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Evaluation of physiochemical properties of Lime (Citrus aurantifolia) leaves tea

تقييم الخواص الفيزوكيمائية لشاي أوراق الليمون (البلدي)

Adissertation submitted to Sudan University of Science and Technology in partial Fulfillment for the requirement of the Degree of B.Sc. (Honors) in Food Science and Technology

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DEDICATION

I dedicate this

Work to my father, mother, brothers and sister.

With love.

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All praise and thanks are due to Almighty Allah who always guides me to the write path and has helped me to complete this Research.

First and foremost I would like to record my graduate to may supervisor **Dr: MahaFadulMohmmed** for her supervision , advice , encouragement, patience and guidance me from the early stage of this research .

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ABSTRACT

This study was conducted to make caffeine- free tea from lime (citrus aurantifolia) leaves (LLT). Leaves were obtained from the farm of university of Khartoum faculty of agriculture. Then thy washed by water and divided into four samples (250 gm.) for each sample , then three samples were exposed to withering process for fourteen hours to reduce moisture content of leaves , because withering enhance color and teats of the tea. Then leaves exposed to fermentation process in closed plastic containers, because fermentation of tea very important for color, test and quality of tea .the fermentation were done to three samples for (four, six and eight hr.) . Fermentation of tea is very important process in tea manufacture for color and quality of tea .and the rest sample was crushed and dried without fermentation. Results of proximate analysis of lime leaf tea (LLT) showed that (LLT) non-fermented(15.1%) and (LLT) fermented for six hours(16.24%) and (LLT) fermented for eight hours (14.78%)are better in ratio of carbohydrates and there were no significant between them .While (LLT) fermented for four hours(11.27%) occupied the second place. And there are no significant differences between ash (3.7% , 3.6% , 3.5%, 3.8%) , moisture (1.5% , 1.4% , 1.6% 1.5%) , protein (6.3%, 6.7% 5.4%, 6.4%) , fat (0.3%, 6.6% , 6.2% , 6.5%) and fiber(6.3%, 6.6% , 6.2% , 6.5%)respectively. The results of minerals content showed that that non-fermented (LLT)(169.8 mg/100g) and (LLT) fermented for six hours(167.6 mg/100g) had high calcium content compared to the rest of samples , while the (LLT) fermented for eight hours(154.3 mg/100g) occupy the second class and the lowest percentage in calcium was in (LLT) fermented for four hours(135.6 mg/100g) and there is no significant between it and the (LLT)fermented for eight hour((154.3 mg/100g). Also the results shows there were no significances differences between (Fe, Mg, k, p) in (non-fermented (LLT) , (LLT) fermented for

four hr. , (LLT) fermented for six hr. , (LLT) fermented for eight hr.) (1.2 mg/100g, 0.8 mg/100g, 1.3 mg/100g, 0.7 mg/100g) (66.9 mg/100g, 55.7 mg/100g, 61.17 mg/100g, 54.58 mg/100g) (11.8 mg/100g, 108.9mg/100g, 113.1 mg/100g , 111.6 mg/100g) respectively. The results of sensory evaluation showed that the red tea was better in color, while there were no significant different between non-fermented (LLT) , (LLT) fermented for four hours , (LLT) Fermented for six Hours , (LLT) fermented for eight hours (3.4 B) , (3.7B) , (3.7 B) , (3.7 B) . red tea(4.4A) and (LLT) fermented for four hours(4.1 A) was better in flavor compared the rest of the samples, while the (LLT) fermented for eight hours (3.4 AB) ranked middle the samples however (LLT) fermented for six(3.0 B ,) hours and (LLT) non-fermented (3.0 B) measured on late order.) that the red tea(4.3 A) was significantly higher in taste but not significantly different from the (LLT) fermented for four hours(4.0 AB) , while the non-fermented (LLT)(3.2 BC) and (LLT) fermented for eight hours(3.7 ABC) ranked second and the (LLT) fermented for six hours(2.8 C) was last. Overall acceptability of (LLT) red tea(4.5 A) was better but not significantly different from the (LLT) fermented for four hours(3.4 AB) , while the (LLT) fermented for eight hours(3.2 BC) and non-fermented (LLT)(3.3 BC) ranked second .and (LLT) fermented for six hours(2.7 C) ranked last.

The results of this study revealed that It possible to produce caffeine-free tea from lime (citrus aurantifolia)leaves -(LLT) fermented for four hours can be alternative for red tea and it is good drink for people who suffering from high level of calcium due to its lowest calcium content.

المخلص

اجريت هذه الدراسة في جامعة السودان للعلوم والتكنولوجيا كلية الدراسات الزراعية قسم علوم وتكنولوجيا الاغذية ، لتصنيع شاي خالي من الكافيين من ورق الليمون (البلدي).اوراق الليمون تم الحصول عليها من مزرعة جامعة الخرطوم كلية الدراسة. تم غسل الاوراق وتقسيمها الى اربعة عينات 250 جرام لكل عينة ، ثم تعريض ثلاثة مجموعات منها لعملية الذبول لمدة اربعة عشر ساعة لخفض كمية الرطوبة في الاوراق لان عملية الذبول تساعد على تحسين اللون والطعم في الشاي. بعد اكتمال فترة الذبول تم تخمير الاوراق في اواني بلاستيكية محكمة القفل ، لان عملية تخمير الشاي مهمة بالنسبة للون والطعم والجودة الكلية للشاي. وكان التخمير لثلاثة مجموعات لمدة (4 ساعات , 6 ساعات ، 8 ساعات) على التوالي وتركت العينة الرابعة من غير تخمير حيث سحنها وتجفيفها.

