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Evaluation of carbohydrate and phenol content of citrus fruits species

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Abstract
Carbohydrate content (Nutritional) and Phenol content (Anti-nutritional) of six varieties of citrus fruit species: Sweet orange (*Citrus sinensis*), Lemon (*C*itrus *limonum*), Mosambi (Citrus *limetta*), Pomelo (*C. maxima*), wild orange (Citrus aurantium) and mini orange (*C. microcarpa*) were analysed in this study. The results of the analysis revealed the presence of carbohydrate and phenol contents. Carbohydrate contents were found to be higher in Citrus maxima with the range of 3.690±0.705. In *Citrus sinensis* and *Citrus limonum*, carbohydrate content is low (2.983±0.0026 and 3.073±0.001) when compared with other citrus fruits. The total phenol content was higher in *Citrus sinensis* with the range of 1.3036±0.0031.Carbohydrate and Phenols have been responsible for the biological, antioxidant activities.

Keywords: Carbohydrate, phenol content, citrus fruits

Introduction
Citrus fruits are one of the most important fruit crops, which belong to the family of rutaceae, and are grown throughout the world. All citrus fruits are acidic in nature. Citrus fruits are identified as having a pleasant or sweet smell, and a juicy pulp. Pulp is a soft, wet, shapeless mass of material. Citrus fruits include oranges, lemons, grapefruits, limes, and tangerines those with a tough skin and juicy pulp inside. Oranges are a great spring time snack with a refreshing taste. It is a good source of carbohydrate with high antioxidant potential. Citrus fruits contain a variety of vitamins, minerals, fiber and phytochemicals such as carotenoids, flavonoids, and limonoids, which appear to have biological activities and health benefits. There is considerable evidence that citrus fruit have antioxidant and anti-mutagenic properties and positive associations with bone, cardiovascular, and immune system (Franch and Valls-Belles,*et al.,* 2010) [5]. Taxonomic identification of these species of fruit trees is difficult because there are many spontaneous and commercial hybrids, but citrus can be generally classified into the following categories; sweet oranges and clementines (*C. clementine*), sour/bitter oranges (*C. aurantium*), lemons (*C. limon*) and limes (*C. aurantifolia* and *C latifolia*) pummelos (*C. grandis*). Many citrus fruits, such as oranges, tangerines, grapefruits, and clementines, are generally eaten fresh. They are typically peeled and can be easily split into segments. These fruits are rich in essential nutrients, vitamins and minerals and hence very important for the immune system of out body.
Citrus sinensis, orange or sweet orange is a small tree belonging to Rutaceae that originated in southern China. Oranges are now grown commercially worldwide in tropical, semi tropical and some warm temperate regions. They have become the most widely planted fruit tree in the world (Bailey *et al.,* 1976) [3]. Fruit typically contains juicy and sweet pulp, divided into 10 to 14 segments and ranges in color from yellow to orange to red. Lemon Fruits are citrus fruits dark green in colour and with thick pulp, moderately juicy and acidic. *Citrus limetta* is a sweet lemon. Fruit, light yellowish orange in colour, surface rough with prominent streaks on the rind, well-defined segments (9 to 12), and pulp is light yellow in colour and juice is sweet. The sweet lime (*Citrus limetta*), is commonly known as Mosambi in Indian subcontinent. It is native to Asia and best cultivated in India, China, southern Japan, Vietnam, Malaysia, Indonesia and Thailand. This fruit is eaten fresh or squeezed to make juice, a rich source of vitamin C and replenish energy, (Arias and Ramon, 2005) [2]. Lemon is one of the most widely used citrus fruits worldwide.

Lime, is other close relative, is comparatively smaller and possesses thinner skin. The fruits in this study are good sources of B complex such as pantothenic acid, pyridoxine and folates which forms the basis for their anti cancer property.

