AN ATTEMPT TO PREPARE A CARBONATED BEVERAGE FROM ROSELLE (*Hibiscus sabdariffa* L.) CONCENTRATE

By

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بسم الله الرحمن الرحيم
To the soul of my father
Omer Abd Allah
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Above all, my special praise and unlimited thanks are to Allah, who helped me and gave me health and patience to complete this study.
The purpose of this study is to determine the nutritional and industrial value of carbonated roselle beverage produced from roselle calyces. Calyces of Alrahad cultivar were in the present investigation.

Proximate chemical analysis for roselle calyces was carried out to determine the levels of the various nutrients in the calyces. The results showed that the roselle calyces contained 7.6% moisture, 10% crude protein, 10% crude fiber, 0.6% fat, 9.2% ash and 62.6% carbohydrate. Roselle calyces were found to contain 0.548% Calcium, 0.201% Magnesium and 0.016% Iron. The results of present work indicated that the roselle calyces is nutritional acceptable since it can provide both nutrients and minerals.

Carbonated roselle beverage was prepared from concentrated calyces extract, sugar (sweetener), gum Arabic (stabilizer) and sodium benzoate as preservative.

The effect of storage at room temperature (30-35°C) for 4 months on pH, acidity, total soluble solids and color intensity of the roselle carbonated beverage was investigated. The results indicated that storage slightly increased the pH value and decreased the acidity, total soluble solids and color intensity.
The microbial analysis for this beverage was carried out to determine the effective role of the preservative (0.05g/litre sodium benzoate) against microorganism. The results of the study showed that the beverage was devoid from yeast and mould and has low bacterial count.

This beverage can compete with other carbonated beverages. The panel test confirmed the good quality, palatability and overall acceptability of roselle carbonated drink compared to similar types of drinks.
خلاصة الأطروحة

أجريت هذه الدراسة بغرض تحديد القيمة الغذائية والصناعية لزهرة الكركدى. تم استخدام إزهار الكركدى (صنف الرهد) في هذه الدراسة. أجريت التحاليل الكيميائية التقريبية لهذه العينة لتحديد نسب العناصر الغذائية المختلفة الموجودة بها. حيث أثبتت الدراسة احتواء إزهار الكركدى على 7.6% رطوبة, 10% بروتين, 10% ألياف, 0.6% دهون و62.6% كربوهيدرات. كذلك أثبتت الدراسة احتواء أزهار الكركدى على 0.548% كالسيوم, 0.201% مغنيسيوم و0.016% حديد. حيث أوضحت الدراسة أن إزهار الكركدى مقبولة من ناحية تغذوية لإمدادها للعناصر الغذائية والتغذوية معاً.

تم تصنيع مشروب الكركدى الغازى من مستخلص إزهار الكركدى بعد تركيزه مضاف اليه.

سكر (مادة محلية), صمع عربي (مادة مثبتة) وبنزوات الصوديوم كمادة حافظة.

اعتمدت الدراسة على معرفة اثر التخزين لفترة 4 أشهر في درجة حرارة الغرفة (30-35 °C) على الأس الهيدروجيني, الحموضة, المواد الصلبة الذائبة الكلية وعلى كثافة اللون لمشروب الكركدى الغازى. حيث أوضحت الدراسة حدوث ذبذة في قيمة الأس الهيدروجيني ونسبة نقصان في كل من درجة الحموضة, المواد الذائبة الكلية وكثافة اللون.

اجريت تحاليل ميكروبية لتقدير فعالية بنزوات الصوديوم (0.05 جم/لتر) كمادة حافظة وقد كانت النتيجة خلو المشروب تماماً من الخسائر وعفن مع وجود عدد ضئيل من البكتيريا.

هذا المشروب يعتبر منافساً للمشروبات الغازية الأخرى وقد استثنى الاختبار الحسي جودة مشروب الكركدى الغازى وتقبل المستهلك له بعد مقارنته مع انواع أخرى من المشروبات الغازية.
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**CHAPTER ONE**

**INTRODUCTION**

*Hibiscus Sabdariffa* L. is the botanical name for the annual herb which belongs to the family malvaceae. While Roselle is the most widely used name for the crop plant, in the Sudan it is commonly known as karkade. The principal production areas in the Sudan are eastern kordufan sands, Elrahad and Ummruaba. karkade is however, grown on smaller scale around Elobeid, in western kordukan near Elfashir and Nyala (Percy, 1971).

Roselle is a source of a red beverage, containing citric acid and salt serving as a diuretic, also containing ascorbic acid. The flower contains anthocyanin and glucoside hibiscin, the drink made by placing, the calyx in water (Duke, 1978).

During 1960 the Sudan became the world major supplier of Roselle dried calyces. In 1968 about 1,500 metric tons were exported (Mclean, 1973). In African countries, it is considered as a medicinal plant and used for the treatment of cough and for wound dressing (Watt and Breyer *et al.*, 1962). Also roselle water extract is used both as a hot or more popular as cold refreshing drink (Ismail *et al.*, 1985). Earlier trial for preparation of carbonated beverage from roselle calyces were done by Saeed and Ahmed,
1972. According to them a very good carbonated beverage was produced from roselle calyces. Recently products from roselle calyces, such as, powder and concentrates, are developing in the Sudan. The objective of this study was to investigate the potential of roselle concentrate for the preparation of carbonated beverage.
2.1. Soft Drinks

2.1.1. Back Ground

Soft drinks are enormously popular beverage consisting primarily of carbonated water, sugar, and flavorings. Soft drinks rank as America's favorite beverage segment, representing 25% of the total beverage market. Two thousand years ago Greeks and Romans recognized the medicinal value of mineral water and bathed in it for relaxation, a practice that continues to present. The first imitation mineral water in the U.S was patented in 1809; it was called "soda water" and consisted of water and sodium bicarbonate mixed with acid to add effervescence (Louis, 1980). Beverages containing carbon dioxide are, nowadays, very popular products. Consumers enjoy their "pleasurable and sought after" sensation, despite the fact that they can be irritating, or even painful, for some people. The sensations elicited by carbonated drinks are either of mechanical origin, due to the bursting CO₂ bubbles stimulating mechanoreceptors on the tongue, or of chemogenic origin by formation of carbonic acid (H₂CO₃) in a reaction catalysed by carbonic anhydras (Dessirier et al., 2000).
2.1.2. Soft Drinks and Health

Pharmacists in America and Europe experimented with myriad ingredients in the hope of finding new remedies for various ailments. Already the flavored soda waters were hailed as brain tonics for curing headache, nervous afflications (Louis, 1980).