وبينت نتيجة التحليل الكيمائي لشاي ورق الليمون بان لا توجد فروقات معنوية في عينات شاي ورق الليمون: الغير مخمر ، المخمر لمدة اربعة ساعات ، المخمر لمدة ستة ساعات والمخمر لمدة ثمانية ساعات في نسبة كل من (الرماد ، الرطوبة ، البروتين ، الدهن ، الالياف)((3.7% , 1.5% , 6.3% , 0.3% 6.3%) ،(3.6% , 1.4% , 6.7% , 6.6% , 6.6%) ،(3.5% , 1.6% , 5.3% , 6.2% , 6.2%) ,((3.8% , 1.5% , 6.4% , 6.5% , 6.5%) على التوالي.اظهرت نتيجة تحليل المعادن ان هناك فروقات معنوية بين العينات في نسبة الكاسيوم ، ونجد ان عينة شاي ورق الليمون الغير مخمر (169.8 مللغم/100ج) وعينة شاي ورق الليمون لمدة ستة ساعات (167.6 مللغ/100ج) كان نسبة الكالسيوم فيهما عالية مقارنة ببقية العينات ، في حين احتلت عينة شاي ورق الليمون المخمر لمدة اربعة ساعات (135.6 ملغ/100ج) على المرتبة الثانية ، بينما عينة شاي ورق الليمون المخمر لمدة ثمانية ساعات (154.3 ملغ/100 ج) كان تركيز الكالسيوم فيها بنسبة متوسطة .ولا توجد فروقات معنوية بين نسبة كل من (الحديد ، البوتاسيوم ، الماغنيزيوم ، الفسفور) في شاي ورق الليمون : الغير مخمر ، المخمر لمدة اربعة ساعات ، المخمر لمدة ستة ساعات ، المخمرو لمدة ثمانية ساعات (169.8 مللغرام/100 جرام , 1.2 ملغرام /100ج , 66.9 ملغرام/100ج , 118.8 ملغرام/100جرام , 7.8 ملغرام/100 جرام) ، (135.6% ملغرام/100ج , 0.8 ملغرام/100ج ، ملغرام/100ج , 108.6ملغرام/100ج , 6.0 ملغرام/100ج) ، (167.6 ملغرام/100ج , 1.3 ملغرام/100ج , 61.17 ملغرام/100ج , 113.1 ملغرام/100ج , 6.5 ملغرام/100ج) ، (154.3 ملغرام/100ج , 0.7 ملغرام/100ج , 54.58 ملغرام/100ج , 111.6 ملغرام/100ج , 5.6 ملغرام/100ج) على التوالي. واضحت نتائج التقييم الحسي ان هناك فروقات معنوية بين العينات في صفة اللون ونجد ان عينة الشاي الاحمر (4.6 A) تفوقت معنويا على العينات الاخرى واحلت المرتبة الاولى في حين ان لا توجد فروقات معنوية بين شاي ورق الليمون : الغير مخمر ، المخمر لمدة اربعة ساعات ، المخمر لمدة ستة ساعات والمخمر لمدة ثمانية ساعات (3.4 B ، 3.7 B ، 3.7 B ، 3.7B) . ايضا بينت نتائج التقييم الحسي ان كل من عينات الشاي (4.4A) وشاي ورق الليمون المخمر لمدة اربعة ساعات (4.1 A) تفوقا في صفة النكهة عن بقية العينات ، في حين ان عين شاي الليمون المخمر لمدة ثمانية ساعات (3.4 AB) احتلت مرتبة وسطية بين العينات ، بينما شاي اوراق الليمون الغير مخمر (3.0 B) وعينة شاي اوراق الليمون المخمر لمدة ستة ساعات (3.0 B) تحصلتا على مرتبة متاخرة في صفة النكهة. كذلك بينت نتائج التقييم الحسي ان الشاي الاحمر (4.3 AB) تفوق معنويا في صفة الطعم ولكن لا يختلف معنويا عن شاي ورق الليمون المخمر لمدة اربعة ساعات (4.0 AB) ، في حين ان عينات شاي ورق الليمون الغير مخمر (3.2 BC) وشاي ورق الليمون المخمر لمدة ثمانية ساعات (3.7 ABC) تحصلتا على

المرتبة الثانية في حين نجد ان عينة شاي ورق الليمون المخمر لمدة ستة ساعات (2.8 C) احتلت المرتبة الاخيرة .و واضحت ايضا نتائج التقييم الحسي ان كل من عينة عينات الشاي (4.3A) وشاي ورق الليمون المخمر لمدة اربعة ساعات (3.8 AB) تفوقا في درجة القبول العام على بقية العينات ، بينما عينة شاي ورق الليمون الغير مخمر (3.3BC) وعينة شاي ورق الليمون المخمر لمدة ثمانية ساعات(3.2BC) تحصلتا على المرتبة الثانية ، في حين ان شاي ورق الليمون المخمر لمدة ستة ساعات (2.7C) تحصلت على المرتبة الاخيرة.

ومن هذه الدراسة يمكن ان نخلص الى إمكانية صناعة شاي خالي من الكافيين من وراق الليمون .اعتماد عينة شاي ورق ورق الليمون كبديل للشاي الاحمر وايضا هذه العينة مناسبة للذين يعانون من حصاوي الكلى لان تركيز نسبة الكاسيوم فيها منحفض.

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CHAPTER ONE

INDRDUCTION

Tea is manufactured from the young shoots of the tea plant Camellia sinensis, which consist of two or three leaves and an unopened terminal bud. Tea is a non-alcoholic aromatic beverage and it is generally accepted that, next to water, it is the most frequently consumed beverage in the world. It is also one of the lowest cost beverages and is consumed by a large number of people around the world (Katiyar and Mukhtar, 1996; Heneberry, 2006). The tea plant C. sinensis is native to Southeast Asia but is currently cultivated in more than 30 countries around the world (Chaturvedula and Prakash, 2011). In India, tea was used as a medicinal plant before the British arrived and introduced it as a drink for pleasure in the 19thcentury (Bhagat et al., 2010). India is the second largest producer of tea next to China. It occupies an important place in the worldwide production of tea and plays a very vital role I India's national economy. Tea is perhaps the only industry for which India has retained its leadership over the last 150 years (Gupta and Dey, 2010; JolvisPou, 2016; Kumar and JolvisPou, 2016).

Lime (*Citrus aurantifolia*) is a small perennial evergreen tree in the family Rutaceae grown for its sour fruit. The lime tree is irregularly branched and possesses sharp spines. The leaves of the tree are elliptical with small rounded teeth around the edge. The leaves can grow 4–8 cm (1.6–3.2 in) in length. The tree produces small, cupped white flowers and yellowish-green fruit which is round or egg-like in shape. Lime trees can reach 5 m (16 ft) in height and can produce fruit for many years. Lime may also be referred to as sour lime, key lime, Mexican lime, acid lime or West Indian lime and originated in southeast Asia .(Morton , J. 1987).

Citrus aurantifoliais an important medicinal and food plant widely cultivated in many parts of the world .It is valued for its nutritional qualities and numerous health benefits. The plant is used in traditional medicine as an antiseptic, antiviral, antifungal, anthelmintic, astringent, diuretic, mosquito bite repellent, for the treatment of stomach ailments, constipation, headache, arthritis, colds, coughs, sore throats and used as appetite stimulant. These health benefits of Citrus aurantifoliaare associated with its high amounts of photochemical and bioactive compounds such as flavonoids, limonoids, phenols, carotenoids, minerals and vitamins..(The Parma Innovation Journal, 2015).

General objective:

To produce an alternative to tea, which is caffeine free using lime leaves, the new tea is low cost, and has medical benefits.

Specific objectives:

1- To produce a fermented lime leaves tea
2- To evaluate the chemical composition of fermented lime leaves
3- To measure the mineral composition of fermented lime leaves
4- To assess the sensory evaluation of the of fermented lime leaves

CHATER TWO

LITRITURE REVIWE

2.1. Tea

Tea, a traditional beverage originally from China, is the oldest, most popular, non-alcoholic caffeine containing beverage in the world, and its infusion is prepared by brewing of processed leaves of the tea plant, Camellia sinensis(Kumar and Shruthi, 2014). Tea is the second most widely consumed beverage in the world following water. The most commonly consumed teas are black, green, and oolong which are all derived from the plant Camellia sinensis, a member of the Theaceaefamily. Approximately 3.0 million metric tons of dried tea is produced annually, 20% of which is green tea, 2% is oolong, and the remainder is black tea. Green tea and oolong tea are predominantly consumed in Asian countries, whereas black tea is widely consumed in India and Western countries (Anonymous, 2002). .

Tea is the most widely drunk beverage in the world (Stagg, 1980). Green tea, popular in the far east, differs from black tea familiar in the west in that an oxidation step (called fermentation) occurs in the processing of the latter compound but not the former compound . Although it has little nutritional value per se, tea refreshing, mildly stimulant and produces a feeling of well-being. The latter two properties have been assumed to be due to caffeine, about 50 mg of which is present in a cup of tea; caffeine is known to have ``stimulant and increase capacity of work`` (Rall*et al* , 1990). However, other components of a cup of tea, notably, the polyphenols, may also contribute to be the effects of tea, in view of their known pharmacological properties (Stagg and Millin,1975). The complex of

oxidized polyphenols in tea is often called ``tannins`` (Kirk andOthmer, 1980). It should be stressed, however, that unlike some compounds from other plants also given this generic name, tea tannins are not harmful. (Stagg,1980 ;Wheeler,1976).

