

ORIGINAL ARTICLE

Estimate the Vitamin C and Antioxidant Activity of Pomelo (*Citrus maxima* (Burm.) Merr) Fruit Juices Using Different Extraction Methods

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ABSTRACT Pomelo (*Citrus maxima*) is a citrus fruit native to Southeast Asia, and is considered a good source of dietary fibre and antioxidant materials. The outside of the pomelo fruit (the peel and the pith) contains high levels of pectin, which has been shown to lower blood sugar and cholesterol (Tocmo *et al.*, 2020). Citrus Fruits such as Pomelo and its by-products are considered under-utilized. These by-products are high in dietary fibers and carotenoids and are rich source of value-added compounds such as polyphenols, essential oils, pectin which can be utilized in food, cosmetic and pharmaceutical industries to develop novel products with numerous health benefits (Panwar *et al.*, 2021). So, a study was put through in order to raise the value of Pomelo fruit in India. Hence the present study on the topic "Estimate the Vitamin C and Antioxidant Activity of Pomelo (*Citrus maxima* (Burm.) Merr) Fruit Juices using Different Extraction Methods" was executed. **Methods:** Pomelo fruit (Red and White variety) were used for the study. Extraction of pomelo juice by different methods like cold-press, manual squeeze and blender were conducted. Vitamin C analysis was done using iodometric titration. Total antioxidant activity of juices was done using DPPH assay. **Results:** Organoleptic evaluation of Cold-pressed Pomelo juice, Red and White variety was carried out in which Sample A2 (Red) has the highest overall acceptability with mean score (3.8 ± 0.447). Vitamin C content in Cold-pressed juice was the highest 45.5 ± 7.07 , and decreased in the order Manually Squeezed > Blender with a range between 44.5 ± 7.21 and 31.85 ± 6.01 mg/100 mL. Sample A4 (Manually Squeezed White Pomelo juice) had the lowest IC₅₀ value of 57.51 (µg/ml) that shows a high antioxidant activity in Manually Squeezed White Pomelo juice. **Conclusion:** Citrus Maxim a which is an unrecognized under utilized fruit without considering its nutritive properties and health benefits. From this study it can be concluded that pomelo fruit can be utilized effectively by developing novel products that will have good health benefits as well as having therapeutic potential in terms of significant antioxidant activity and vitamin C content which will increase the demand of the fruit. Red variety pomelo was found to be superior in the therapeutic effects and acceptability. Large amount of waste is produced from pomelo mainly when they are juiced these by-products can be utilized effectively in food application.

Keywords: *Citrus maxima*, Pomelo, Cold-press, DPPH assay, Vitamin C, Organoleptic evaluation, Underutilized fruit

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INTRODUCTION

Citrus is one amongst the largest plant species that consists of forty species which are distributed worldwide. Citrus is one

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of the foremost vital fruits, which is consumed principally fresh and has been used as a flavoring medication or additive or food supplement. Citrus is believed to possess bioactivities like antioxidant, anti-inflammatory, antimicrobial, and is usually recommended to be accountable for the interference of cancer and chronic diseases (Karimi *et al.*, 2012). Citrus fruits are best-known to contain bio-active compounds like phenolics, flavonoids, vitamins, and essential oils that are believed to be liable for a variety of protecting health benefits together with anti-oxidative, anti-inflammatory, anti-tumour, and anti-microbial activities (Oikeh *et al.*, 2016). A number of studies have proven the presence of polyphenols, vitamins, minerals, dietary fibres, essential oils and carotenoids content in citrus fruits which make it a health-benefit promoting fruit (Rafiq *et al.*, 2018a). Citrus juices are consumed majorly attributable to their biological process and special flavour. Fruit juice consumption is beneficial for the upkeep of better health and hindrance of diseases. The positive health advantages of juices have been ascribed partially to vitamin (ascorbic acid), the most important vitamin found in fruits and vegetables (Hafid Boudries, 2012). The word “underutilized” refers to the categories of plant species in which the potentiality has not been fully realized. Underutilized fruits and vegetables, which are only grown in some regions possess immense nutritional potential, but are not consumed or distributed world wide (Mann, 2016). Lifestyle changes and raised awareness among health-conscious consumers have driven the beverage industry to develop and introduce purposeful drinks with additional nutritional values and health-promoting benefits. Moreover, the invasive demand for freshly squeezed un-pasteurized fruit and vegetable juices reflects changes in client preferences for beverages which does not undergo processing (Khaksar *et al.*, 2017). Regular fruit juices are extracted by centrifugal juicers that utilize a fast-spinning metal blade close against a mesh filter. This method separates the juice extract from fruit flesh by centrifugal force. Once the metal blade spins at a relatively high speed, it generates heat, which may negatively have an effect on the bio-active compound present in the fruit juice (Khaksar *et al.*, 2017). Cold-pressed juices are extracted by cold-pressed extractors that initially crush then press the fruit to extract its juice at a very low speed. This extraction method generates nearly no heat and preserves the nutritional quality of the fruit juice (Khaksar *et al.*, 2017). Cold pressed juice can help in reducing the stress of detoxification on the body. These juices are loaded with nutrients that aid in fighting toxins and boosting immunity and health. Cold pressed juice is so potent and instantaneous because it removes the fiber of the whole fruits. Body does not need to break the fiber down in the digestive system before it is to be used, cold pressed juice is like a kick start to the whole system. The