Woodroof and Phillips (1974) reported that soft drinks help settle upset stomachs, modern theory holds that the carbonation is responsible for this settling effect. Many doctors recommend adding $\frac{1}{2}$-1$\frac{1}{2}$ liters of water daily when fever is present. Chilled carbonated beverages provide the additional fluids in a form that renders them "more acceptable than ordinary water in fever and other diseases accompanied by intense thirst". Woodroof and Phillips (1974) also reported that many doctors recommend soft drinks to children and adults when other foods and liquids cannot be tolerated. Besides increasing liquid intake, they are effective in lessening nausea and gastric distress. According to Woodroof and Phillips (1974) a survey of 380 hospitals showed that over 85% of them used soft drinks routinely; 271 use carbonated beverages to alleviate post-operative and pregnancy nausea, 244 use carbonated beverages when no other food can be tolerated; 188 use carbonated beverages to insure adequate liquid intake; 185 use carbonated beverages as a between-meal beverage, 126 use carbonated beverages to aid digestion, 56 use carbonated beverage to facilitate administration of
milk in febrile cases. They also, reported that doctors even prescribe soft drinks some times for colds and prevent dehydration from virus infection.

2.1.3. Main Production Stages

A flow diagram of soft drinks production (potter and Hotchkiss, 1995) is shown in figure 1.

2.1.4. Incoming Ingredients

The major ingredients of soft drink beverages in addition to water are carbon dioxide, sugar or sweeteners, flavorings, preservatives, colorings, and acidifiers (Potter and Hotchkiss, 1995)

2.1.4.1. Water

Water is basic to the finished beverage. Like flavor, it's an integral part of the drink. In the beverage it is the vehicle or liquid portion that carries the sugar, flavor, acid, color, and carbon dioxide. Over 85% of total volume is water. Water must be of sufficient quality to maintain the correct balance of flavoring ingredients and, at the same time not contribute substances which will affect the taste or appearance of the drink. To produce a uniform and consistent high quality, it is essential to use treated water, that mean water must be purified from undesirable materials like algae, protozoa, yeast, molds, iron, manganese, alkalinity, turbidity, sulfur, and carbohydrate, and standardized for those that are desirable (Woodroof and Phillips, 1974).
2.1.4.2. Carbon Dioxide

Pure carbon dioxide is colorless and has a very faint odor. When dissolved in water, carbon dioxide produces carbonic acid and the solution is chemically active because of its acid properties. Only a small part of the dissolved CO₂ unites chemically with the water to form the acid, its pH value is in the range of 3.7 to 3.2 (Woodroof and Phillips, 1974).
Figure 1: Process flow diagram of soft drinks production
2.1.4.3. Sugar

The second main ingredient is sugar, which makes up 7-12% of soft drinks and deserves close attention to its quality control. Sugar is used in either dry or liquid form (Riley, 1972). Sugar is derived from cane or beet and the product from either source is acceptable. In European Economic Community (EEC), there are currently no microbiological standards set for sugar, contrary to the USA where the National Soft Drink Association has set standards for microbiological (mesophilic bacteria, yeasts, molds) control of the product (Muller and Hersch-doerfer 1986; Bartins et al., 1998 and Kotzamanidis et al., 2000). The sugar content of heavy metals (As<1, Cu<2, Pb<0.5mg\Kg) and pesticides residue should be compatible with the standards set by codex (1994).

2.1.4.4. Sweeteners

Sweeteners are used alternatively to sugar in order to reduce the content in the drink without affecting sweetness. Sugars differ not only in chemical properties and sweetness but in viscosity and non-sugar ingredients. Thus in a food system, the added sugar (or that already present in the product) is capable of undergoing reaction with itself or with other chemicals, thereby yielding final products having more or less sweet taste, more or less viscosity and/or flavor properties (Woodroof and Phillips, 1974).
Goldenberg and Herschdoerfer (1987) claimed that Chemical analysis to ensure purity is essential to avoid health or spoilage hazards to the product.

2.1.4.5. Chemical Preservative

A variety of chemical compounds may be added to foods to prevent their microbial decomposition. In some instances this is done merely to extend the storage life of foods which must also be held at refrigeration temperature above freezing. Even under these conditions more than one chemical compound may be added. Chemical compounds are sometimes added to food to stabilize these products indefinitely at ambient or room temperature. When this is done other factors than the chemical itself, such as high soluble-solids content or drying to comparatively low moisture content, are used in combination with addition of chemicals to prevent microbial decomposition (Nickerson and Sinskey, 1972).

Some carbonated drinks, notably those with low pH, can be satisfactorily manufactured without preservatives. For other soft drinks, and particularly those containing fruits, employment of a chemical preservative such as benzoic acid and sorbic acid is essential (Giese, 1994; Luck and Jager, 1997). They are used widely in a variety of food products to inhibit the growth of bacteria, yeast, and molds associated with food spoilage (Bui and Cooper, 1987).
2.1.4.5.1. Benzoic Acid

Benzoic acid is used as soluble salts (sodium benzoate and potassium benzoate). Sodium benzoate was the first chemical permitted in foods by the U.S. Food and Drug Administration, and it continues in wide use today in a large number of foods (Jay, 1986).

The antimicrobial activity of benzoate is related to pH, the greatest activity being at low pH value. Benzoate is used to act as moulds and yeasts inhibitor although it is effective against some bacteria (Jay, 1986).

2.1.4.6. Colors

Coloring matters are a special case of food additive necessary to preserve an attribute of quality. Coloring matters cannot successfully obscure an undesirable off-color. For the manufacturer of food products the principle should be to use artificial coloring matters to enhance the quality of his products and not to attempt to mask any defect, and to use the minimum concentration consistent with this objective (Herschdoerfer, 1972).

Caramel from heated sugar, a non-synthetic color, is commonly used in dark beverages such as cola drinks. These coloring materials are much preferred over natural fruit colors because of their greater coloring power and color stability. Even when natural fruit extracts are used, their colors are generally supplemented with synthetic (Potter and Hotchkiss, 1995).
2.1.4.7. Acidifiers

Acidifiers constitute essential and universal substances present in the final product. The most common is citric acid which can be obtained in its anhydrous form or as monohydrate. Phosphoric acid is used in cola drinks. Malic, lactic, tartaric, and acetic acids are rarely employed (Price-Davies and Houghton, 1987).

2.1.4.8. Flavors

Flavors are substances capable of giving desirable taste and/or odor to a food. Flavoring materials may be of natural origin; either processed or unprocessed, or synthetically manufactured (Goldenberg and Herschdoerfer, 1987; Luck and Jager, 1997). Flavor is one of the properties of food about which very little is known. It is due always to a balance of constituents and the effect of an additive can rarely be forecast. As an example may be quoted the effect of adding a small concentration of saccharin to a mixture of natural lemon juice and sugar. The result is not merely an intensification of sweetness, but an enrichment of the flavor as a whole (Herschdoerfer, 1972).

2.1.4.9. Gum Arabic

Gum Arabic is a mixture of principally polysaccharides and proteoglycans, the latter being arabinogalactan proteins (AGPs). Gum Arabic, exudates from acacia trees, has a unique combination of excellent emulsifying
properties and low solution viscosity. These properties make gum Arabic very useful in several industries but especially in the food industry where it is used as a flavor and stabilizer of citrus oil emulsion concentrates in soft drinks (Woodroof and Phillips, 1974). Gum Arabic also contains trace levels of lipids (Yadav et al., 2006). Gum Arabic was grouped into several grades including crystal gum Arabic, powdered gum Arabic and spray dried gum Arabic. The most popular grade used in beverage is the spray dried gum Arabic because its being cleaner product, contains no sediment and its moisture content is less than 9 % (Woodroof and Phillips, 1974).