2.1.1Tea – Plant varieties

There are mainly three tea plant varieties according to geographical distinction such as China, Assam and Cambodia. These three varieties have a number of hybrids.

The Chinese variety is a multi- stemmed bush growing as high as 9 feet and is a hardy plant, able to with stand cold winters and has an economic life of at least 100 years. The Assam variety is a single stem tree ranging from 20-60 feet in height and includes several sub varieties, having an economic life of 40 years with regular pruning and plucking. The Cambodian variety is a single stem tree growing to about 16 feet in height and is not cultivated but has been naturally crossed with other varieties (ICRA, 2006).

2.1.2 Types of made tea

Tea is traditionally classified based on the degree of fermentation (oxidation) the leaves have undergone. The quality of the final product depends upon the quality of leaves plucked and preservation during processing. Two leaves and a bud are the raw material for all types of made tea but the difference lies in the method of processing as explained below:

White tea: made from young leaves that have undergone no oxidation. White tea is produced in lesser quantities and is more expensive than tea from the same plant processed by other methods. It is not sowell-known in countries outside China.

Green tea: the oxidation process is stopped after a minimal amount of oxidation by application of heat. The tea is processed within one to two days of harvesting. The fresh green appearance of made tea can be procured only if pure China jat leaf is used. The manufacturing process involves heat treatment of the freshly plucked leaves through rolling and drying in stages to inactivate the enzymes responsible for fermentation and every carries taken to retain the color and the chemical make-up of the tea leaf (UPASI, 2003).

Oolong: Oolong is semi fermented tea where the oxidation process takes 2-3 days.

Black tea: in back tea production, the tea leaves are completely allowed to oxidize. In the manufacture black tea, conditions are regulated to encourage the change of color of tea leaf to blackish brown. Orthodox and CTC are the two methods of manufacturing of black tea.

Orthodox is the older method of producing tea with enhanced flavor and emphasis of briskness, rather than color, thicknesses and strength. Preservation of tips lends an attractive look to orthodox tea. Tea leafs in the CTC method are subjected to more vigorous and harder processing, leading to more rapid and intensive oxidation and fermentation. The liquor is thick, strong and more intensive in colour. Higher ‘cuppage’ of per unit weight of made tea is the plus point of CTC(ICRA, 2006).

2.1.3 Chemical constituents

Different classes of compounds found in tea discussed: ploy-phenols, amino acid, caffeine, nucleotides, carbohydrate, lipids, organic acid, chlorophyll, carotenoids, unsaponifable compounds, saponins, and volatile compounds. (Food Reviews International , 1995).

2.1.4 Health Benefits of tea

Green tea: Ant cancerous, cardio protectant, regulate body temperature, blood sugar and promote digestion. Black tea: Decrease blood pressure, ant mutagenic, cardioprotectant. White tea: Anti-obese, ant mutagenic, anti-oxidative potential.(A. jain*et al* , 2013).

2.1.5 Manufacturing Process

Tea leaves are first plucked from the gardens which are normally spread o hundreds of acres of land and then transported to the factory. In the factory, it goes through various stages of processing to get made tea.

2.1.5.1Plucking:

Most green tea types are produced using young, tender leaves. The leaf unit harvested varies with the specific type of green tea. Many green tea types include the terminal bud, the internodes and 1e3 leaves immediately below the bud . For some other green tea types, the harvested unit consists of a single young leaf. Older leaves are generally not used to produce high-quality green teas because these leaves are rough with an astringent flavor. Tea leaves are plucked by hand, or are mechanically harvested by a plucking machine. Many high quality green teas are hand plucked. Farmers may hand pluck up to 30 kilos of fresh tea leaf per day. Hand plucking is the most labor intensive and expensive process in tea cultivation and has been replaced in many instances by mechanized pluckers to increase labor productivity and decrease labor costs. Leaves are plucked from the same plant at intervals of four days to two weeks.(castle.L. 1997).

2.1.5.2 Withering

The process of withering involves partial removal of moisture from fresh leaf and is carried out in order to condition the leaf physically for subsequent processing. Besides, some chemical changes also take place during withering and these are independent of the physical process. Thus, withering involves (a) Physical wither and (b) Chemical wither. While the physical wither can be completed even in 3-4 hours, however for completion of the chemical wither, a period of 12-16 hours is required as such the withering trough can't normally be used more than once a day. Withering is carried out either by Natural Withering or by Trough withering system. The green leaves that are spread upon the wire mess of withering trough are charged with cold and warm air through an axial flow fan so that the moisture content is reduced to the desired level. Generally the level of reduction in moisture depends upon the grades and quality of tea, which is to be manufactured.(NEDF and ISO , 2008).

2.1.5.3 Rolling:

The withered leaf are rolled to rupture the leaf cells and release of enzymes and to give a twist to the leaf. It is achieved by processing withered leaf in Rotor vane. During rolling operation chemical changes among the principal constituents of leaf start as soon as the juice of leaf is squeezed out in contact with the air. The

chemical changes are caused by the enzyme present in the leaf. The enzyme brings about chemical changes but it does not change itself. Generally, leaf is rolled in Rotorvane before send to further processing.(NEDF and ISO ,2008).

2.1.5.4 Operating on C.T.C Machines:

After the leaves are rolled, they are put into the C.T.C machine (i.e. cutting, tearing and curling machine). This machine cuts the leaf into uniform size with maximum cell distortion leading to quicker and more even oxidation during fermentation. The C.T.C machine is comprised of two rollers rotating in opposite directions at the arranged speed. The speed of the two rollers are different, one of the roller is fast rotating at a speed of around 675 revolutions per minute, whereas the slow roller rotates at a speed of 60 to 73 revolutions per minute. Generally, a constant clearance between the rollers is maintained. The roller segment is in sharp condition, which cut the leaves three times. During the process it is specifically seen that leafs are not heated as it destroys briskness and quality. (NEDF and ISO, 2008).

2.1.5.5 Fermentation:

Fermentation of tea is the most significant step in tea manufacturing since in this Step the most important properties of tea i.e. Liquor characteristic develops. The term 'Fermentation' is rather historical and does not accurately describe the process that occurs during the manufacture of black tea. This process involves enzymic oxidation/ degradation of polyphenols, lipids, carotenoids and tarpene-glycosides , and their subsequent condensation/degradation leading to formation of coloured polymers and aroma and flavor compounds. (Goswami*et al.*, 1999).After processing in the C.T.C machine the leaf are fermented.

Fermentation of the tea leaf is a very important process in Tea manufacture for briskness, strength,colour and quality largely depend upon it. The duration of fermentation varies according to rise and fall of temperature. A temperature of 76’F to 78’F represents the ideal temperature of the fermenting room and it takes

roughly between 1 to 2 hours in the fermenting process. The leaf processed in C.T.C is spread on the fermenting floor or fermenting bed of fermenting machine. Generally, they are spread at a thickness of half an inch. Fermentation begins as soon as the juice of the leaf come into contact with the air enzyme present in the leaf being about chemical changes among the constituents of the leaf cell such as latechins(polypheno's) and caffeine. When the leaf become bright red in the fermenting room it is the best time to transfer to the drying room for firing.(NEDF and ISO, 2008).

