juices. For a longtime fruit juices were not recognized as food born illness because of their low pH and high organic acids levels. In the year 2001 FDA estimated 16000 to 48000 cases per year can be attributed to juices, most of it is due to consumption of unpasteurized juices.

Spoilage by microorganisms is limited to acid-tolerant organisms, which are predominantly lactic acid bacteria, yeast and molds. Lactic acid bacteria, are associated which spoilage problems during processing operations, and therefore were reported to have thermal death times. The thermal time sufficient to destroy most microbial population range from

0.1-0.3 min. in orange juice at 65°C (Juven, 1967; Murdock *et al*., 1953), while Kopelman and Schger (1976) gave them less than 1 min. at 60^{o}C.

Monitoring of fruit juice concentrates for the presence of heat-resistant molds was advised by Murolock and Hatcher (1976) since pasteurization treatments may not adequately control this type of spoilage. Some heat resistant mold species are quite capable of surviving pasteurization treatments even with low oxygen levels in pack.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Collection of samples:

Fruits:

Grapefruit and pummelo fruits were purchased from central market of Khartoum state. The origin of these fruits is " Elshamalia" the North state of Northern Sudan and both fruits are of the season (2006). The fruits were kept in polyethylene bags and stored at room temperature until used.

- Citric acid powder, reagent grade, one and a half gram citric acid have been added as a preservative.
- Commercial sugar represented as sucrose for e.g. Kenanna Sugar

3.2 Preparation of Samples:

Collected fruits were used for the preparation of a concentrated juice that may be later reconstituted by addition of water to the required dilution.

3.2.1 Preparation of concentrate:

The grapefruit and pummelo fruits were squeezed mechanically by using hand. The natural juice was sieved using a wire mesh to remove seeds and cellular materials. The juice was also filtered twice using a cloth filter to ensure removal of cellular material and result into clear natural juice.

The juice obtained was then evaporated to (45) brix at 100°C in a stainless steel open pan with addition of sugar (4.25pound sugar +3litter

juice) for 10-15 minutes the total soluble solids raised to (53-57) brix. And then one and half gram citric acid were added as a preservative and to maintain bitter taste, when the concentrate was relatively cool. Then stored on a deep freezer (temp. -18^{o}C) for 12 hours. After that the concentrated juice had been packed on plastic bottles and then distributed for storage at refrigerator and room ambient temperatures.

Assessment includes proximate composition, titratable acidity, vitamin C content, pH values, soluble solids (brix) and microbiological analysis (lactic acid bacteria–yeast and mold–total viable count).

3.3 Proximate composition:

Moisture, protein, fat, fiber and ash content: of grapefruit juice and pummelo juice were determined according to the AOAC (1995) procedures.

The carbohydrate content was determined by difference.

3.3.1 Moisture determination:

Ten ml of sample was measured in a clean crucible using sensitive balance. The crucible with the sample was placed in an air-dry oven at 105°C and left to stay over night. Then crucible was transferred to oven again and weighted after 2 hours, this was repeated until constant weight was obtained.

Calculation:

$$\text{Moisture Content\%} = \frac{(w_2-w_1)-(w_3-w_1)}{w_2-w_1} \times 100$$

where:

w_1 = weight of empty crucible

w_2 = weight of crucible + sample

w_3 = weight of crucible +dry sample

3.3.2 Determination of ash:

An empty crucible was accurately weighed, and then 10ml of sample were weighed in it using sensitive balance. The sample in crucible was placed in muffle furnace at 550°C for more than 3 hours until white to grey ash was obtained, then crucible was removed from furnace to a desiccators to cool, then weighed.

$$\text{Ash content \%} = \frac{w_2 - w_1}{W_3} \times 100$$

where :

w_1=weight of empty crucible

w_2=weight of crucible with ash.

w_3 =weight of sample

3.3.3 Crude protein:

Crude protein content was determined using Kjeldahl method and calculated by multiplying the amount of nitrogen by 6.25 .10ml of sample was weighed in kjeldahl flask, half a tablet of catalyst mixture (10 parts K_2So_4 to one part of $CuSo_4$) and 25 ml of concentrated H_2SO_4 were added. The ash content of the flask was digested under boiling at maximum heat for 2-3 hours till clear, and then the flask was distilled using NaOH 40% ,the ammonia was received in 100ml conical flask containing 10ml of 0.1NHCl and crude protein percentage was calculated as follows:

$$\text{Crude Protein \%} = \frac{N \times T \times .10ml \times 14 \times 100 \times 6.2}{1000}$$

Were:

N= Normality of HCl for sample titration.

