EFFECT OF ACTIVE CARBON FILTRATION ON PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF LIME JUICE

Kalugampitiya Arachchilage Dona Lakmini **Bhagya**[a], Sarath **Alahakoon**[b], Katugampalage Don Prasanna Priyantha **Gunathilake**[a*]
[a]Department of Food Science & Technology, Faculty of Livestock Fisheries & Nutrition, Wayamba University, Sri Lanka
[b]Country Style Foods, St Anthonies Rd, Kirimatiyagara, Kadawatha, Sri Lanka
[*]E-mail: (email:kdppgunathilake@yahoo.com)

Abstract
Lime (Citrus aurantifolia) is widely used as a flavor enhancer in Food Industry. Extracted lime juice cannot be preserved for long time due to the development of delayed bitterness. Therefore, industry has introduced artificial alternatives for lime juice. The aim of this research was to evaluate effect of active carbon filtration on physico-chemical properties of lime juice. Extracted juice was kept in PET bottles for one month at ambient conditions to facilitate sedimentation and the development of bitterness and then the juices were filtered through activated carbon columns. Columns of three heights (5cm, 10cm and 15cm) were used. Filtered juices were analyzed for physiochemical (pH, total soluble solids (TSS), titratable acidity-TA), total phenolic content (TPC), antioxidant activity (DPPH radical scavenging) and sensory quality (simple ranking test, 5 point hedonic scale). Based on evaluated quality of the juice obtained after filtered through 10cm activated carbon column was selected for further studies. The pH, TSS, and TA values for selected juice were 2.2, 4.8°, 5g citric acid equivalent /100mL respectively, and those values were significantly different compared to the natural lime juice (pH 2.01, TSS 7and TA 6.29g citric acid equivalent/100mL). Total phenol content of selected lime juice was 0.029mg galic acid equivalent GAE/g, which is significantly lower than natural lime juice (0.611mg GAE/g). Sensory results revealed that the sour taste and the overall acceptability of the filtered lime juice were similar to that of natural lime juice. Sensory results revealed that the natural lime juice showed higher "bitterness" compared to filtered juice. That is realized the activated carbon filtration and de-sedimentation effective for minimizing delayed bitterness. However, phenolic content of the treated juice has been significantly reduced compared with natural lime juice.

Keywords: Delayed bitterness, limonin, sedimentation, activated carbon, phenolic compound

Received: 09.04.2018 Reviewed: 22.09.2018 Accepted: 21.09.2018

1. INTRODUCTION

Lime is a citrus fruit and *Citrus aurantifolia* **(Chrism and fanz) Swingle is the type of lime cultivated in Sri Lanka**. Limes are a good source of vitamin C, and are often used to accent the flavors of foods and beverages. Limes contain acids, prominently two types of organic acids called citric acid and malic acid. Tartaric, benzoic, succinic acid, etc. are coming as traces amounts. Citric acid accounts for the largest portion of the organic acid. It's about 60% of the total soluble constituent of the edible part (Bermejo and Cano, 2012). Lime is well-known as "dehi" in Sri Lanka which is used for enhance taste of foods like sambal, curies, salads, etc., sometimes it is used for prepare thirst quenching juices. Lime fruit juice uses in the industry as a taste enhancer of mixed fruit juice; RTS (Ready to Serve) beverage making is possible with lime. It has very own smell, flavors, and also colours which improves consumer appeal. Lime is popular as a seasonal fruit and available in surplus amounts when it comes to season. But at the off season it increases the value of little number of limes and cannot be found in enough amounts. Ancient Sri Lankans are tend to keep these lime using several method like pickling, lime with salt but not give fresh taste. Though the production of lime is around 150millions in annually and about 50% of lime has been wasted due to in-proper handling, transportation and also lacking of the technology to preserve lime. Because of the increasing market demand of lime at the off season private sector tend to made artificial mimics of lime to taste consumers curies, sambals, etc. but they are not able to give taste, flavor same as fresh lime extraction.