Citrus maxima are the largest citrus fruit. Citrus maxima commonly known as Pomelo, Chinese grapefruit, Pommelo, Jabong, Shaddock, a crop plant of India, China, Japan, Indonesia, United state of America, Philippine, Thailand, (Bailey *et al.,* 1976) [3]. The tree has large evergreen elliptic leaves, 10.5 to 20 cm (4 to 8 in) long, with winged petioles. The flowers and fruits are borne singly, in contrast to grapefruits, in which they grown in clusters of 2 to 20. The hot leaf decoction is applied on swellings and ulcers. The fruit juice is taken as a febrifuge. The seeds are employed against coughs, dyspepsia and lumbago, (Morton and Pummelo,*et al*.,1987) [15]. The fruit include treatment of coughs, fevers, cardiotonic, cancer and gastrointestinal disorders (Van Wyk, *et al.,* 2005) [20]**.**

Citrus aurantium is the scientific name for the plant commonly referred to as bitter orange, sour orange. Bitter orange is a member of the Rutaceae family, a hybrid between Pummelo (*Citrus grandis)* and Maderin (*Citrus reticulate).*This plant is native to Asia. Various parts of the plant are used worldwide for a variety of indications. Bitter orange and its components are commercially available in herbal weight loss supplements, (Preuss *et al.,* 2002) [17] often in combination with other ingredients hypothesized to promote weight loss. Calamondin (*Citrus microcarpa*) is a small citrus fruit. The peel is thin and smooth, yellow to yellow-orange and easily separable. It is moderately drought-tolerant.

Citrus Fruits are also important sources of Carbohydrate and phenolic compounds and other kinds of phytochemicals. Citrus fruits are rich sources of antioxidants such as ascorbic acid, phenolics, pectins etc.which are important for human nutrition (Fernandez *et al*., 2005) [9] Carbohydrate is the main energy-yielding nutrient in citrus. It also contains the simple carbohydrates; fructose, glucose and sucrose as well as citric acid which can also provide a small amount of energy. They also contain a variety of vitamins, minerals, fiber, and phytochemicals such as carotenoids, flavonoids, and limonoids etc. which have health benefits. There is considerable evidence that citrus fruit have antioxidant and anti-mutagenic properties and positive associations with bone, cardiovascular, and immune system health (Codoner and Valls, 2010) [5]. Phenolic compounds are excellent antioxidants due to their ability to donate an electron or hydrogen from phenolic hydroxyl groups.

Materials and Methods

Sample collection

The experimental citrus fruits comprising sweet orange (*C sinensis*), Lemon (*C. limonum*), mosambi (*C. limetta*), pomelo (*C. maxima*), wild orange (*C.aurantium*) and mini orange (*C. microcarpa*). The citrus fruits were purchased from a local market and were evaluated for the carbohydrate and total phenolic components.

Taxonomical Information of selected Citrus Fruit Species

Citrus sinensis
Kingdom: Plantae
Order: sapindales
Family: Rutaceae
Genus: *Citrus*
Species: *C. sinensis*

Citrus limonum
Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: *Citrus*
Species: *C. limon*

Citrus limetta
Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: *Citrus*
Species: *C. limetta*

Citrus maxima
Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: *Citrus*
Species: *C. maxima*

Citrus aurantium
Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: Citrus
Species: *C. aurantium*

Citrus microcarpa
Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: Citrus
Species: *C. microcarpa*

Sample preparation

Six different species of citrus fruits were peeled out and for reducing the surface area the endocarps and mesocarps were cut into smaller pieces with a sharp clean knife. The juices from six citrus fruit samples were pressed out from the fruit, preserved and stored in airtight bottles in a refrigerator until analysis.