2.1.5.6 Drying:

The fermented tea practices are dried or fired to arrest the fermentation and also to reduce the moisture to about 3%.clean and odorless hot air varies between 90-160c depending on the type of dryer. Drying or firing is thermal energy intensive operation that also consumes electronic energy to drive blowers and dryers.(university of moratuwa Sir lank)

2.1.5.6 Grading:

Black tea is usually graded on one of four scales of quality. Whole leaf teas highest quality followed by broken leaves, fanning, and dusts. Whole leaf te are produced with little or no alteration to the tea leaf. This results in a finish product with a coarser texture than that of bagged teas. Whole leaf teas are wide considered the most valuable, especially if they contain leaf tips. Broken leave are commonly sold as medium grade loose teas. Smaller broken varieties may included in tea bags. fanning's are usually small particles of tea left over from t production of larger tea varieties, but are occasionally manufactured specific for use in bagged teas. Dusts are the finest particles of tea left over from product of the above

varieties, and are often used for tea bags with very fast, very har brews. Fanning's and dust are useful in bagged teas because the greater surfer area of the many particles allows for a fast, complete diffusion of the tea into t water. Fanning and dusts usually have a darker color, lack of sweetness, a stronger flavor when brewed.(Agriculture, forestry and fisheries- republic of south Africa).

2.1.5.7 Packaging:

After the tea has been sorted into respective grades, it is necessary to pack these in suitable packages/sacks so that the quality of made tea does not deteriorate in transit. Tea is packed by packing machines having magnetic attachment to prevent possible pieces of iron metal fillings from mixing with tea. Efforts are made to prevent tea from absorbing moisture. Tea chests used for packing tea are moisture-proof.

In most of the tea gardens in Assam, the present mode of packing is not absolutely air-tight and as a result tea absorbs more than 3% moisture by the tiine it reaches market. It has been found that plywood tea chests with lining of alumina and tissue paper, metalized polyester or cellophane are suitable for packing tea. (Das A.K, 1999).

2.2 lime(Citrusaurantifolia)

Citrus aurantifolia (Christm) Swingle (C. aurantifolia) is a polyembryonic plant cultivated inmany countries all over the world and grows in hot subtropical or tropical regions such as Southern Florida, India, Mexico, Egypt, and the West Indies (Patil RJ,2009) The plant belongs to the kingdom: Plantae; Phylum: Magnoliophyta; Class: Magnoliopsida; Order: Sapindales; Family: Rutaceae; Genus: Citrus and Species: Citrus aurantifolia(Sethpakdee R, 1992) C.

aurantifoliais commonly called Lime (Nigeria), Key lime, Mexican lime, Sour lime, Dayap, bilolo, Indian lime, Egyptian lime (USDA,2013).

2.2.1. citrus cultivation in Sudan:

The Sudan grows are diversity of citrus species and varieties for local consumption. The most rootstock in sudan is sour lime orange (citrus aurantium) (khairy et al, 1969, Dawoud , 2004). The agro-climatic conditions are in favor of lime and grapefruit production (Dafalla, G, 2004). The yield in Sudan is low (appr. 6-7/ tons hectare) compared to the actual potential (Abdilrouf, 2004). This might be attributed to the following factors : low nutritional inputs and inadequate cultivation , the use of infect planting materials , planting of unimproved cultivars of sections and rootstocks (Elamin , O, 2004) and inadequate use of chemical for pest and diseases control (Dafalla, G, 2004). However limited exports of lime and grapefruit to the gulf states and Europe have been reported (Dafalla, G, 2004).

2.2.2Plant Description:

C. aurantifoliais a small shrubby tree, about 5 m tall. It is an evergreen and ever bearing tree that is densely and irregularly branched and possesses short and stiff spines (thorns). The leaves are alternate; elliptical to oblong-oviate (4-8cm×2-5cm) shaped and has a crenulate margin. The flowers are 1 inche in diameter and are yellowish white with a light purple tinge on the margin. The fruits are globose to ovoid berry of about 3 - 6 cm in diameter and sometimes have apical papilla. It is yellow when ripe but usually picked green commercially. The fruits and flowers appear throughout the year but are most abundant from May to September in the Northern hemisphere. The fruit peels are very thin with densely glandular segments with yellow-green pulp vesicles. The fruit juice is acidic and fragrant, sour as lemon juice but more aromatic. It is usually valued for its unique flavor

compared to other limes. The seeds are small, plump, ovoid, pale, and smooth with white embryo.(Sethpakdee R, 1992).

2.2.3 Brief history and geographical distribution of C. aurantifolia:

C. aurantifolia is believed to have originated from the south East Asia around 4000 BC and is native to the Indo-Malayan region. It was assumed to have been carried to North Africa anther near east by the Arabs; and was taken by crusaders from Palestine to Mediterranean Europe. It was introduced to the Caribbean Island and Mexico by the Spaniards and redial became naturalized in the West Indies and Mexico (Morton J 1987) . The current major citrus producing countries in the world are Spain, USA, Israel, Morocco, South Africa, Japan, India, Brazil Turkey and Cuba(Patil RJ,2009) .In Nigeria, about 930,000 tons of citrus fruits are produced annually from an estimate million hectares of land . The major citrus producing States in Nigeria are: BenueNassarawa, Kogi, Ogun, Oyo, Ebonyi, Kaduna, Taraba, Ekiti, Imo, Kwara, Edo and Delta. .(Taiwo TA,2005).

2.2.4 Domestic Uses of Citrus aurantifolia

Citrus fruits are consumed worldwide in form of fresh fruit or are processed into citrus products and citrus-by-products. Approximately, one third of total citrus production is utilized for processing (Okwu DE, 2008). C. aurantifoliajuice is used as flavorings in beverages; and it is also used by juice producing companies to produce fruit juice which are presented inform of freshly squeezed orange juice or frozen concentrated orange juice. The juice is squeezed from fresh fruit and packaged in paper cartons, glass or plastic containers, without being pasteurized. In addition, C. aurantifoliafruits can be processed to obtain other food products such as dehydrated citrus products or marmalades, jams, sorbet, pickles, jellies, candies and sugar boiled. Citrus essential oils are another by-product of citrus fruits.

Essential oils are volatile oils obtained from the citrus fruit peels' sacks. They are used by the food industry to give flavor to drinks and foods. They are also a component for the pharmaceutical industry for the preparation of drugs, soaps, perfumes, hair cream, body oil and other cosmetics as well as for home cleaning products (Yano M,1999). C. aurantifoliajuice and sometimes fruit peels are combined with vinegar to produce disinfectant; it is combined with salt to clean copper pot. It is used to polish brass, aluminum and copper. It is combined with peroxide or used alone to bleach cloth, remove ink, rusty and mineral stains from cloths; whitening tannin shoe and soften fabrics (Yano M,1999) . C. aurantifoliafruit juice has been used to preserve the quality of "zobo" drink in Nigeria. It was reported to decrease the total coliform and other bacterial counts in "zobo" drink. Thus, the plant can be used to prolong the shelf life of "zobo" drink (Nwachukwu E, 2007) C. aurantifoliafruit peels have been found very useful domestically. In the kitchen, it is used for cooking, to addflavour to food, cakes and roasted chicken. It is used to garnish salad and to add flavor to drinking water. It is used for bathing and washing of hair, and also, used as a deodorizer to freshen up smelly garbage and composite pile. It also serves as natural room freshener (Effiom OE, . 2012).