2.1.5. Bottle Washing

Returnable bottles must be given a thorough cleaning and sanitizing before being refilled. Bottles are washed by soaking or flushing with caustic soda solutions, followed by a thorough scrubbing inside and outside. Then they are carefully rinsed with potable water before filling. Each state has legal requirements governing sanitation in food processing plant including bottle washing. Machines which wash returnable bottles are fully automatic and bottles need not touched by workers before filling. After washing, the bottles are fully inspected manually, or by electronic devices designed for that purpose (Herschdoerfer, 1972).

If the plant is filling one-trip bottles or cans which will not be returned, caustic washing is not necessary. These containers come from bottle and can manufacturing companies in a sanitary wrapping, and at most need
only to be rinsed by air or water before filling (Woodroof and Phillips, 1974).

2.1.6. Preparation of Bottling Syrups

The bottling syrup contains all the ingredients of the finished product except in the case of carbonated drinks the carbon dioxide and most of the water. Sugar in the form of syrup is the first ingredient to be added to the mixing tank; the amount added is controlled by means of a metering pump. If the sugar is delivered in granulated form, there is the additional step of dissolving the sugar and producing syrup. Because of the synthetic additives potential toxicity, accurate and reliable methods are required for their determination in order to ensure food safety. Once the bottling syrup is prepared, it may be necessary to filter or pasteurize it before pumping it down to storage tanks or directly to the filling line (Price-Davies et al., 1987). Each batch of bottling syrup should undergo through quality control tests before being released. The bare minimum consists of a taste test, making up the syrup as in the finished product, measurement of the Brix, and detection of yeast (Varnam and Sutherland, 1994).

2.1.7. Carbonation

Carbon dioxide is a colorless, non toxic gas with slight pungent or biting odor, produced by (I) burning carbon compounds, such as coke, oil, gas;
(Π) heating limestone to form lime and carbon dioxide, (мыш) fermentation to produce alcohol and carbon dioxide, and (IV) trapping from gas wells.

The gas is distributed as (I) liquid in cylinders under high pressure, or (Π) as a liquid in tank trucks or rail cars under low pressure, or (мыш) solid in insulated containers or trucks at atmospheric pressure (Woodroof and Phillips, 1974).

Carbonation is the process of saturating the beverage with carbon dioxide. Popularity of carbonated drinks is due to the unique taste, zest, and sparkle imparted by the carbon dioxide. The dissolved gas also plays a major part in inhibiting and/or destroying harmful bacteria.

Most beverages are carbonated in a range of 1.5-4 volumes. This is carried with carbonators of various designs where carbonation speeded by providing intimate contact between the liquid and the CO2. The liquid is cooled because the solubility of CO2 in water is greater at lower temperature and extra pressure is applied to force more CO2 into the solution. In practice, the entire flavored drink may be carbonated or only the water may be carbonated for subsequent mixing with the flavored syrup (Potter and Hotchkiss, 1995).

2.2. Roselle

Roselle (Hibiscus Sabdariffa L.), commonly known in the Sudan as karkade, is a tropical crop. The plant is grown as a fiber crop in many
countries; however, in the Sudan it is grown for calyces. The calyces are used for extraction of soluble matter which is used as beverage.

**2.2.1. Classification**

Roselle is classified as follows:-

- **Kingdom**: Plantae
- **Division**: Magnoliposida
- **Order**: Malvelas
- **Family**: Malvaceae
- **Genus**: Hibiscus
- **Species**: sabdariffa

**Binomial Name**: *Hibiscus sabdariffa*.

**2.2.2. Botanical Description**

Roselle, termed karkade, is Erect, mostly branched, annual; stems to 3.5m tall, variously colored dark green to red; leaves alternate, divided into 3-7 lobes; flowers large, short, red to yellow with dark center; capsules 5cm long, 5.3cm wide; root a deep penetrating tap root (Duke, 1983).

**2.2.3. Ambient Conditions**

The plant is relatively tolerant to most soil types; the best being heavy retentive (Hassan, 1988). The plant is best cultivated at the beginning of rainy season at temperature range between 20°C and 35°C. The crop has
overall growing period of six months. The plant can be propagated from cuttings, they grown from seeds.

2.2.4. Food Uses and Nutritional Value

The food uses and nutritional value of Roselle were reported by Duke (1983). Many parts of Roselle including seeds, leaves, fruits and roots are used in various foods. Among them, the fleshy red calyces are the most popular. They are used fresh for making wine, juice, jam, jelly, syrup, ice cream, cakes and flavors. It is also dried and brewed into tea. The young leaves and tender stems of Roselle are eaten raw in salads or cooked with meat and other vegetables. The seeds contain about 17% of oil. Seeds also used as an aphrodisiac coffee substitute, excellent feed for chickens, the residue after oil extraction is valued as cattle feed when available in quantity. The red calyces which contain antioxidant are rich in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron that are nutritionally important.

2.2.5. Medicinal Uses

Roselle is used in many folk medicines. It is valued for its mild laxative effect and for its ability to increase urination, which is attributed to two diuretic ingredients, ascorbic acid and glycolic acid. Because it contains citric acid, it is used as a cooling herb, providing relief during hot weather by increasing the flow of blood to the skin's surface and dilating the pores
to cool the skin. The leaves and flowers are used as a tonic tea for digestive and kidney functions. A lotion made from Roselle leaves is used on sores and wounds (Duke, 1983).

Perry (1980) showed that in Philippines the biter root is used as an operative and tonic. Watt and Breyer-Brandwijk (1962) showed that the plant extract decreases the rate of absorption of alcohol, lessening the intensity of alcohol effects in chickens.

2.2.6. Chemical Composition of Roselle

2.2.6.1. Moisture Content

Generally the moisture content varies according to the humidity, cultivar, area and environmental factors. Ibrahim et al., (1971) reported that the moisture content is about 9-14% of the dry calyces.

Reaubourg and Monceaux (1940) reported that Roselle dried fruits of American origin contained about 15% moisture.

2.2.6.3. Protein Content:

Busson et al., (1957), determined the protein content of Roselle samples from West Africa. Their analysis showed 7.9-9.3 % protein on dry weight basis.

In the study made by Ibrahim et al., (1971), 13 amino acids were given by the protein hydrolyzates of the whole and spent calyces, six of which were
essential amino acids. While the protein hydrolyzate from the water extract gave only nine amino acids.

2.2.6.2. Fats Content

Lipids are a group of heterogeneous compounds, which are classified together because of their solubility in organic solvents. This solubility differentiates them from other constituents such as protein, carbohydrates and nucleic acid in seeds. They include free fatty acids, mono, di and tri-glycerides, phospholipids, steroles and glycerols.