Plate 1: Pomelo

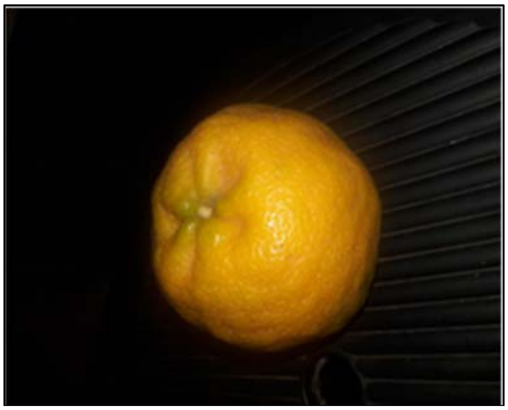

Plate 2: Sweet orange

Plate 3: Lemon

Plate 4: Mosambi

Plate 5: Bitter orange

Plate 6: Mini orange

Determination of Carbohydrate

The fruits were cut into smaller pieces with a sharp clean knife. One gram of the fruit sample was weighed and grinded. This was transferred into extracting tube and the extracts were mixed with 10 mL of distilled water and shaken for few minutes. This was filtered into 100 mL flask and transferred into a centrifuge tube and centrifuged at 10000 rpm for 10 minutes. A known quantity (0.1 mL) of the extract was pipetted into boiling tube and filled with distilled water (0.9mL) and colour was developed by addition of 4 mL of anthrone reagent. This was allowed to stand over a hot water bath for about 10 minutes. The absorbance was measured at 620 nm in a spectrophotometer after colour change of solution.

Determination of Phenol

One gram of sample was taken and reflexed it in 80% methanol for 20 minutes. It was ground thoroughly and filtrate was taken. The filtrate was subjected to centrifugation at 1000 rpm for 10 minutes and the supernatant was collected. The filtrate was made it to a known volume by the addition of methanol to it. An aliquot of 0.1mL from this was taken in to a tube. Folins reagent (0.5mL) was added to the mixture followed by the addition of 2mL 20% Na_2CO_3 and kept in a boiling water bath for 5 minutes. The white precipitate formed was centrifuged at 5000 rpm for 5 minutes. Absorbance was measured at 650 nm in a spectrophotometer.

Result and Discussion

This study was undertaken to compare the carbohydrate content and phenol content of different varieties of fruits of *Citrus* species. The values for carbohydrate and phenol content are shown in Table 1. The carbohydrate content was found to be highest in *Citrus maxima* with the range of 3.690±0.705 g GAE/g. In *Citrus* sinensis and *Citrus limonum*, carbohydrate content was found to be relatively low (2.983±0.0026 g GAE/g and 3.073±0.001 g GAE/g respectively) when compared to other citrus fruits. The total phenol content was higher in *Citrus sinensis* with the range of 1.3036±0.0031 g GAE/g. The presence of phenol indicates that the citrus fruits could act as anti-inflammatory, anti-clotting, antioxidant, and hormone modulators. Phenols have been responsible in having the ability to block specific enzymes that causes inflammation.

Table 1: Carbohydrate and phenol content

Citrus fruit	C. *sinensis*	C. *limonum*	C. *limetta*	C .*maxima*	C. *aurantium*	C. m*icrocarpa*
Carbohydrate	2.983±0.0026	3.073±0.001	2.911±0.0134	3.690±0.705	2.544±0.0026	2.342±0.0022
Phenol	1.3036±0.0031	1.871±0.0017	1.551±0.0015	2.332±O.0021	2.050±0.0024	0.053±0.0015

Citrus fruits have high calorific value. This may be due to the carbohydrate content of the fruits of this species. The carbohydrate content also acts as mild natural laxative for human beings. The carbohydrate content of citrus fruits comprises mainly of dietary fiber, sucrose, glucose and fructose. With the exception of dietary fiber, all other carbohydrate (glucose, fructose and sucrose) are water soluble and sweet tasting sugars. The results are comparable with the works of earlier works. People with diabetes must always use sugars in moderation, remembering that the same amount of sugars is better tolerated when taken in its natural form in fruits containing organic acids and phytochemicals (Roger, 2002) [18].