nutrients immediately enter the body and start its work (Franklin, 2018). *Citrus maxima* commonly known as shaddock, a citrus variety usually referred to as “pomelo” is a common fruit widely fully grown and consumed fresh or processed into juice (Tocmo *et al.*, 2020). It is a tropical plant species which originated from Southeast Asia and are widely cultivated in some parts of West Africa. *Citrus maxima* contains two parts; the peel and pulp which can be easily separated out. The pulp is light coloured or pink and coarse with large, spindle-shaped juice sacks (Ani and Abel, 2018). Pomelo contains a history of cultivation and consumption geological dating back 4,000 years ago. Pomelo fruit reaches ten to thirty cm in breadth and may weigh up to one to two kilo normally and can also depend on the cultivar (Tocmo *et al.*, 2020). Pomeles are rich source of antioxidant flavonoids, and are good source of potassium, phosphorus, folic acid, citric acid, etc. The peel is made into processed products such as marmalade and candy or sometimes dipped in chocolate and is also used for cooking (Kore *et al.*, 2017). Based on recent investigatory studies, hesperidin, a bioactive flavonoid found in citrus peel, which is well known for its high binding affinity to the main cellular receptors of SARS-CoV-2. Which is said to be beneficial for prophylaxis and treatment of COVID-19, along with other coexistent flavonoids such as naringin, which could help restrain the pro-inflammatory over-reaction of the immune system (Meneguzzo *et al.*, 2020).

OBJECTIVES

This research article entitled “Estimate the Vitamin C and Antioxidant Activity of Pomelo (*Citrus maxima* (Burm.) Merr) Fruit Juices using Different Extraction Methods” was focused on the following objectives: To analyse the physical properties of Pomelo (*Citrus maxima* (burm.) merr) fruit, to estimate the Vitamin C content and anti-oxidant activity in different extraction methods of Pomelo (*Citrus maxima* (burm.) merr) juice, to evaluate organoleptic acceptability of cold-pressed Pomelo (*Citrus maxima* (burm.) merr) (red and white var) juice. It is expected that by-products can be utilized effectively in food application as large amount of waste is produced from pomelo mainly when they are juiced.

MATERIALS AND METHODS

Physical Properties of Pomelo Fruit

One valuating the physical properties of atypical pomelo fruit. The rare two varieties of pomelo available which are red and white. Pomelo fruit is somewhat round in shape with a circumference of around 45cm which was measured by a standard Freeman's measuring tape. On cutting the fruit into equal halves, fruit had a length of 10 cm each and a width of 13 cm. The pomelo fruit has a thick rind (thin flavedo and

thick white albedo) which is about 2 cm in length and breadth which protects the flesh inside from external turbulences (Sirisomboon and Lapchareonsuk, 2012). Inside of the pomelo contain flesh with juicy sacs, and seeds. Seeds are found in large amount in white pomelo when compared with red pomelo.