Subbaram et al., (1964) reported that the fatty acid component percentage of Roselle seed were 18.4% palmitic, 12% stearic, 35.8% oleic, and 38.4% linoleic.

According to Ahmed and Hudson (1982), the oil of Roselle seeds from different cultivars showed difference in fatty acids composition in the following ranges: palmitic (17.4-22.6%), stearic (3.9-5.2%), oleic (34-39.8%), and linoleic (30.1-37.45%).

USDA, (2004) showed that ratio of fat was 0.64% of edible portion in Roselle. Mclean (1973) reported that seeds have been found to keep over a year without variation in the oil fatty acid content. The oil claimed to have good cooking properties compared to cotton seed oil.

Haarer (1952) reported that the nutritive value of Roselle seeds exceed that of cotton seed cake because of its high fat content.
2.2.6.5. Ash Content

The ash content of foodstuff represents the inorganic residue remaining after the organic matter has been burned. Mclean (1973) reported that ash represents about 9% of the dry calyces, and that the analysis of ash showed the presence of sodium, potassium, calcium, magnesium, aluminium, iron, sulphate, phosphate, chloride and carbonate.

2.2.6.4. Carbohydrates

Brand (1942) and Hulme (1971) reported 3% sucrose in Roselle. Ibrahim et al., (1971) detected glucose and arabinose in the water extract of Roselle from Sudan. Roselle was shown to contain pectin (Riaz, 1969). Hamidi (1966) reported that pectin increases with sepal development during maturation.

USDA (2004) showed in every 100 grams of edible portion in Roselle there are 11.31 grams of carbohydrate.

2.2.6.6. Acid Content

Flavor characteristics of Roselle were found to be mainly in the acid content. The presence of citric, malic, succinic, lactic, tannic and oxalic acids have all been reported in Roselle by Pritzker and Jungunz (1937). Prenesti et al., (2005) reported that the acidity plays a protective role in the phenolic molecules, thus preserving both color and antioxidant power. Ibrahim, et al., (1971) detected malic, oxalic, and citric acids in Sudanese
Roselle. Gerieble (1939) isolated an acid which reacted in some respects identically to well known acids such as citric and tartaric, his test suggested that it was allocation of hydroxyl citric acid C₆H₇O₇. He named it hibisic acid.

Ascorbic acid had been reported in roselle by many analysis that showed Ascorbic acid concentration varying from 21 to 89.4mg/100g in both fresh and dried samples (Mclean, 1973). Also in Roselle dried calyces from Sudan vitamin c with a concentration of 13.4mg/100g was reported by Eltinay and Ismail, (1985).

2.2.7. Hibiscus Anthocyanin:

Anthocyanins, the biggest group of water-soluble natural pigments of plant, are responsible for the attractive colors of flowers (Gradinu et al., 2003). According to them roselle anthocyanins exhibited good antiradical activity throughout storage in all humidity environments studied, despite of a substantial loss in color intensity.

Aqueous extracts from the dry calyces of Hibiscus, contain two main anthocyanins: delphinidin-3-sambubioside or cyanidin-3-xylosyl glucoside or hibiscin and cyanidin-3-sambubioside or cyanidin-3-xylosyl glucoside and gossypicyanin, and two minor anthocyanins, delphinidin-3-glucoside and cyaniding-3-glucoside (Du and Francis, 1973). The dry calyces of *Hibiscus sabdariffa* yield as much as 1.5% (w/w d.b) pigment that has
transmission spectral features very similar to those of Red No2 amaranth (Francis, 1975).

2.2.7.1. Factors Affecting the Stability of Anthocyanin

Esselen and Sammy (1973) and Esselen and Sammy (1975) have first attempted to study the stability of Hibiscus sabdariffa anthocyanins in different food formulations (jellies, drinks, carbonated beverages, and freeze dried powders). While Pouget et al., (1990) examined the effects of different chemical compounds (ascorbic acid, BHA, propyl gallate, di-sodium EDTA, sodium sulfite) on Hibiscus sabdariffa anthocyanin stability.

2.2.7.1.1 Co-Pigmentation

Co-pigmentation is a phenomenon widely seen in plant tissues and their aqueous extracts. Molecules acting as co pigments, such as flavonoids, alkaloids, organic acids, usually have no color by themselves, but when added to an anthocyanin solution, they greatly enhance the color solution (Mazza and Brouillard, 1990). The studies of Maccarone et al., (1985) and Maccarone et al., (1987) and Teh and Francis, (1988) have supported the view that co pigmentation and self-association influence color intensity and stability of the anthocyanins. However, Teh and Francis claimed that there have been no kinetic studies on thermal degradation of co-pigmented anthocyanins solution. Levine and Slade (1992) reported that such
information would be relevant to thermally processed food products containing anthocyanin pigments. Moreover, for low and intermediate moisture content foods, the physical state of the product (glassy or ruby state) has been claimed to be a major determinant of the diffusion rates of reactants, thus affecting the rate of deteriorative reactions in food including color degradation. Water availability is important for anthocyanin breakdown (Erlandson and Wrolstad, 1972). Two hydrolytic mechanisms of degradation at limited water concentrations were postulated by Markakis et al., (1957). One being hydrolysis of the glycosidic linkage to yield unstable aglycone and other involving opening of the pyrilium ring to form a substituted chalcone and finally degradation products; the later is consistent with the view that heating favors the formation of the primary anthocyanin breakdown products (most of which are colorless ) leads to formation of brown polymetric compounds.

2.2.7.1.2. Oxygen

‘Oxygen and heat have been reported as the most important factors affecting the destruction of anthocyanins (Jackman and Smith, 1992). The kinetics of the degradation of anthocyanins are generally first order at temperature up to 100ºc (Daravingas and Cain, 1968). Oxygen and temperature were considered the most specific "accelerating agents" in degradation of anthocyanins (Nebesky et al., 1949). It is reported by Adams (1972) that several highly purified 3-glucosides of cyanidin were
degraded at 100°C in dilute aqueous hydrochloric acid at various pH values between pH 1.0 and pH 4.0 under both nitrogen and oxygen atmospheres.

2.2.7.1.3. Storage Temperature

Palmidis and Markakis (1975) studied the effect of storage temperature in carbonated beverages colored with grape anthocyanins. They reported that increasing of storage temperature accelerated greatly the pigment destruction. Saeed and Ahmed (1977) studied the stability of stored carbonated beverage from roselle at different temperatures (ambient, 68 and 45°F). They found that the keeping quality was 90 days at ambient temperature during the summer time when the ambient temperature reaches its maximum.

2.2.7.1.4. pH

Anthocyanins in aqueous solution of pH values ranging from pH 1.0 to pH 14.0 result in the production of all the colors of the rainbow (Brouillard, 1982). At the prevailing room temperature anthocyanin coloring is really stable only in acidic media. In alkaline media, cleavage of pyrylium ring takes place 'more or less' rapidly. For pH values ranging from pH 5.0 to pH 12.0, intensively colored solutions, blue or greenish, (Brouillard, 1982).