2.2.5 Ethnomedical Uses of Citrus aurantifolia:

C. aurantifoliain its natural state is widely used in West Africa, particularly in Nigeria where it is employed in herbal medicine to treat several illnesses. It forms an essential ingredient in the preparation of most herbal concoctions (Odugbemi T,2007) Different parts of the tree have been used traditionally to cure some illnesses:

2.2.6Leaves:

The decoction of pounded leaves is drunk for stomach ache, used as eye wash and to bath feverish patient. Poultice of leaves are applied to ulcer wounds, used for skin disease and also applied to abdomen after child birth. Crushed leaves are applied to forehead to treat headache and it is squeezed near the nostril for irritant inhalation to treat nausea and resuscitate fainting individuals (Khan IA, 2010) . Infusion of C. aurantifolialeaves have been given to treat fever with jaundice, sore throat and oral thrush(Khan IA, EA ,2003). A decoction of the flower is believed to help induce sleep for those with insomnia (Khan IA, 2010) . In southwest Nigeria, thelimoniods and flavonoids are among the major phytoconstituentinC.aurantifolia responsible for the anticancer activity (Jayaprakasha GK,2008) . The essential oil of C.aurantifolia has 78% inhibition of human colon cancer cells, DNA fragmentation and apoptosis induction as revealed from a study and it is suggestive of the potential use of the plant for prevention of cancer especially colon cancer(Poulose SM, Harris ED, Patil BS,2005) .

2.2.7 Antioxidant activity:

Studies of the juice and fruit peels (Boshtam M, Moshtaghian J, Naderi G, Asgary S, Nayeri H, 2011) as well as leaves(Loizzo MR, Tundis R, Bonesi , M, Menichini F, De Luca D, Colica C, Menichini,2012) of F

C.aurantifolia revealed that the plant has a concentration-dependent effect on low density lipoprotein (LDL) oxidation. The antioxidant activity of C(Boshtam M, Moshtaghian J, Naderi G,2011). Aurantifoliawas ascribed to their hydrogen

donating ability which may be due to the presence of flavonoids, carotenoids and Vitamin C . Flavonoids present in C. aurantifoliafruit juice and peels demonstrate their antioxidant activity by inhibiting the enzymes responsible for superoxide anion production such as xanthine oxidase and protein kinase C(Hanasaki Y, Ogawa S, Fukui S,1994) .

2.2.8Anti-obesity activity

Studies by(Asaasnri, A, B , R , S , 2010) on the effect of essential oil of C. aurantifoliaon weight gain in mice showed that mice treated with the extract displayed a reduction in both the amount of food intake and body weight compared with the control group. Another interesting finding was that co-administration of C. aurantifoliaessential oil and ketotifen caused a significant suppression of weight gain and also decreased weight gain in mice. The weight loss was ascribed to the fact that C.aurantifoliamay possibly promote anorexia which may play significant role on weight loss.

2.2.9Effects on the bone

The efficacy of C. aurantifolia and C. sinensis against osteoporosis was evaluated in an ovariectomized rat model, and the result revealed that the administration of citrus extracts increased the trabecular bone mineral content, and bone mineral density of tibia, improved the levels of phosphorus and calcium. The result demonstrates that C. aurantifoliapossesses the ability to reduce bone loss. (Shalapy NMM, A , HA , B , 2011).

Chapter Three

MATERIAL AND METHODS

3.1 Materials

Samples of lime (citrusaurantifolia) leaves was obtained from the farm of university of Khartoum faculty of agriculture. and all chemicals are of analytical grade .

3.2 Preparation of Lime Leaves

Most green leaves are plucked by hands , then the leaves were washed thoroughly with water to remove dust and any foreign materials , and then the leaves were divided into four groups and exposed to wilt process for 14 hours , the withered leaves are rolled , and then three groups were exposed to fermentation process:

A-Non-fermented sample.

B- Fermented for four hours.

C-fermented by sunlight for six hours.

D-fermented for eight hours.

All samples were fermented in plastic containers. The fermented lime leaves tea are dried and they are ready to use.

3.3 Methods

3.3.1 Chemical methods

3.3.1.1 Moisture content

Moisture content was determined according to the AOAC (1990) as

Follows:

Two grams of sample were weighted using sensitive balance in clean dray and pre-weighted crucible and then placed in an oven at 105 ^{o}C and left overnighted. The crucible was transferred to desiccators and allowed to cool and then weight. Further placements in the oven were carried out approximately constant weight was obtained.

Moisture content was calculated using the following formula:

MC %=(W2-W1)-(W3-W1)/(W3-W1) *100

Where:

MC=moisture content

W1=weight of empty crucible.

W2= weight of crucible with sample.

W3 = weight of crucible and the sample after drying .

3.3.1.2 Crude protein

The crude protein in the sample was determined by the micro-Kjeldahl method following the method of the AOAC (2003).

Principle

Lime leave tea sample was digested with a strong acid (sulphuric acid) so that the sample releases its nitrogen content which can be determined by a suitable titration technique. The amount of protein in the sample is then can be calculated from the nitrogen concentration of the sample. A conversion factor of 6.25 (equivalent to 16 g nitrogen per 100 grams of protein) was used in this study. The kjeldahl method is divided into three steps which can be summarized under the following:

A) Digestion

The lime leave tea samples (0.2 grams) was transferred into a digestion flask and then digested by heating the sample for 2-3 hours in (3.5N) sulphuric acid. The digestion process was catalyzed by a mixture 0.4 of 10 parts k_2so_4 to one part of $Cuso_4$. The heating was continued till the black color turned to pale blue and the fumes disappeared.

B) Distillation

After the digestion has been completed the digestion flask was cooled and transferred to a distillation unit using a minimum volume of water. The solution in the distillation unit was then turned alkaline by addition of 20 ml of sodium hydroxide (40%) to release the ammonia. The released ammonia was distilled into 20 ml of 2% boric acid in a conical flax, adding to it 2 to 3 drops of Bromochresol Methyl red as indicator.

C) Titration

The nitrogen content in the sample was then estimated by titration of the ammonium borate formed with a standard hydrochloric acid (0.1N).The titrations continued till the color of the solution turned to red-pink. The protein concentration as per-cent was determined by using the following equation:

$$\%\text{Crude protein} = \frac{TV \times N \times 14.00 \times F \times 100\%}{\text{Sample weight(g)} \times 100}$$

Where:

TV: actual volume of HCL used for sample titration (ml sample- ml blank).

N: normality of HCL.

F: Protein conversion factor = 6.25

3.3.1.3 Fat content

The sample oil content was determined by using a continuous extraction apparatus (soxhlet type), as described by Pearson (1970).

About five gram (5±1) samples were weighed and transferred to an extraction thimble covered with a piece of glass wool and then placed in the soxhlet apparatus .After that, the solvent (petroleum ether) was added into a dried weighted soxhlet flask and the extraction process was continued for about six hours. Then, the oil sample was dried in an oven (Carblite, Sheffield, England) for a 30 min to eliminate any remaining amounts of the solvent and the flask was reweighted. The fat per-cent was calculated by using the following equation:

$$\%\text{Crude fat} = \frac{(W1 - W2) \times 100\%}{\text{Sample weight (g)}}$$

Where:

W1= weight of the empty soxhlet flask (g).