Selection of Fruit

Pomelo an under-utilized citrus fruit (Dewi *et al.*, 2016) was selected for the study. It is a fruit with a potential for commercialization. Pomelo fruit for the study was procured from nearby localities of Kollam and Alappuzha. Two varieties of pomelo were taken for the study, commonly classified as white flesh or pigmented one with red flesh. The fruit used were sound and was good quality.

Quality Evaluation

The quality of fruits and vegetables constitutes a dynamic composite of their physicochemical properties and consumer perception (Kyriacou and Roupheal, 2018). Quality of the fruit was assessed by analyzing their size, colour, ripeness. The process of external ripening of citrus fruit mainly depends on the conversion of chloroplast to chromoplasts and also reduction of chlorophylls and addition of carotenoids which change peel colour from yellow to green. Changes associated with external ripening are comparable to the senescence of vegetative chlorophyllous tissues and it is also influenced by environmental changes, availability of nutrients and hormones (Iglesias *et al.*, 2007).

Extraction of Pomelo Juice (Red and White Var) by Different Extraction Methods

Citrus Juices are mainly the fruit juices consumed worldwide, many essential nutrients and health-related bioactive molecules such as vitamin C, limonoids, flavonoids, minerals, fatty acids, sugars and carotenoids are found in citrus juices (Xu *et al.*, 2014). Pomelo fruit is rich in naringin content, it is a bitter tasting fruit with flavanone glycoside and reported antioxidant. They are generally consumed as fruit as it tastes sweet when ripe, and is slightly acidic with a point of bitterness. Pomelo shows properties such as appetizing, cardiac stimulant and antitoxic. Due to the bitterness of juice, *Citrus maxima* have low commercial value (Kumar *et al.*, 2017). Different methods of extraction of Pomelo juice have been tried to find the best method to extract pomelo juice and to increase its commercial value.

Different Extraction Methods

Pomelo's (Red and White Var) that are ripe and matured were washed and peeled by hand. The thick fruit rind was peeled off after incision of longitudinal cuts with a sharp knife onto the spongy rind to reveal the juicy segment of fruit flesh. The fruit was separated into individual segments and the lamella was peeled and discarded. Care must be taken to avoid any seeds or peel because only fruit flesh was used for the study (Dewi *et al.*, 2016). Juice was prepared by three different methods such as Cold-Pressing, manual squeezing and blenderized.

Figure 1: Cold-Pressed Pomelo Juice (Red and White Var)



Cold-Pressed Pomelo Juice

Cold-pressed pomelo juice was procured from a juice shop at Ernakulam, Vytilla named Raw Squeezed-Kerala's first made to order cold-pressed juice. Two Pomelo of both the varieties were given for juicing. 300 ml of Juice each was given in glass bottles. There was no addition of water or any other sweetener to the juice.

Hand Squeezed Pomelo Juice

For this method Apex Fruit and Vegetable Hand Juicer was used to extract the juice. The fruit segments were filled in the feeding tube and pressed with the pressure cup. There is a rotator handle which will move the produce to ash redder plastic mesh. This extracts maximum juice and leaves the rind behind giving clear fruit juice in the collection glass. 100ml fresh pomelo juice was obtained from both the varieties of Pomelo fruit without adding water and sugar.

Blenderized Pomelo Juice

Preethi mixer grinder was used for juicing. Pomelo segments were directly put into the juicer jar and blended till fully grinded without adding water or sugar then the juice were filtered using a fine mesh. 100 ml clear Pomelo juice from both the varieties was obtained.

Vitamin C Analysis of Pomelo Juices

Vitamin C or ascorbic acid, is a nutrient which is considered as the safest and most effective water-soluble vitamin. Vitamin

C is found mostly in fruits and vegetable (Nweze *et al.*, 2015). Vitamin C content of the Pomelo Juices extracted by different methods were analyzed titrimetrically by Iodometric titration method. Test was carried out in CEPCL Laboratory & Research Institute, Kollam.