Lukton et al., (1956) showed that the rate of destruction of pelargonidin-3-glucoside both in buffer solution and in strawberry juice at 45°C was virtually pH dependant in the range of pH 2.0-4.5 and in absence of
oxygen. In the presence of oxygen however, anthocyanin degradation increase dramatically with pH.

2.2.7.1.5. Light

Palmidis and Markakis (1975) found that light accelerated the destruction of anthocyanins, extracted from grape pomace. They reported that after 135 days of storage at 20°C in the dark, approximately 30% of the hot water extracted pigment was lost while up to 50% of the pigment was lost with exposure to light at the same temperature.

2.2.7.1.6. Presence of Ascorbic Acid

Beattie et al., (1943) show that changes in color occur concurrently with progressive losses of ascorbic acid. Markakis et al., (1957) postulated that an interaction between ascorbic acid and anthocyanin may occur, resulting in color loss. Tamenn (1973) noticed that the addition of ascorbic acid discolored the extract proportional to its dose. The influence of ascorbic and dehydro-ascorbic acid on pigment degradation of strawberry was reported by Everett (1953).
2.2.7.1.7. Metals

The discoloration of anthocyanin pigmented fruits by reaction with tin of can has long been known to the canning industry. Tamenn (1973) observed that contact with iron, copper, zinc, aluminum and tin spoiled the color.

2.2.7.1.8. Enzymes:

Erlandson and Wrolstad (1972) reported that enzymetic degradation of anthocyanins attributed to glycosidases freeing the anthocyanin from their sugar, they also reported that peroxidase catalyzed anthocyanin degradation.

2.2.8. Processing of Roselle as Carbonated Beverage

Saeed and Ahmed (1972) have developed a formula for the preparation of carbonated beverage from Roselle calyces. The organoleptic studies have showed that the carbonated drinks acceptable with excellent color and flavor.

2.2.9. Extraction

The extracting solvent of roselle calyces is water. Preliminary trials carried out on methods of extraction, had shown that extraction with hot or boiling water produced cloudy extract. Part of the cloud precipitate on standing and apparently contained pectin and gum. Extraction with water at ambient temperature produced a clear sparkling extract. If however, old extractor
was prolonged to 24 hours a cloudy extract again resulted (Jackson and Bashir, 1968a).

It was observed that there was an increase in percentage extraction as the ratio of water to karkade was increased. Extraction of about 60% of the weight of the raw material was obtained when 100 part of karkade was used. The ratio of 4:1 extraction gave only about 15% also; a decrease in particle size increased percentage extraction and required less water (Bashir, 1969).

2.2.10. Preparation of the Beverage

Twenty Kg of the Roselle calyces (Rahad variety) were soaked in tap water. The extract was filtered and the syrup was prepared in the Food Research Centre pilot plant according to the procedure described by Saeed and Ahmed (1972).

The syrup was taken in stainless steel tank to a commercial carbonated soft drink bottling plant. The syrup was transferred to the mixing tank of the plant and mixed with a filter aid (Hyflo) and pumped through the filter unit of the plant to the syrup tank. The bottling machine was adjusted to drop the recommended dose of syrup per bottle. The bottling line was thoroughly cleaned prior to bottling of the product. Before taking samples the first 70 bottles were discarded in order to be sure that the samples were free from traces of the commercial product (Saeed and Ahmed, 1977).
CHAPTER THREE
MATERIALS AND METHODS

3.1. Materials

Roselle Samples: Dry Roselle calyces, ELRAHAD cultivar, were obtained from ELNASR Company. They were kept in cleaned bags at room temperature.

Sucrose (granulated cane sugar) was obtained from local shops.

Carbonated water was obtained from Mamoon Elberair carbonated beverages plant.

All chemicals used were of analytical grade.

3.2. Preparation of Sample

3.2.1. Extraction

Roselle liquid extract was prepared by soaking the calyces in soft water (softened by water softener instrument and sterilized by ozone and Ultra Violet UV) in a ratio of 7: 1 (water: calyces) for three hours at 50°C to produce roselle extract at concentration of 7% (total soluble solids) (Annon, 2006). The soft water was obtained from ELNASR Company.

3.2.2. Concentration

Roselle extract (7% total soluble solids) was concentrated to 30% total soluble solids in the forced circulation vacuum evaporation concentrator at pressure -85 and steam temperature 58°C (Annon, 2006).
3.3. Manufacturing Procedure

One liter of roselle soft drink was prepared as follows:-

Two hundred milliliters syrup with Brix degree 40 was made by dissolving sugar (175g) and gum Arabic (2g) in 100ml soft water. Then it was carefully sterilized in oven in 80°C for 30minutes.

Sodium benzoate (0.05g), dissolved in 20 ml of concentrated roselle, were added to the syrup. Then carbonated water (780ml) was added to the solution, until the total soluble solids (T.S.S) of the beverage were 14 Brix degrees. Then the beverage was filled into sterile bottle, sealed and kept for further analysis.

3.4. Proximate Analysis

3.4.1. Moisture Content

The moisture content of the samples was determined according to AOAC (1984). Two grams samples (in triplicate) were weighed in an empty, dry and clean crucible with a known weight. The crucible containing the sample was placed in an oven at 105°C for 24 hours. After that the crucible was removed and placed in a dissicator containing dry silica gel and weighed three times at 10 minutes intervals and the weight was calculated as average. Then the moisture content as percentage was calculated according to the following equation:

\[
\text{Moisture Content} \% = \frac{(W1- W2) \times 100}{S}
\]

Where:

W1: Weight of crucible and sample before drying.
W2: Weight of crucible and sample after drying.

S: Original weight of sample.

3.4.4. Crude Protein Determination

Crude protein content of the sample was determined according to AOAC (1984). A 0.2g of dried sample was placed in 100ml Kjeldahl digestion flask, 0.4g of Kjeldahl catalyst tablets and 3.5ml of concentrated sulfuric acid were added. The flask was heated in an electrical heater for 2 h. The sample was cooled and diluted with distilled water and then placed in the distillation apparatus. Twenty milliliters of 40% sodium hydroxide (NaOH) were added and the distillation was applied for 10 min. The ammonia evolved was received in 10ml of 2% boric acid contained in a 100ml conical flask attached to the receiving end. The trapped ammonia was titrated against 0.02N hydrochloric acid (HCL) using universal indicator (Bromocresol green and methyl red in alcohol). The protein content as percentage was calculated according to the following equation:

\[
\text{Protein content \%} = \frac{\text{Volume of HCL} \times 0.02 \times 14 \times 3.25 \times 100}{\text{Sample weight} \times 1000}
\]

3.4.3. Crude Fiber Determination

Crude fiber content of the sample was determined according to AOAC (1984). Two grams of defatted sample (left after hot extraction of the fat) were taken in triplicate and placed in a 200ml beaker and 20ml of 1% (v/v) of sulfuric acid were added. Then the contents were brought to boil for 30min, with constant stirring
using a glass rod. Then the contents of the beaker were filtered using a cloth filter of a known weight. Hot water was used to wash the beaker, the glass rod and the filtrate for several times till the filtrate became neutral (free from acid by using litmus paper to insure that it became neutral). The filtrate was removed from the filter using a spatula and placed again in the beaker, 20ml of 1% sodium hydroxide were added to the beaker and the contents were brought to boil again for 30min, with constant stirring. Hot water was used to wash the filtrate till it became neutral (free from alkali by using litmus paper to insure that it became neutral). Then the filter containing the crude fiber was placed in an oven at 105°C for 24hours. After drying, the filter was removed and cooled in a dissicator and then weighed. The crude fiber as percentage was calculated according to the following equation:

\[
\text{Crude Fiber Content } \% = \frac{(W2-W1) \times 100}{S}
\]

Where:

W1: Weight of empty filter.
W2: Weight of the filter containing the crude fiber.
S: Original weight of sample.