T=Titration figure.

10ml= weight of sample.

1000:Number of milligrams in one gram.

14:Equivalent weight of nitrogen.

6.25: Protein conversion factor.

3.3.4 Fat Content:

The fat content was determined using Soxhelt method .10ml of sample was placed in a thimble, the thimble was covered with cotton wool .An empty dry and clean round flask with a known weight was connected to the siphoning apparatus and heater was switched onto start extraction. After the extraction was completed, the solvent was evaporated from the round flask .The round flask containing the extracted fat was weighed and fat content was calculated according to this equation:

$$\text{Fat Content \%} = \frac{w_2 - w_1}{w_3} \times 100$$

where:

w_1=weight of empty flask

w_2= weight of flask and oil

w_3 =original weight of sample

3.3.5 Crude fiber:

Determination of crude fiber content was achieved using glacial acetic acid and nitric acid 10:1 solution on 10 ml .

3.4 Titratable acidity:

Five grams of concentrated juice were taken in a 50 ml beaker diluted by adding 100 ml distilled water and 10 ml of the dilution was used as a sample. Phenolpthalein was added as an indicator. The sample was titrated carefully with 0.1 N NaOH and titratable acidity was determined as citric acid by the following formula:

$$\text{Acid (\%)} = \frac{\text{(ml. of NaOH) (N. NaOH) (dil. factor) (equ.wt. of acid)}}{\text{1000 (wt. of sample g)}} \times 100$$

$$\text{Dilution factor} = \frac{\text{Sample total volume}}{\text{Sample volume used in titration (10 ml)}}$$

3.5 Vitamin C content:

Thirty grams of the sample blended with reasonable amount of 0.4% oxalic acid. (4g/liter) and filtered by Whatman (No.1) filter paper. The ample volume completed to 250 ml with 0.4 oxalic acid. Twenty ml of filterate pipeatted into a conical flask and titrated with a known strength 2-6-dichlorophenol indophenol until a faint pink color appeared. The dye strength determined by taking 5 ml oxalic acid 10% (50mg/00ml) and added to a standard ascorbic acid (0.05/250ml) oxalic acid 10% titrated with 2-6-dichloerophenol indophenol (0.2g/500ml) till faint pink color expressed in mg/100g.

Determination of dye strength:

Add 5 ml of solution in a beaker and titrate with dye solution to faint pink color.

$$\text{Dye strength} = \frac{1}{\text{titer}}$$

Calculation:

$$\text{Ascorbic acid} = \frac{\text{Titer (ml) X dye strength X 100}}{\text{Factor}}$$

$$\text{Factor} = \frac{\text{Sample (wt.) X sample volume for titration (20 ml)}}{\text{Total volume sample}}$$

3.6 pH measurement:

The pH was determined by immersing pH electrode in the juice placed in a 50 ml beaker, sufficient amount of juice covered the tips of the electrode of pH meter device consort P407, Schoft Gerate, Belgium.

3.7 Total Soluble solids (brix):

Soluble solids were determined at 20^{o}C using a hand refactometer, (Vippon Optical Work Co., Ltd., Tokyo).

3.8 Microbiological analysis:

3.8.1 Preparation of serial dilutions:

Ten milligrams of each sample were added to a conical flask containing 90 ml of sterile 0.1% peptone water and shaking well to give a dilution (10^{-1}). One ml of dilution (10^{-1}) was transferred by using sterilized pipettes to a test tube containing 9 ml of sterile dilute to give (10^{-2}). The serial decimal dilution up to (10^{-6}) were prepared as described by Harrigan (1976).