Anti-Oxidant Activity of Pomelo Juice

Anti-oxidant activity of Pomelo Juice extracted by different methods was carried out in CEPCL Laboratory & Research Institute, Kollam. Anti-oxidant activity of the juices was estimated by *in vitro* method-DPPH radical scavenging assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is commonly used to determine antioxidant activity of plant extracts (Chen *et al.*, 2020). The DPPH radical scavenging activity is normally classified in terms of inhibition percentage of the pre-formed free radical by antioxidants, the parameter to express the antioxidant capacity is the IC₅₀ value (concentration required to obtain a 50% antioxidant effect). This value is applied to compare the activity of different compounds (Chen *et al.*, 2013). Linear regression analyses was used to estimate the IC₅₀ values. Use of standardized units resulted in similar curves and IC₅₀ values for the same natural antioxidant under different concentrations of the DPPH radical (Martinez-Morales *et al.*, 2020b). Radical scavenging activity was calculated by using the following formula:

Inhibition (%) = $\frac{(A_0 - A_t)}{A_0} \times 100$ A₀ represents absorbance Control and A_t is for absorbance sample respectively.

Figure 2: Commercial Cold-Press Juicer



Organoleptic Analysis of Cold-Pressed Pomelo Juice (Red and White Var)

Sensory analysis play an important role during various stages of food product development (Cruz *et al.*, 2010). Main objective of sensory analysis is obtaining responses in relation to foods on how they are accepted by human beings using their senses such as visual, olfactory, taste, touch, and auditory (Stone and Sidel, 2004). Organoleptic evaluation of the cold-pressed pomelo juice was carried out using an evaluation card. The sensory attributes such as appearance, colour, mouth feel, flavour, taste, sweetness, bitterness, astringency, citrus odour, fruit taste, fresh taste, pleasant, viscosity and overall acceptability were determined.

RESULTS

Estimation of Vitamin C Content

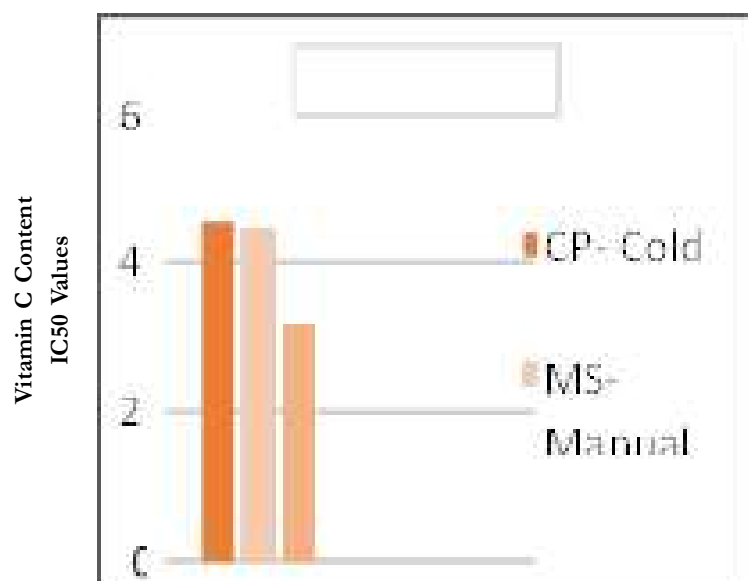
Pomelo (Red and White Var) was juiced by different juicing methods like cold pressing, manual squeezing and in blender. Vitamin C content of pomelo juice was estimated by Iodometric Titration Method. The method involved in iodometric titration is the reaction of iodine with ascorbic acid, which oxidizes it to dehydro ascorbic acid. The end point of redox titration is determined by the first iodine in increased amount that is complexed with starch, giving a deep blue-violet colour. (Silva *et al.*, 1999). The content of Vitamin C in Cold-pressed juice was the highest 45.5 ± 7.07 , and decreased in the order Manually Squeezed > Blender with range between 44.5 ± 7.21 and 31.85 ± 6.01 mg/100 mL. The lower contents in Blender juice are mainly due to the thermolysis and oxidation of Vitamin C, after blending with high-speed

rotating blades (Khaksar *et al.*, 2017; and Kim *et al.*, 2017) compared the nutritional value of grape juice which was juiced by using different juicers: low-speed masticating juicer, high-speed centrifugal juicer, and a blender. It was investigated that the juice extracted from a low-speed masticating juicer had a higher nutritional quality than the other types. In a study by (Carr and Maggini, 2017) vitamin C is an essential nutrient which is not synthesized by human body due to loss of a key enzyme in the biosynthetic pathway.