**3.4.5. Fat Determination**

The fat content of the sample was determined according to AOAC (1984) using Soxhelt apparatus as follows:

Two grams sample were taken in triplicate and placed in thimble. The thimble was covered using cotton wool. An empty, dry and clean round flask with a known weight was connected to the siphoning apparatus. The thimble containing the
sample was placed in the siphoning apparatus and 200ml of petroleum ether (with a boiling point 40-60°C) were added. Then the condenser was connected to the siphoning apparatus and the heater was switched on and extraction was applied for 8 hours. After the extraction was completed the petroleum ether was evaporated from the round flask. The round flask containing the extracted fat was weighed and the fat content as percentage was calculated according to the following equation:

\[
\text{Fat Content\%} = \frac{(W_2 - W_1) \times 100}{S}
\]

Where:

W1: Weight of the empty round flask.

W2: Weight of the round flask containing the extracted fat.

S: Original weight of the sample.

3.4.2. Ash Determination

The ash content of the sample was determined according to AOAC (1984). Two grams of the sample (in triplicate) were weighed in a clean and ignited ashing dish with a known weight. The ashing dish containing the sample was ignited in a muffle furnace at 550°C for 3hours. Then the ashing dish was removed and cooled in a dissicator and weighed again. Then the ash content as percentage was calculated according to the equation:

\[
\text{Ash Content\%} = \frac{(W_2 - W_1) \times 100}{S}
\]

Where:

W1: Weight of the empty ashing dish (before ignition).

W2: Weight of the ashing dish containing the ash (after ignition).
S: Original weight of the sample.

3.4.6. Total Carbohydrate

The total Carbohydrate was determined by the difference:

100 - (c.p% + c.f% + crude oil% + total ash% + moisture%)( AOAC, 1984).

Moreover, calyces were analyzed to determine the following:-

3.5. Mineral Analysis (Ash Composition)

3.5.1. Mineral Extraction

The mineral of defatted samples were extracted according to Pearson method (1981). Each sample was burnt in muffle furnace at 550°C for 2 hours, then cooled and 5ml of 5N HCL were added, and warmed in sand bath for 10min. Then the solution was carefully filtered in a 100ml volumetric flask and finally distilled water was added to complete the volume. The extracts were stored in bottles for analysis.

3.5.2. Mineral Content

Three minerals, namely iron, calcium and magnesium in roselle herb, were estimated by atomic absorption spectrophotometer (Perkin-Elmer Model 2380) using air acetylene flame at specified absorption wave length as follows (1) Calcium (Ca) 422.7nm; (2) Iron (Fe) 252.7nm; (3) Magnesium (Mg) 285.2nm (Perkin-Elmer, 1994).
3.6. Determination of Total Soluble Solids (T.S.S)

The Total soluble solids were determined using hand refractometer (AOAC, 1984) as follows:

Two drops of syrup or paste were placed on the main stage of sloping prism of the refractometer.

The plastic cover was closed and read against a good light source to read the soluble solids as Brix degree.

3.7. PH Determination

PH of 10% solution was determined using a glass electrode fisher pH meter. The pH meter was calibrated with a buffer solution at pH 4.0 (Ruck, 1963).

3.8. Total Acidity

Total acids of the sample were determined according to Ruck (1963) by titrating 100ml of 10% against 0.1N NaOH to pH 8.2. Total acids were expressed as citric acid according to the following equation:-

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

Where:

Equivalent wt. of citric acid = 64.0

\[\text{Dilution Factor} = \frac{\text{Sample total volume}}{\text{Sample volume used in titration (10)}}\]
3.9. Microbial Examination

3.9.1. Preparation of media

3.9.2. Plate Count Agar

Plate count agar, obtained in a dehydrated form, was used for enumeration of bacteria. It was prepared according to the directions of the manufacturers by suspending 6.2g in 100ml distilled water. The medium was allowed to boil until it was completely dissolved and then was sterilized in an autoclave at 121°C for 15 minutes under pressure of 15 lb/inch².

3.9.3. Sabouraud Dextrose Agar

This medium was used for enumeration of yeast and moulds. It was obtained in dehydrated form. It was prepared according to the manufacturers instructions by suspending 4g in 200ml distilled water and boiling to dissolve completely and then it was sterilized in an autoclave at 121°C for 15 minutes under pressure of 15 lb/inch².

3.9.4. Methods

Beverage samples were shaken and duplicate samples of 1ml each, were taken by pipette in tubes containing 9ml of sterile peptone water. Serial decimal dilutions up to $10^{-6}$ were prepared; 0.1cm³ aliquot of each dilution was pour-plated in duplicates into PCA and surface plates of SDA. The plates were incubated at 37°C for 24 hrs while the SDA plates were incubated at 28°C for 72hrs. And then were taken and
counted. The colonies were counted using colony plate count device (Nickerson, John 1974)

3.1.0. Organoleptic Evaluation of Carbonated Roselle Beverage

Fourteen judges, from Dept of Food Sci and Technol, Faculty of Agric, U of K evaluated the Roselle carbonated beverage, (compared to other red-colored natural beverage prepared from fruits), for color, flavor, taste and overall acceptability. The scores were 1 (poor), 2-3 (fair), 4-5 (good), 6-7 (very good) and 8-9 (excellent). The results are shown in table (3).

Statistical Analysis

Data were assessed by Analysis of Variance (ANOVA) as shown by Sendecor and Cochran, 1987 and by Duncan’s Multiple Range Test (DMRT) with probability p ≤ 0.05 (Duncan, 1955).


4.1. Proximate Analysis of Roselle Calyces

The results of the proximate chemical composition were shown in Table 1. The parameters determined were moisture, crude protein, crude fiber, fat, ash and carbohydrate.

4.1.1 Moisture Content

The moisture content of the dry roselle calyces was found to be 7.6% (Table 1). This result is not in agreement with the results obtained by Ibrahim *et al* (1971) who reported a range of 9.20-14.90% for calyces of some cultivars grown in Sudan. Alshoosh (1997) reported 5.40 to 9.93% moisture content for calyces of six karkade cultivars grown in the two consecutive seasons 94/95 and 95/96. A higher value of moisture content (12.60) was detected by Ismail (1980) for dry calyces. Adam (2005) reported that the moisture content of the dry karkade calyces is 11%.