3.8.2Total viable count:

Total viable count was carried out using the pour-plate method as described by Harrigan (1976). One ml of each dilution was transferred aseptically into sterile Petri dishes to each dilution 15 ml of melted and cooled (45^{o}C) plate count agar was added. The inoculums was mixed with medium and allowed to solidify. The plates were incubated at 37^{o}C for 48 hrs. by using a colony counter the viable bacterial colonies were counted. The result was expressed as colony forming units (c.f.u.) per serum.

3.8.3 Mold and yeast:

From suitable dilutions of sample 0.1 ml was aseptically transferred onto solidified potato dextrin agar (PDA). The sample was spread all over the plates using sterile bent glass rod. Plates were then incubated at 28^{o}C for 48 – 72 hours.

3.8.4 Lactic acid bacterial count:

MRS media was used for counting lactic acid bacteria by using sterile pipette 0.1 ml from every dilution was transferred to a Petri dish containing solidified MRS medium and it was spread by using sterile (L) shape glass rod on the surface of the solid media.

Anaerobic system was used for incubation in an anaerobic jar using gas-generating pits at 37^{o} for (2 – 3) days.

3.9 Sensory evaluation:

A 6-member sensory panel, consisting of the members of Food science and Technology department, Faculty of Agriculture U. of K. were selected to measure particular parameters including taste, visual color, aroma, and overall acceptability of the juice.

3.9.1 Statistical analysis:

Data generated was subjected to Statistical package for Social sciences (SPSS). Means$\pm$SD were tested using two designs. The first is 'One–Factor Complete Randomized Design' (type of juice) to analyze 1. proximate composition and 2. Physicochemical properties according to Mead and Gurnow, (1983). And the second design is 'Three-Factors Complete Randomized Design'; to analyzed (1), total soluble solids, (2) pH- values, (3) vitamin C and (4) titratable acidity according to Steel and Torrie (1980), then the means were separated using Duncan's Multiply Range Test (DMRT).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Juice yield:

Upon extraction of the juice from the two fruits (pummelo-grapefruit) it has been recognized that the flesh of pummelo fruit was harder and the sugar content was high. The fruit yield about 2.8 lit. from 24 pieces pummelo fruit one fruit weighed 550 g. and yield 116 ml for each piece while a grapefruit yield a higher juice content from a less amount of grapefruit, about 125 ml for each piece from one and a half dozen. This describes that pummelo fruit higher in weight bigger in size gave a less juice content.

The extraction of juice was done by a hand squeezing mechanism using a hand presser.

4.2 Visual features:

Although raw grapefruit juice has a light pink color it's concentrate had a light brown pink color resembles the color of traditional "Aradaibe" juice or a dilution of honey in water, pummelo raw juice resembles orange juice in color, appearance and texture but this color had been changed to a dark yellow to brown color with a heavy texture (Fig. 5). The color of the two concentrated juices had been developed during the storage period to a brown color, but it could be recognized stronger in room conditions storage where color changed to a dark brown color (Figure 6). A similar change had been recognized in refrigerator storage of less effect (Fig. 7).

(A) (B)

(A) (B)

Fig. (5): Pummelo and grapefruit concentrates after processing (at zero time)

A: Pummelo

B: Grapefruit

Fig. 6: Color changes (browning development)of grapefruit concentrates during storage at room temperature

Fig. (7):

(A) (B)

Comparison of grapefruit concentrates (A) at cold storage and (B) at room storage (after half period).

The brown color of the two concentrates is submitted to the carmaliziation of the added sugar upon the heat treatment. Browning in citrus juices had been studied by a number of investigations and the subject had been reviewed by Handwerk and Coleman (1988). The general consensus of this review is that citrus browning compounds require the interaction of reducing sugars and/or sugar degradation products with non sulfur containing amino acids and browning is effected by heat, storage temperature and acidity content. Eskin (1990) mentioned that development of browning in lemon and grapefruit juice concentrates may be attributed to their higher acidity, while Huelin *et al*. (1971) stated that since ascorbic acid has a control role in the browning of citrus juices and concentrates it decomposes easily in strong acid solutions and the development concentrates is rapidly accelerated.