W2= weight of soxhlet flask with oil content (g).

3.3.1.4 Determination of carbohydrates:

Carbohydrates were determined by difference according to the following equation:

Total carbohydrates = (MC+AC+FC+CF+CP)

Where;

MC=moisture content

AC= ash content

FC= fat content

CF= crude fiber

CP=crude protein

3.3.1.5 Crude fiber

The crude fiber of the lime leave tea was determined following the standard method described by the Association of Official Analytical Chemists (AOAC, 2003).

Principle

The crude fiber is determined gravimetrically after the sample is chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as crude fiber.

A sample of grams 2.0 gram was weighted and two hundred (200) ml of sulphuric acid (0.26N) were added, boiled for 30 min and then filtered. The residue washed three times by using hot water and after that 200 ml of NAOH (0.26N) was added, boiled again for 30 minutes and filtered. Then, the reside was carefully washed three time with hot water and until it was free from alkali. After that, the sample was dried in an oven (Carblite, Sheffield, England) at 105 ^{o}C(overnight) and reweighted. The residue was ached in a muffle furnace (LEF- 103S, serial No. 07033002, Korea) at 550 C^{o} for three hours till a light grey ash was formed and a constant weight was obtained .Then, the total crude fiber per-cent was calculated using the following equation:

$$\text{Crude fiber \%} = \frac{(W1 - W2) \times 100\%}{\text{Sample weight (g)}}$$

Where:

W1= weight of the sample before ignition (g).

W2= weight of sample after ignition (g).

3.3.1.6 Determination of ash content

The ash content of the sample was determined according to the **AOAC (2003).**

Procedure:

The empty crucibles were accurately weighed and then two grams of powder of lime leaf tea were transferred to each crucible by using a sensitive balance. Then, the crucibles and their content were placed in muffle furnace at 550^{o}C to 700^{o}C for more than 6 hours until while to grey ash was obtained. After that, the crucibles

were transferred from the furnace to desiccators to cool to room temperature and re-weighted. The ash content was calculated by following equation:

$$\text{Ash content (\%)} = \frac{(W1 - W2) \times 100\%}{\text{Sample weight (g)}}$$

Where:

Wt1= weight of crucible with the remaining ashed sample (g).

Wt2= weight of empty crucible (g).

3.3.2. Determination of minerals

Ten milliliters (10 ml) of HCL (2N) were added to the remaining ash sample and placed in hot sand path for about 10-15 min. After that, the sample was filtered and diluted to 100 ml in a volumetric flask. Then, the trace elements ferrous (Fe^{++}) and were determined according to Perkin Elmer (1994) by using Atomic Absorbance Spectroscopy (JENWAY 3110, UK). Sodium (Na) and potassium (K) were determined by using Flam Photometer (Model PEP7 JENWAY). While, calcium (Ca), magnesium (Mg), and phosphorus (P) were determined as described by Chapman and Parratt (1961).

3.4. Panel test

The sensory test was done by using affective (Hedonic) method for fifteen semi-trends panelist.

3.5Atatisticalanalyses

The results were analyzed using Anova with one factor. The mean were separated by Duncan's multiple range test with aid of Mstatc.C computer program .

Chapter Four

RESULTS AND DISCUSSION

4.1 Chemical Composition of Lime Leaf Tea:

The results of statistical analysis in table (1) figure (1) showed that there were significant differences between the samples. The results show that the sample of lime leaf tea (LLT) non-fermented(15.1%) and (LLT) fermented for six hours(16.24%) and (LLT) fermented for eight hours (14.78%)are better in ratio of carbohydrates and there were no significant between them .While (LLT) fermented for four hours(11.27%) occupied the second place. And there are no significant differences between ash (3.7% , 3.6% , 3.5%, 3.8%) and moisture (1.5% , 1.4% , 1.6% 1.5%) and protein (6.3%, 6.7% 5.4%, 6.4%) and fat (0.3%, 6.6% , 6.2% , 6.5%) and fiber(6.3%, 6.6% , 6.2% , 6.5%) this indicates that fermentation effect was in color , taste , flavor and did not affect the chemical components.

Table No (1) : Chemical Composition Of Lime Leaf Tea

Samples	Ash	Moisture	Protein	Fat	Fiber	CHO
Non-fermented	3.700 A	1.500 A	6.300 A	0.3160 A	6.367 A	**15.1 A**
Fermented four hours	3.600 A	1.420 A	6.733 A	6.660 A	6.600 A	**11.27 B**
Fermented six hours	3.500 A	1.630 A	5.433 A	6.200 A	6.267 A	**16.24 A**
Fermented eight hours	3.800 A	1.500 A	6.433 A	6.533 A	6.500 A	**14.78 A**
LSD	2.259	0.9339	3.766	0.3766	2.824	**3.427**
C.V	32.88	32.81	32.13	61.16	23.32	**12.68**

Means followed the same letter are not significantly different.

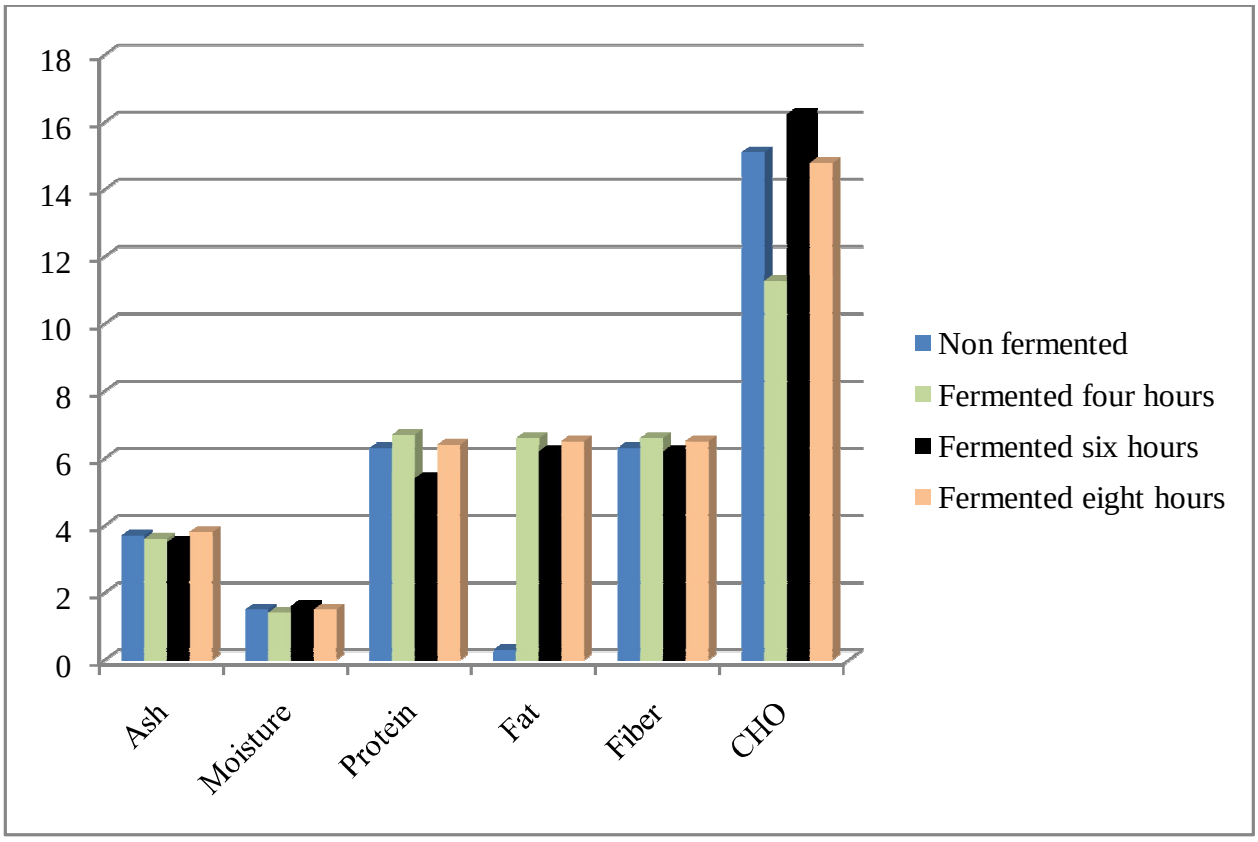


Figure (1) chemical compsotion lime (citrus aurantifolia) leaf tea

4.2 minerals content of lime(citrusaurantifolia) leaf tea:

4.2.1 Calcium:

The results statistical analysis in table (2) figure (2) figure(3) , showed that non-fermented (LLT)(169.8 mg/100g) and (LLT) fermented for six hours(167.6 mg/100g) had high calcium content compared to the rest of samples , while the (LLT) fermented for eight hours(154.3 mg/100g) occupy the second class and the lowest percentage in calcium was in (LLT) fermented for four hours(135.6 mg/100g) and there is no significant between it and the (LLT)fermented for eight hour((154.3 mg/100g).

Also the results shows there were no significances differences between (Fe, mg, k, p) Fe (1.2 mg/100g, 0.8 mg/100g , 1.3 mg/100g , 0.7 mg/100g) Mg (66.9 mg/100g , 55.7 mg/100g , 61.17 mg/100g , 54.58 mg/100g) K(11.8 mg/100g , 108.9mg/100g , 113.1 mg/100g , 111.6 mg/100g) because the effect of fermentation was in the color and test and flavor.

Table (2) showed the minerals content of lime citrus (aurantifolia) leaf tea:

Samples	**CA**	**Fe**	**Mg**	**K**	**P**
Non-	169.8 A	1.2 A	66.9 A	118.8 A	**7.8 A**

fermented					
Fermented 4 hours	135.6 B	0.8 A	55.7 A	108.9 A	**6.0 A**
Fermented 6 hours	167.6 A	1.3 A	61.17 A	113.1 A	**6.5 A**
Fermented 8 hours	154.3 AB	0.7 A	54.58 A	111.6 A	**5.6 A**
LSD	18.83	0.04800	13.18	18.83	**2.824**
C.V	6.38%	21.74%	11.77%	8.84%	**23.08%**

Means followed the same letter are not significantly different.

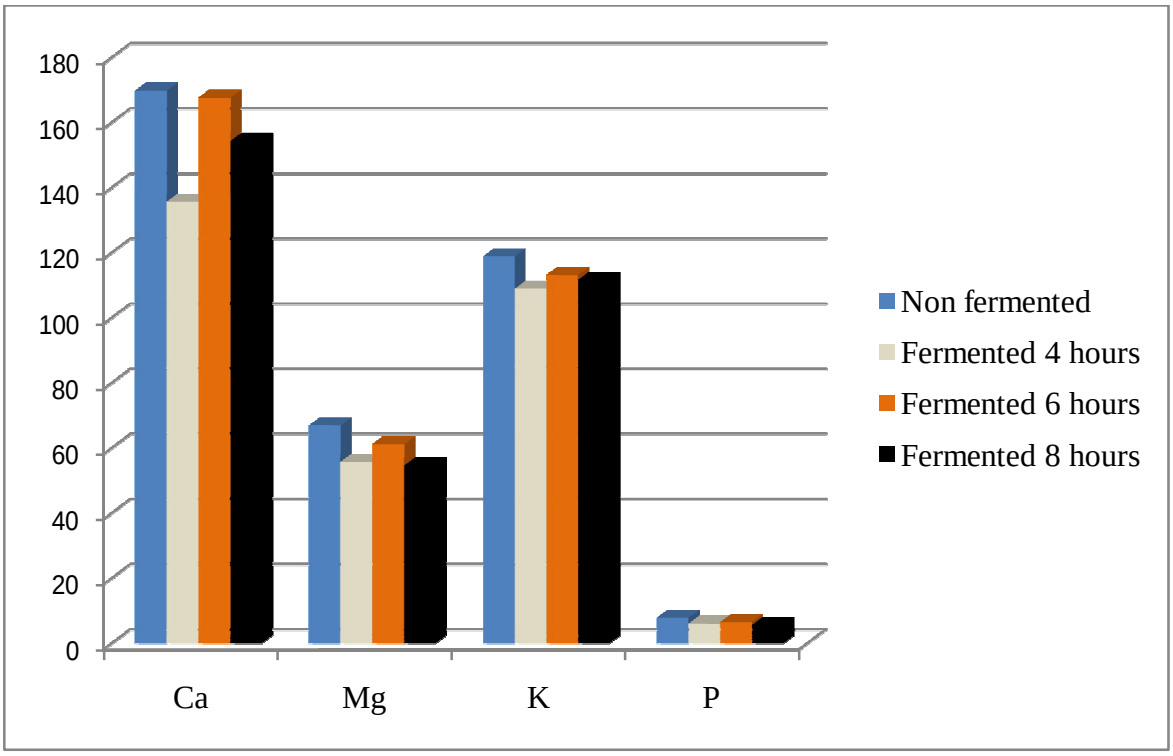


figure (2) shows minerals content in lime (citrus aurantifolia) leaf tea

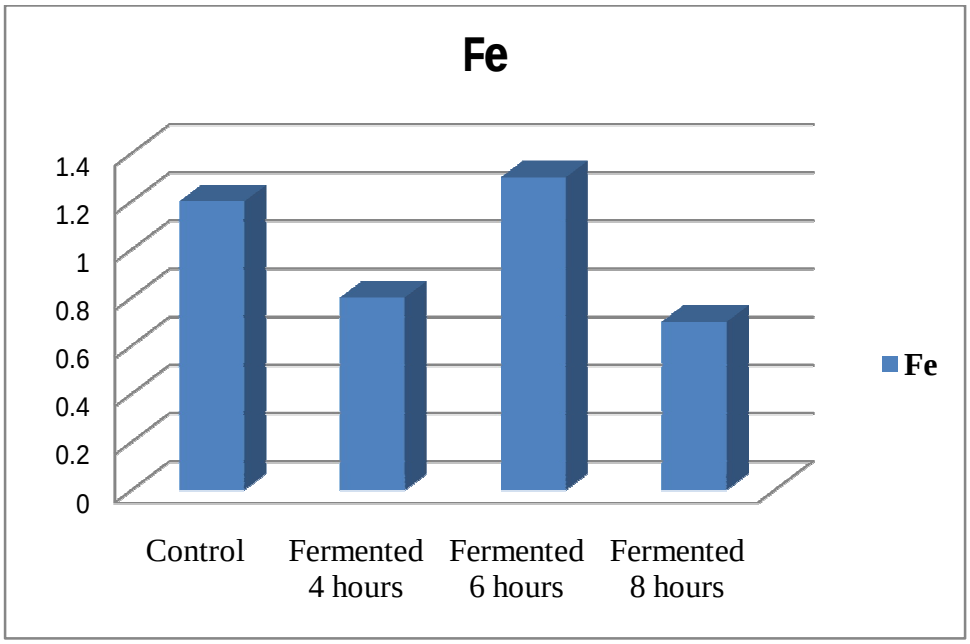


Figure (3) shows Fe content in lime (citrus arantifolia) leaf tea:

4.3 Sensory Evaluation of Lime (Citrusaurantifolia) Leaf Tea:

4.3.1 Color:

The results of statistical analysis table (3) figure (4) showed that there were significant differences between samples (3.4 B , 3.7 B , 3.7 B , 3.7 B) the red tea(4.6 A) sample was significantly better than other samples in color and ranked first. However the rest of the samples did not have significant differences between them and ranked second.