4.1.2 Crude Protein Content

The crude protein of the dry roselle calyces was found to be 10% (Table 1). Alshoosh (1997) who studied the protein content of six cultivars in two consecutive seasons 93/94 and 94/95 reported a range of 8.70 to 13.57%. A slightly lower protein value for dry calyces (9.44%) was reported by Ismail

### 4.1.3. Crude fiber content

The crude fiber content of dry calyces was 10% (Table 1), a result which is lower than the value of 15.22% reported by Mclean (1973) for dried calyces. A slightly higher value of 11.32 to 12.24% was reported by Alshoosh (1997) for six Roselle cultivars for the two consecutive seasons 93/94 and 94/95. A value of 13.2% reported by Adam (2005). Ibrahim et al., (1971) reported that Roselle grown in the Sudan contains 14% crude fiber.

### 4.1.4. Fat Content

The fat content of roselle calyces was found to be 0.6% (Table 1). This result lies within the range of 0.91% to 0.93% reported by Alshoosh (1997). A slightly lower fat content of 0.16% for red calyces and 0.12 for white calyces were detected by Adam (2005). The lower percent of fat in roselle calyces could be attributed to the accumulation of fat in the seed germ.

### 4.1.5. Ash content

The ash content of roselle calyces was found to be 9.2% (Table 1). This result lies within the range of 7.13 to 14.60% reported by Alshoosh (1997). A range of 8-11.6% ash content for roselle calyces was reported by Ibrahim
et al., (1971). Slightly higher values of 10.6% (red) karkade and 9.5% (white) karkade calyces were reported by Adam (2005).

4.1.6. Total Carbohydrate content

The total carbohydrate of roselle calyces, calculated by difference, was found to be 62.6% (Table 1). Slightly lower values of carbohydrate detected by Adam (2005) were 57.16% for red karkade and 61.55% for white karkade calyces. Abubaker (2006) detected a value of 59.31% for roselle calyces.

4.1.7 Total Acidity

The total acidity (as citric acid) of roselle extract was 16.1%. This value is higher than the value obtained by Adam (2005) who detected a value of 9% for karkade calyces. A lower value of 2.1% was reported by Sharief (2004).

4.1.8. pH Value

The pH value of roselle calyces was found to be 2.8. This value is a slightly higher than the value obtained by Abubaker (2006) who detected 2.49 for roselle crushed calyces. The result obtained in this study lies within the range of 2.7-3.42% reported by Alshoosh (1997). A slightly lower value of 2.55 was detected by Sharief (2004) for roselle calyces.
### Table (1) Proximate Chemical Composition of Roselle Calyces

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Percentage* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.6</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>10</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>10</td>
</tr>
<tr>
<td>Fat</td>
<td>0.6</td>
</tr>
<tr>
<td>Ash</td>
<td>9.2</td>
</tr>
<tr>
<td>Carbohydrate**</td>
<td>62.6</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

* Each value is a mean of three determinations.

** Carbohydrate was determined by difference.
4.1.9 Mineral Content

4.1.9.1 Calcium Content

Calcium content of roselle calyces was found to be 50.43mg/l which representing 0.548% of the total ash content (Table 6). Calcium content ranging from 0.53 to 3.35% was reported by Alshoosh (1997) for six roselle cultivars grown in seasons 94/95, 95/96. A value of 60mg/100g (w/w) calcium content for roselle calyces was detected by Adam (2005).

4.1.9.2 Magnesium Content

The magnesium content of roselle calyces was 18.5mg/l representing 0.201% of the total ash content (Table 6). This result is lower than that obtained by Alshoosh (1997), who reported 0.24- 0.25% for six cultivars of roselle grown in the Sudan. It is also lower than the value of 0.24% detected by Abubaker (2006) for roselle calyces.

4.1.9.3 Iron Content

The iron content of roselle calyces was found to be 1.49mg/l (w/v) i.e 0.016% (Table 6). Alshoosh (1997) reported iron content of 147.3mg/100g (w/w). Adam (2005) detected values of 25mg/100g for red karkade and 20mg/100g for white karkade calyces.
### Table (2) Mineral Content of Roselle Calyces

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Mineral Content * (mg/l)</th>
<th>Mineral Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>50.43</td>
<td>0.548</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>18.5</td>
<td>0.201</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>1.49</td>
<td>0.016</td>
</tr>
</tbody>
</table>

*Each value is a mean of three determinations.*
4.1.1.0. Roselle Carbonated Beverage (RCB)

Roselle beverage was prepared as described in sections 3.3.1, 3.3.2 and 3.4. The beverage obtained was stored at room temperature (30-35°C). The effect of storage on pH, Acidity, total soluble solids (T.S.S.), and intensity of color was then investigated.

4.1.1.1. pH Changes During Storage

Figure 2 shows changes of roselle carbonated beverage pH on storage at room temperature (See Appendix 1). The pH value of roselle carbonated beverage was 3.68 at zero time. A four months storage had increased the pH value to 3.85. The percent increase in pH after one month storage was 0.005%. This was followed by a further 0.025% increase in second month of storage. A further increment of 0.01% increase was observed when the samples are stored for three and four months so that the total increase reaches 0.05% in the four months of storage. Saeed and Ahmed, (1977) observed an increase in pH of roselle carbonated beverage during 135days storage at room temperature (30-35°C). However they obtained a slight drop in the pH value when the beverage was stored at 68°F and 45°F for the same time length. The slight increase in pH value during the four months storage of roselle carbonated beverage could be attributed to the decrease in total beverage acid contents.
Figure 2
4.1.1.2 Acidity Changes During Storage

Figure 3 shows changes of roselle carbonated beverage acidity on storage at room temperature (See Appendix 2). The acidity of roselle carbonated beverage was 9.6 at zero time. Four months storage decrease acidity to 2.98. The percent decrease in acidity after one month storage was 0.18%. This was followed by a further 0.27% decrease in second month of storage. A further increment of 0.40% decrease was observed when the samples are stored for three and four months so that the total decrease reaches 0.69% in the four months of storage. Clegg, (1964) attributed the decrease in acidity of Roselle beverage to consumption of citric acid during the non–enzymatic browning reaction. However, the decrease in acidity observed in the present study could be due to loss of the organic acids original present.

4.1.1.3. Total Soluble Solids (T.S.S.) Changes During Storage

Figure 4 shows the effect of storage period on the total soluble solids of the roselle carbonated beverage stored at room temperature (30-35°C). See Appendix 3. At zero time, the total soluble solids content for roselle carbonated beverage was 14 Brix. No changes were observed on T.S.S. during storage for two months after three months of storage the total soluble solids content had showed a 0.07% decrease to a value of 13 Brix.
Acidity Changes During Storage

Figure 3
T.S.S. Changes During Storage

% Changes

Storage Time in Months

Figure 4
This value remains unchanged after four months storage. This result is contrast to that obtained by Saeed and Ahmed (1977) who found an increase in T.S.S. for roselle carbonated beverage stored at room temperature for 135 days. The same trend was observed by Saeed and Ahmed (1977) when roselle carbonated beverage were stored at 68°F and 45°F. The decrease in total soluble solids during storage may be due to pectin precipitation during storage.