4.3 Proximate analysis:

Results in table (4) describe the proximate analysis of grapefruit and pummelo juice as a comparison between raw juice and its concentrated form. All results reported on raw juice agreed with the results obtained from the National Public Health Institute of Finland (2003-2006)

4.3.1 Moisture content:

Moisture constitutes the major portion of the two juices and a non significant difference was recorded between the two raw juices while significance was obtained between the two concentrated forms.

According to the obtained results the moisture content 89.67% and 89.70% for the two juices agrees with the results obtained from the national public health institute of Finland, which reported that the moisture content of grapefruit juice ranges from 89-90% obviously the water content of the raw juice was higher than the water content of it's

concentrate, this is due to the evaporation which helped loss of a great amount of water during the concentrate process and reduced the portion to 42-46% and this reduction was reflected to the increase of other portions such as carbohydrate.

4.3.2 Carbohydrate content:

Results in table (4) showed a sharp increase in carbohydrate content of raw juice and it's concentrated form results had been described as 8.9% and 9.4 % for pummelo and grapefruit juice to 56.5% and 52.8%, respectively to it's concentrates with a significant difference.

4.3.3 Fat content:

The results from table (4) shows a significant increase in the fat content, the increase was observed by <0.2for raw juice to <0.3 to its concentrate.

4.3.4 Fiber content:

A significant difference was observed in fiber content as shown in table (4) results described 0.2 for both pummelo and grapefruit raw juice and 0.1, 0.04 respectively for its concentrates. This might be referred to analysis of fiber by heat on a acidic solution.

4.3.5 Protein content:

Data in table (4) described that protein significantly decreased from 0.16% for both pummelo and grapefruit juice to 0.15% and 0.13% for both concentrates respectively.

4.3.6 Ash Content:

The data in table (4) the results of ash content for the raw juice and its concentrated form. The pummelo and grapefruit raw juice measured 0.4 and 0.3% while pummelo and grapefruit significantly decreased to 0.3% and 0.2%, respectively.

Table (4) Proximate composition of raw juice and its concentrated form

Juice type		Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Vit. C (mg/100g)
Raw juice	Pummelo	0.16±0.01^{a}	0.15±0.01^{d}	0.23±0.01^{a}	0.45±0.01^{a}	89.67±0.58^{a}	8.99±0.00^{d}	39.63±0.06^{a}
	Grapefruit	0.16±0.00^{a}	0.18±0.01^{c}	0.21±0.01^{b}	0.32±0.00^{b}	89.70±0.00^{a}	9.37±0.06^{c}	39.60±0.00a
Concentrated juice	Pummelo	0.15±0.01^{b}	0.28±0.01^{c}	0.11±0.00^{c}	0.35±0.01^{b}	42.57±0.06^{c}	56.54±0.00^{a}	38.53±0.01b
	Grapefruit	0.13±0.01^{c}	0.31±0.01^{a}	0.04±0.00^{d}	0.20±0.00^{d}	46.47±0.06^{b}	52.82±0.01^{b}	37.43±0.06^{c}

Any two mean values having different superscript letters in a column differ significantly ($P \leq 0.05$).

Chemical properties:

Chemical properties of grapefruit and pummelo concentrates are shown in table (5). The table describes the difference between raw juice and its concentrates, the results explained as pH, titratable acidity and total soluble solids (T.S.S.)

as a comparison between the two juices. Among the two juices. The pH parameters where high and significantly increased after processed to concentrated juice. However the citric acid treatment affected the titratable acidity, data from table (5) reveal that titratable. Acidity sharply increased after concentration from 1.8% to 2.9% for pummelo juice and from 1.3% to 3.8% for grapefruit juice with a high significance and differentiated by 1.1 and 2.5 for both juice respectively.

Degradation of vitamin C content :

Vitamin C content of raw pummlo and grapefruit juice was 39.7 mg/100g and 39.6 mg/100g respectively (table 4). After process concentrated juice gave 38.5% mg/100g. and 37.4%mg/100g. respectively a loss of only 1.1-2.2% with a relative stability. This is due to certain substances, which retard oxidation of ascorbic acid (Inagkai 1943, 1944, 1947).