Estimation of Anti-Oxidant Activity in Pomelo (*Citrus maxima (burm.) merr*) Juice (Red and White Var) by Different Extraction Method

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a free radical based antioxidant assay on electron-transfer which produces a violet-coloured solution in ethanol. It also provide an easy and rapid way to determine the antioxidant activity (Garcia *et al.*, 2012). Antioxidant capacity has been used widely as a tool to evaluate the quality of fruit juices after processing and during storage (Khaksar *et al.*, 2017). The IC₅₀ values is estimated from nonlinear and linear regression analyses. Similar curves and IC₅₀ values were obtained with the use of standardized units for the same natural antioxidant, under different concentrations of the DPPH radical (Martinez-Morales *et al.*, 2020a). The Samples A1 to A6 are compared with the Standard BHT. BHT was used as a control standard antioxidant. Least IC₅₀ Value depicts the highest antioxidant activity and high scavenging activity. Control BHT has the lowest IC₅₀ value of 50.89 (µg/ml) when compared with the IC₅₀ Value of the Samples. Sample A4 (Manually

Figure 3: Vitamin C Content in Pomelo Juice by Different Extraction Methods



Squeezed White Pomelo juice) had the lowest IC₅₀ value of 57.51 (µg/ml) which has a difference of 6.88 (µg/ml) that shows a high antioxidant activity in Manually Squeezed White Pomelo juice as this method generates no heat and preserves the nutritional quality of the juice. A4 is followed by A2 (Cold-press White) < A1 (Cold-press Red) < A3 (Manually Squeezed White) < A5 (Blenderized Red) < A6 (Blenderized White) in increasing order of Antioxidant Activity. On comparing the antioxidant capacity of cold-pressed pomelo juice with the others it was found that there were no significant differences between different methods of extraction in Red and White variety Pomelo Juices. In a comparative study red-fleshed pomelo juice had a higher content of lycopene, β -carotene, soluble protein, and soluble solid, and also depicted a stronger (DPPH)-radical scavenging activity, reducing power activity and bile-acid-binding activity, as well as higher anti-proliferative activity in cancer cells. High amounts of lycopene and β -carotene in the red-fleshed pomelo fruits will be a lead to high antioxidant, hypolipidemic and anticancer property. Hence, red pomelo juice will be a potential natural resource for multi targeted pharmacological and oncological therapies (Qiu *et al.*, 2020).

DISCUSSION

Citrus fruits are largely cultivated, processed and is consumed globally (Panwar *et al.*, 2021). World wide production of citrus fruit has significant increase during the past few years and has reached 82 million tons in 2010 (Rafiq *et al.*, 2018b). Many citrus fruits and its by-products are going underutilized in India. Underutilized fruits may potentially benefit human health due to their flesh and skin colour. Vitamins C, Vitamin E and Carotenoids will contribute to the antioxidant capacity of under-utilized fruits. Fruits that are sour and tastes bitter as that of citrus fruits are known to exhibit high antioxidant capacity (Sarananda *et al.*, 2017). Citrus Fruit such as Pomelo and its by-products are considered under-utilized. These by-products are high in dietary fibers and carotenoids and are rich source of value-added compounds such as polyphenols, essential oils, pectin which can be utilized in food, cosmetic and pharmaceutical industries to develop novel products with numerous health benefits (Panwar *et al.*, 2021). So, a study was carried out in-order to raise the value of Pomelo fruit in India and to develop nutritionally significant product from it. Red and White varieties of Pomelo's were selected and juice was extracted with different methods such as cold-press, manual squeezing and in Blender. Total antioxidant activity of the juices was done using DPPH assay and Vitamin C analysis using Iodometric titration.

CONCLUSION

Citrus Maxima which is an under utilized fruit which is

unrecognized without considering its nutritive properties and health benefits. From this study it can be concluded that pomelo fruit can be utilized effectively by developing novel products that will have good health benefits as well as having therapeutic potential in terms of significant antioxidant activity and vitamin C content which will thereby increase the demand of the fruit. Red variety Pomelo was found to be superior in therapeutic effects and acceptability. Large amount of waste is produced from pomelo mainly when they are juiced these by-products can be utilized effectively in food application. The utilization of the fruit for the preparation of juice and novel products can solve the issues of under-utilization and related spoilage.

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