4.3.2 flavor:

table (3) figure(3) showed that each of samples of red tea(4.4A) and (LLT) fermented for four hours(4.1 A) was better in flavor compared the rest of the samples, while the (LLT) fermented for eight hours(3.4 AB) ranked middle the samples however (LLT) fermented for six(3.0 B ,) hours and (LLT) non-fermented (3.0 B) measured on late order.

4.3.3 Taste:

According to (table 3 , figure 4) that the red tea(4.3 A) was significantly higher but not significantly different from the (LLT) fermented for four hours(4.0 AB) , while the non-fermented (LLT)(3.2 BC) and (LLT) fermented for eight hours(3.7 ABC) ranked second and the (LLT) fermented for six hours(2.8 C) was last.

4.3.2 Over All Acceptability:

The results of statistical analysis table (3) figure (3) showed that the red tea(4.5 A) was better but not significantly different from the (LLT) fermented for four hours(3.4 AB) , while the (LLT) fermented for eight hours(3.2 BC) and non-fermented (LLT)(3.3 BC) ranked second .and (LLT) fermented for six hours(2.7 C) ranked last.

Table (3) shows the sensory evaluation test:

Samples	color	flavor	Taste	overall acepteblity
Red tea	4.6 a	4.6 a	4.3 a	**4.5 a**
Non-fermented	4.3 b	3.4 b	3.2 bc	**3.3 bc**
Fermented four hours	3.7 b	4.1 a	4.0 ab	**3.8 ab**
Fermented six hours	3.7 b	3.0 b	2.8 c	**2.7 c**
Fermented eight hours	3.7 b	3.4 ab	3.7 abc	**3.2 bc**
LSD	0.8174	0.9315	0.050	**0.8979**
C.V	23.95%	30.52%	3.554%	**27.61%**

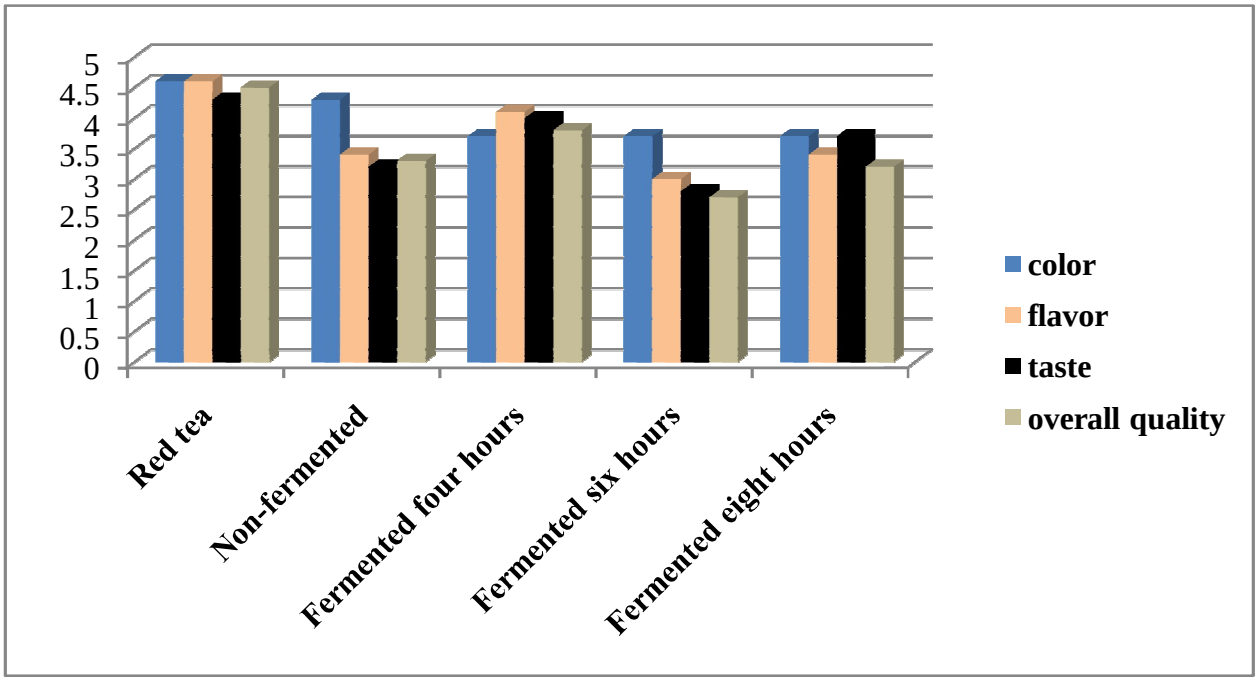


Figure (4) shows the sensory evaluation test of (LLT)

Chapter five

Conclusion and recommendations

5.1 Conclusion

1-The results of this study showed that it possible to manufacture free caffeine tea from lime (citrusaurantifolia) leaf at lower cost and high nutritional value .

2-the results of sensory evolution of lime (citrus aurantifolia) leaf tea showed it was acceptable to the tasters.

3- This study also confirmed that the fermented lime leaf tea for four hours was better than the rest of the sample and ranked first.

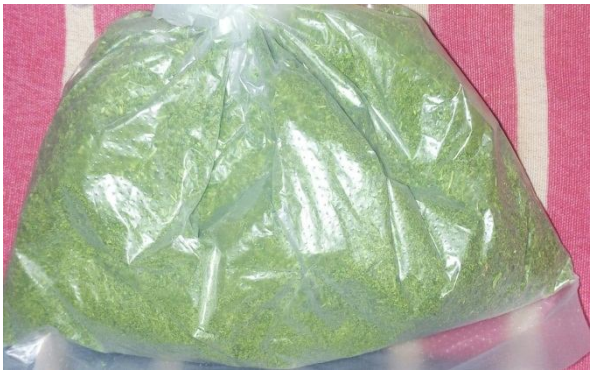
5.2 Recommendations

1. To conduct more studies to manufacture tea from various citrus lavas.
2. To drink lime (citrus aurantifolia) leaf tea every day because of it nutritional benefits.
3. To drink lime (citrus aurantifolia) leaf tea in the evening it contains no caffeine which is found in tea and coffee, caffeine can keep you from sleeping well.

Appendices:

Lime (Citrusaurantifolia) leaves during withering process.

Rolling machine



Powder of non-fermented lime (citrusaurantifolia) leaf tea

Powder of citrus (aurantifolia) leaf tea fermented for four Hours

Powder of lime (citrusaurantifolia) leaf tea fermented for six hours

Powder of lime (citrus aurantifolia) leaf tea fermented for eight hours.

Lime (citrus aurantifolia) leaf tea.

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