Initial vitamin C content of stored pummelo and grapefruit concentrates was 38.5% and 37.3%mg/100g, respectively (table 6). After two weeks a slight decrease has been noticed at refrigerator temperature, while the decrease was significantly higher at room temperature. Parameters of vitamin C content during refrigerator storage had a significant decrease, while room storage was significantly higher and temperature dependence. Degradation of vitamin C content was observed in both refrigerator and room storage and declined during storage period. The degradation depended on many factors such as oxygen ,heat and light (Robertson and Sammaiego, 1986) storage temperature and storage time.

Table (5): Chemical properties of raw juice and its concentrated form

Juice type		PH	T.S.S (%)	Titr. Acidity (%)
Raw juice	**Pummelo**	3.29 ± 0.00^{b}	13 ± 0.00^{c}	1.815 ± 0.00^{c}
	Grapefruit	2.96 ± 0.00^{d}	10 ± 0.00^{d}	1.348 ± 0.00^{d}
Concentrated juice	**Pummelo**	3.48 ± 0.00^{a}	57 ± 0.00^{a}	2.970 ± 0.00^{b}
	Grapefruit	3.18 ± 0.00^{c}	53 ± 0.00^{b}	3.840 ± 0.00^{a}

Any two mean values having different superscript letters in a column differ significantly ($P\leq0.05$).

At the end of storage time the loss of vitamin C was less at refrigerator storage compared to room temperature storage, which ended to half and even less than half of the initial content. A representative table (3) explained degradation on differ citrus concentrate at differ temperature during eight week storage.

Titratable Acidity:

Result presented in table (7) describes titratable acidity decline during storage period affected by the temperature degree. During the refrigerator storage, the first 15 days showed a rapid decrease, after 15days a permanent change may be explained by a significant decrease. However a same result may be explained in room storage temperature except the decrease was higher. Similar results were obtained on table (8) describing pH values during storage period increasing significantly.

Total Soluble Soilds (Brix) :

The effected storage period on total soluble solids (brix) shown in table (9) describes the decrease in the total soluble solids A slight decease has been noticed at refrigerator storage, a higher decrease is noticed in room storage. At the end of storage time some parameters showed an increase from previous parameters. This might be referred to accumulation of deposits shown at the end of the bottles.

Lee and Nagy (1998) mentioned that during storage of grapefruit concentrate over 50% of sucrose was hydrolyzed after 6 weeks at 30^{o}C and the reducing sugar increased in accordance with hydrolysis of sucrose and in juice stored at 50^{o}C more than 98% of the ascorbic acid and bout 9% of total sugar were lost. Similar results had been described on a study submitted to Kenawi, Shekib and El-Shims (1992) on a calcium fortified orange juice concentrate stored for ten weeks at room temperature. They

mentioned in their study that vitamin C content decreased during storage, titratable acidity declined and pH values increased along with storage period.

Table 6: Vitamin C (mg/100g) during storage period

Juice type		Storage period (days						
		Zero	**15**	**30**	**45**	**60**	**75**	**90**
Refrigerator storage	**Pummelo**	38.5 ± 0.000^{a}	36.0 ± 0.000^{c}	30.7 ± 0.058^{g}	29.7 ± 0.577^{g}	28.5 ± 0.100^{h}	28.0 ± 0.100^{i}	25.0 ± 0.000^{k}
	Grapefruit	37.3 ± 0.000^{b}	36.0 ± 0.000^{c}	32.6 ± 0.010^{e}	30.0 ± 0.000^{g}	34.0 ± 0.058^{d}	28.0 ± 0.929^{i}	22.2 ± 0.058^{l}
Room temperature	**Pummelo**	38.5 ± 0.000^{a}	29.7 ± 0.058^{g}	25.7 ± 0.153^{j}	20.5 ± 0.010^{m}	19.5 ± 0.100^{o}	15.4 ± 0.058^{r}	17.8 ± 0.833^{p}
	Grapefruit	37.3 ± 0.000^{b}	25.3 ± 0.306^{k}	20.5 ± 0.010^{m}	19.9 ± 0.100^{n}	17.3 ± 0.115^{q}	17.0 ± 0.100^{q}	12.8 ± 0.208^{s}

Any two mean values having different superscript letters in each column and row differ significantly ($P \leq 0.05$).