Ghasemi *et al.,* (2009) [11-14] in his study used percolation with methanol and found out that the highest content of phenolics was in the orange peel (232.5 mg GAE/g), while the lowest content was found in the use of lemon peel (102.2 mg GAE/g). Petchlert *et al.,* (2013) [16] reported that the content of phenolic compound detected in juices of citruses was lower in comparison with the literature available at the period of the work and reported the values to be ranging from 5.71 ± 0.01 mg GAE/mL to 10.57 ± 0.17 mg GAE/mL for mandarin and orange, respectively. Reason for these differences might be in diverse types of extraction and solvent used, as well as the different origin of the samples. Results of determination of total antioxidant activity using molybdenum reduction method.50 present the concentration of extract to reduce 50% of molybdenum cation (Ghasemi *et al.,* 2009) [11-14].

The sequential order of antioxidant activities of citrus samples were studied from highest to lowest, (Garden *et al.,* 2000) [10] and was found that the order is such that orange, lemon, grapefruit and mandarin juices and from the grapefruit, lemon, mandarin and orange peels. There was no correlation between antioxidant activity and the levels of total phenolic compounds and flavonoids in the citrus samples. (Garden *et al.,* 2000) [10] found 66% and 81% antioxidant activity in orange and Jaffa orange juices, respectively, there was no correlation between antioxidant activity and the phenolic compounds identified in the extracts determined that orange juice is rich in phenolic compounds (naringin, hesperidin) and consequently has a high antioxidant capacity. They also showed that lemon juice had a greater antioxidant capacity than other citrus fruits**.**The antioxidant capacities of orange juices seem to be widely influenced by anthocyanin concentrations (Tripoli *et al*., 2007) [19].

Phenolic compounds are carriers of antioxidant activity in plant extracts, (Zheng and Wang, 2001) [21]. Some phenolic compounds demonstrate antioxidant activity (Majo *et al*., 2005) [6]. They proved hesperidin has a higher antioxidant activity than neohesperidin. They suggested it could be caused by the lack of hydroxyl group in the 7th position orange juice, lemon juice and their peels contained the highest levels of hesperidin; this could be related to their high antioxidant capacities.

A high quality orange is one that is mature with good colour intensity uniformly distributed over the surface. Such oranges must be firm with a fairly smooth texture and shape that is characteristic of the variety, free from decay, defects and other blemishes. The biological activity and the health effects of citrus flavonoids as antioxidants have been reported by earlier workers, (Tripoli *et al*, 2007) [19]. These group of pigments as found in plants and together with anthocyanin play a role in flower and fruit colouration. Also, they are present in dietary fruits and vegetables and exercise their antioxidant activity in several ways, including the activities of metal chelation, (Bombardelli and Morazzoni, 1993) [4]. Studies indicate that flavonoids are excellent radical-scavengers of the hydroxyl radical due to their ability to inhibit the hydroxyl radical and donate hydrogen atom, (Majo *et al.,* 2005, Tripoli *et al.,* 2007) [6, 19]. Oranges contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases, (Ejaz *et al.,* 2006) [8]. Sweet orange contains a variety of phytochemicals like hesperetin and narigenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator. Antioxidants are substances that known to delay or inhibit oxidation, (Halliwell, 1995) [13]. Plant component especially fruit has antioxidant components that are able to reduce oxidative stress, (Agudo *et al.,* 2007) [1]. Fruits are also important sources of vitamins, minerals and other kinds of phytochemicals. Bioactive compounds in fruit that having antioxidant properties are carotenoids, polyphenols, anthocyanins and vitamins (Dillard and German, 2000) [7].

Conclusion

Citrus fruits contain a large variety of nutrient and anti-nutrient components are considered as potential sources of functional components. Presented results reveal that citrus fruits are of good quality and a valuable source of health promoting constituents (carbohydrate as a nutrient and phenol as an antioxidant). Presented results suggest carbohydrate and phenolic compounds that could be responsible for the biological and antioxidant activity respectively. They also provide natural refreshment to the skin. The amount of citrus needed to provide health benefits is quite variable but appears to be within reasonably consumable amounts. One to three glasses of orange juice a day appears to provide improvement in antioxidant, cardiovascular, and insulin sensitivity phenolic component may also be beneficial to health.

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