4.1.1.4. Color Intensity Changes During Storage

The color intensity of the roselle carbonated beverage was read using a DR/3 spectrophotometer.

Figure 5 shows the effect of storage on roselle carbonated beverage color intensity (See Appendix 4). A slight change of 0.001% in color intensity was observed after one month storage. This was followed by 0.060% change after two months. A decrease in color intensity of 0.100% was seen after three months storage a 0.320% change in color intensity was observed. It is therefore, concluded that a continuous decrease in color intensity was observed during the whole period of storage. Saeed and Ahmed, (1977) reported a decrease in color intensity of roselle carbonated beverage on storage at room temperature for 135 days. The decrease in color intensity may be attributed to oxidation reaction of anthocyanin during storage.
Color Intensity Changes During Storage

% Change

Storage Time in Months

Figure 5
4.1.1.5 Microbiological Analysis

The Results showed that the bacterial count in (RCB) was negligible (10per/ml). The results also indicated that the beverage was devoid from contamination of yeast and mould. The results obtained reflect the effective role of the 0.05g/litre sodium benzoate.

4.1.1.6 Organoleptic Evaluation of (RCB)

Roselle carbonated beverage (R) was evaluated for color, flavor, taste and overall acceptability, compared to two other red color beverage designated as F and V.

Analysis of variance (Table 3) showed that there was non-significant difference (P≤0.05) between samples in color (See Appendix 5). A significant difference (P≤0.05) was observed between samples in flavor (See Appendix 6). Where sample R gave a slightly better score than F and V. A highly significant difference between samples in taste and overall acceptability were detected according to the means of squares from the analysis of variance with sample R having better score than sample F and V which gave similar scores (See Appendix 7 and 8).
Table (3): Organoleptic Evaluation of (RCB) Compared to other red-colored Carbonated Beverages

<table>
<thead>
<tr>
<th>Samples</th>
<th>Color</th>
<th>Flavor</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>7.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>7.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>7.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>0.64</td>
<td>0.56</td>
<td>0.70</td>
<td>0.61</td>
</tr>
<tr>
<td>C.V</td>
<td>14.78</td>
<td>12.62</td>
<td>14.78</td>
<td>12.82</td>
</tr>
</tbody>
</table>

-LSD: Least Significant Difference.
-C.V: Coefficient of Variance.
-Means of similar letters are not significantly different at 0.05 and 0.01 level of probability.
-R: Roselle Carbonated Beverage (RCB).
-F and V: Other Red- Color Carbonated Beverages.
4.1.1.7. CONCLUSIONS

The following conclusions were drawn from the present study:

(1) Roselle carbonated beverage with its outstanding overall acceptability can be prepared and bottled in a modern bottling plant.

(2) The prepared carbonated beverage can withstand storage at room temperature (30-35°C) for at least 3 months.

(3) Storage of the beverage at room temperature (30-35°C) for more than 3 months caused slight changes in pH and noticeable changes in acidity, total soluble solids and color intensity.

(4) Although the results of this work showed that the intensity of the red color was reduced, reduction in color intensity was not visualized.

4.1.1.8. Recommendations

From the findings of this study it was recommended that:-

(1) The prepared roselle carbonated beverage should be kept in brown bottles to minimize changes that can take place due to atmospheric oxidation.

(2) Further investigations to cover areas not included in this research are required such as determination of expiry date, stabilizing the color, acidity, pH and total soluble solids for longer periods.
APPENDIX (1): pH Changes During Storage

<table>
<thead>
<tr>
<th>Storage time in months</th>
<th>pH values*</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>3.68</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>3.76</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>3.82</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>3.85</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Each value is a mean of three determinations.
APPENDIX (2): Acidity Changes During Storage

<table>
<thead>
<tr>
<th>Storage Time in Months</th>
<th>Acidity % *</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>9.60</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>7.90</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>7.04</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>5.76</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>2.98</td>
<td>0.69</td>
</tr>
</tbody>
</table>

*Each value is a mean of three determinations.
### APPENDIX (3): Total Soluble Solids (T.S.S.) Changes During Storage

<table>
<thead>
<tr>
<th>Storage Time in Months</th>
<th>T.S.S (Brix)*</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>14</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Each value is a mean of three determinations.*
APPENDIX (4): Color intensity Changes During Storage

<table>
<thead>
<tr>
<th>Storage Time in Months</th>
<th>Color Intensity*</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>1.619</td>
<td>_</td>
</tr>
<tr>
<td>1</td>
<td>1.617</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>1.515</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>1.464</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>1.098</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Each value is a mean of three determinations.
APPENDIX (5): Organoleptic Evaluation of (RCB) Color (ANOVA Table)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F</th>
<th>SS</th>
<th>MS</th>
<th>Fcal.</th>
<th>F tab.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>41</td>
<td>52.12</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>2.90</td>
<td>1.45</td>
<td>1.15</td>
<td>4.09</td>
</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>49.22</td>
<td>1.26</td>
<td></td>
<td>7.33</td>
</tr>
</tbody>
</table>

- NS, * and** non-significant, significant at 0.05 and 0.01 level of probability.
### APPENDIX (6): Organoleptic Evaluation of (RCB) Flavor (ANOVA Table)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F</th>
<th>SS</th>
<th>MS</th>
<th>Fcal.</th>
<th>F tab.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>41</td>
<td>60.00</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>29.71</td>
<td>14.86</td>
<td>19.05**</td>
<td>4.09</td>
</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>30.29</td>
<td>0.78</td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>

- NS, * and ** non-significant, significant at 0.05 and 0.01 level of probability.
APPENDIX (7): Organoleptic Evaluation of (RCB) Taste (ANOVA Table)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F</th>
<th>SS</th>
<th>MS</th>
<th>Fcal.</th>
<th>F tab.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>41</td>
<td>69.90</td>
<td></td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>23.62</td>
<td>11.81</td>
<td>9.92**</td>
<td>4.09</td>
</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>46.28</td>
<td>1.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- NS, * and ** non-significant, significant at 0.05 and 0.01 level of probability.
APPENDIX (8): Organoleptic Evaluation of (RCB) Overall Acceptability (ANOVA Table)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F</th>
<th>SS</th>
<th>MS</th>
<th>Fcal.</th>
<th>F tab.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>41</td>
<td>44.48</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>8.19</td>
<td>4.10</td>
<td>4.41*</td>
<td>4.09</td>
</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>36.29</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- NS, * and ** non-significant, significant at 0.05 and 0.01 level of probability.
REFERENCES


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Figure 1: Process flow diagram of soft drinks production