Table (7): Titratable acidity decline during storage period

Juice type		Storage period (days						
		Zero	15	30	45	60	75	90
Refrigerator storage	Pummelo	2.970±0.000^{b}	1.110±0.010^{e}	0.983±0.006^{f}	0.683±0.015^{s}	0.680±0.000^{t}	0.683±0.006^{s}	0.510±0.010^{v}
	Grapefruit	3.840±0.000^{a}	1.153±0.006^{c}	1.150±0.000^{d}	0.837±0.006^{m}	0.817±0.006^{n}	0.807±0.006^{o}	0.683±0.015^{s}
Room temperature	Pummelo	2.970±0.000^{b}	0.890±0.010^{j}	0.807±0.021^{o}	0.680±0.020^{t}	0.687±0.012^{r}	0.720±0.010^{q}	0.590±0.010^{u}
	Grapefruit	3.840±0.000^{a}	0.940±0.000^{h}	0.943±0.006^{g}	0.897±0.006^{i}	0.860±0.000^{l}	0.863±0.015^{k}	0.790±0.010^{p}

Table 8: pH-values during storage period

Juice type		Storage period (days						
		Zero	15	30	45	60	75	90
Refrigerator storage	Pummelo	3.48±0.000^{k}	3.47±0.006^{l}	3.70±0.017^{f}	3.75±0.025^{e}	3.77±0.010^{c}	3.76±0.006^{d}	3.79±0.006^{b}
	Grapefruit	3.18±0.000^{s}	3.15±0.006^{u}	3.17±0.010^{t}	3.28±0.015^{q}	3.26±0.006^{r}	3.29±0.015^{p}	3.32±0.006^{n}
Room temperature	Pummelo	3.48±0.000^{k}	3.30±0.006^{o}	3.36±0.006^{f}	3.70±0.020^{f}	3.77±0.000^{c}	3.76±0.006^{d}	3.80±0.000^{a}
	Grapefruit	3.18±0.000^{s}	3.50±0.006^{j}	3.52±0.006^{g}	3.50±0.006^{j}	3.51±0.010^{i}	3.51±0.006^{h}	3.51±0.044^{i}

Any two mean values having different superscript letters in each column and row differ significantly ($P \leq 0.05$).

Table 9: Total soluble solids of concentrates during storage period

Juice type		Storage period (days						
		Zero	15	30	45	60	75	90
Refrigerator storage	Pummelo	57.0±0.000^{a}	57.0±0.000^{a}	56.0±0.000^{b}	50.0±1.000^{g}	52.0±0.000^{ef}	54.7±0.577^{c}	49.7±0.577^{gh}
	Grapefruit	53.0±0.000^{d}	51.0±0.577^{ef}	52.0±0.000^{ef}	52.0±0.000^{ef}	48.3±0.577^{j}	49.7±0.577^{gh}	52.3±0.577^{de}
Room temperature	Pummelo	57.0±0.000^{a}	51.3±0.577^{a}	49.3±0.577^{ghi}	41.0±1.000^{m}	40.0±0.000^{n}	36.7±0.577^{o}	47.3±0.577^{k}
	Grapefruit	53.0±0.000^{d}	49.3±0.577^{ghi}	49.0±0.000^{hij}	45.0±0.000^{l}	39.6±0.577^{n}	40.0±0.000^{n}	48.7±0.577^{ij}

Any two mean values having different superscript letters in each column and row differ significantly ($P \leq 0.05$).

Microorganism analysis:

Table (10) shows the effect of storage temperature and storage period on total viable count,(T.V.C.) yeast and molds and lactic acid bacteria at the first month of storage. A high development of microorganisms had been noticed during suitable conditions.

At zero day most parameters of microorganisms growth were negative or negligible at both refrigerator and room storage. At refrigerator storage a slight growth, almost negligible at the first two weeks may be described.

During room storage the pummelo concentrate microorganisms growth was low at 7 days and increased at 15 days then send to a decrease at 30 days, while grapefruit concentrate showed permanent growth at the first two weeks for the T.V.C. (8.7×10^5) and increased to (9.0×10^7) at 30 days. Yeast and mold growth was graded from 3.2×10^5 at 7 days to $8. \times 10^5$ at 30 days. Lactic acid bacteria at 7 days calculated (8.0×10^5) then decreased to the half at 15 days and the increased again after 1 month.

The microorganism activity was high at room storage fermentation had been noticed from the first 7 – 9 days and increased with temperature increase. After 4 weeks from storage mold growth on the surface of foster concentrate at room storage.

After 15 days no color change and a high fermentation had been noticed. Appearance of plastic bottles filled immediately after process were normal and after 15 days storage period they were plunged and seemed blowing and when open made an explosure noise. microorganisms reached their peak after two months at room storage and then start to decease, while at refrigerator storage fermentation had been noticed after two months.

Table (10):Microbial assay

I. Refrigerator storage

Juice type Microbial analysis	Zero day	7 day	15 day	30 day
	Pummelo concentrate			
T.V.C c.f.u/ml	15^{-1}	No bacterial growth	8^{-1}	8.0×103
Yeast and mold c.f.u/ml	- ve	- ve	7^{-1} neg	1.5×10^{2} yeast
Lactic acid bacteria c.f.u/ml	- ve	- ve	- ve	8.5×10^{2}
Grape fruit concentrate				
T.V.C c.f.u/ml	No bacterial growth	No bacterial growth	3^{-1}	3.0×10^{2}
Yeast and mold c.f.u/ml	- ve	1.4×10^{3} yeast	- ve	No l growth
Lactic acid bacteria c.f.u/ml	- ve	- ve	- ve	No growth

II. Room Storage

Juice type Microbial analysis	Zero day	7 day	15 day	30 day
	Pummelo concentrate			
T.V.C c.f.u/ml		3.6×10^{5}	7.2×10^{6}	2.4×10^{7}
Yeast and mold c.f.u/ml		5.6×10^{5}	8.7×10^{6}	6.0×10^{6}
Lactic acid bacteria c.f.u/ml		9.6×10^{5}	1.20×10^{6}	1.80×10^{6}
Grape fruit concentrate				
T.V.C c.f.u/ml		8.7×10^{5}	8.7×10^{5}	9.0×10^{7}
Yeast and mold c.f.u/ml		3.2×10^{5}	7.8×10^{5}	8.9×10^{5}
Lactic acid bacteria c.f.u/ml		8.0×10^{5}	4.2×10^{5}	7.5×10^{5}

T.V.C = Total viable count of bacteria
Cfu/ c =colony f= forming u = unit

CHAPTER FIVE

CONCLUISION AND RECOMMENDATIONS

5.1. Conclusion:

- ✓ The two homemade concentrates were sensorial acceptable only for 3days at ambient room temperatures while at refrigerator storage were acceptable only for 2 months.
- ✓ The concentrates developed an off flavor on standing, which was destenic from the cooked flavor produced during evaporation process.
- ✓ Although the exact nature of the constituents in the juice that produce flavor deterioration was unknown, the flavor characteristics of the juice are primarily due to the volatile compounds, which can be removed by vacuum distillation.
- ✓ By measuring the rate and temperature dependence of browning reactions it is possible to determine the period of storage at a given temperature with out any quality change.
- ✓ The rise of the total soluble solids was due to the amount of sugar added and the moisture decreased because the juice was saturated with sugar, which can conclude that the evaporation procedure wasn't sufficient enough.

5.2Recommendations:

- ☒ The objective of this study was to make a concentrated juice in a easy home made economical preparation, but unfortunately this procedure wasn't sufficient enough to maintain nutritional benefits and acceptance of natural flavor and appearance that's why I recommend for using a more advanced technology for production of grapefruit juice.
- ☒ Evaporation procedures should be done by indirect heat such as using a water bath or a vapor heat evaporation at a minimum time.
- ☒ As the result shows less degradation and deterioration in nutrient content had been recognized at low temperature so it may be regarded that best results would be at frozen and chilled temperatures.

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