When preserving of lime juice need to address several issues, for instance delayed bitterness is one of the important condition should be avoid during the extraction and storage. There are two bitter principles in citrus juices, the limonoids and flavanone neohesperidosides. Bitterness in intact fruit tissues and freshly prepared juice is caused by flavanone neohesperidosides in citrus such as naringin in grapefruit (Hasegawa, et al 1996**).** Limonin bitterness is developed after the juicing process and it is called as delayed bitterness. Limonin bitterness is found with all citrus species. Fruit tissues not normally containing bitter limonin; but do contain non bitter precursors of limonin, limonoate A- ring lactone. Limonin bitterness develops several hours after the juice is extracted at room temperature, or overnight if kept in a refrigerator (Hasegawa, et al 1996**).** Limonin bitterness is prominent in each and every citrus fruits which should be avoided to obtain better product. Limonoate A-ringlactone is the predominant limonoid aglycone present in fruit tissues of most citrus species, and it is gradually converted to limonin after the juice is extracted. This conversion proceeds under acidic conditions below pH 6.5 and is accelerated by the enzyme, limonin D-ring lactone hydrolase. There are several methods to remove limonin from the juice and this research was mainly focus to minimize the development of bitterness in lime juice through sedimentation and activated carbon based filtration.

2. MATERIALS AND METHODS

Materials

Properly cleaned lime fruits were purchased from a local lime supplier in Gampaha district, Sri Lanka. All the chemicals used for this study were of analytical grade.

Preparation of filtered juice

Cleaned limes were referred for the crushing process. In this process all the parts of the lime (even peel, lime seed, flavedo, albedo) were crushed to get the lime juice out from the fruit and then the juice was filtered through a cleaned cotton wool and removed the suspended particles. Extracted juice was added 75 mg/L potassium metabisulphate and was kept in PET bottles for one month at ambient conditions (30 °C) to facilitate sedimentation and the development of bitterness. Then lime juice filtered through cotton wool to remove sediments. Lime juice was then filtered using three different activated carbon columns (5cm, 10cm, and 15cm in height) as shown in Figure 1 and the filtered juices were stored at -18 °C for physico-chemical analysis.

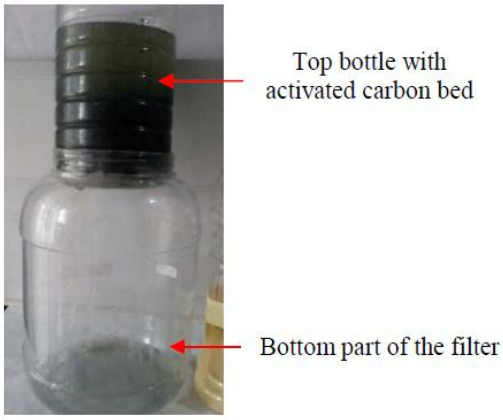

Figure 1. Apparatus used for active carbon filtration

Physico-chemical analysis

pH value was determined by using a digital pH meter (Model AA cells, pH meter, Japan). Total Soluble solid content was determined using a Brix meter (Model ATAGO, N-50B brix 0-50%, Japan). Titratable acidity of the lime juice was measured according to the methods described by Gunathilake (2012).

Preparation of methanolic extracts

One gram of juice sample and 10 mL of methanol sample were mixed together. Then the mixture was put in to the shaking water bath 50rpm at 60°C for 25 minute period. Then the sample was centrifuged for five minutes. Then the supernatant was collected and stored at -18 °C until analysis of total phenolic content and antioxidant activity.

Total phenolic content

Total phenolic content of the samples was spectrophotometrically analysed using Folin-Ciocalteau method (Singlton, 1999) with some modification as described in Gunathilake et al. (2018).

Antioxidant activity

Antioxidant activity of the lime juices was evaluated using DPPH radical scavenging assay as described in Gunathilake and Ranaweera, 2016.

Sensory analysis

Lime juices filtered with different height of carbon columns were evaluated for their organoleptic properties. Fresh lime juice was used as the control. Simple ranking test was carried out to determine whether the samples are significantly different from each other in the means of appearance (colour), taste (sour taste and bitter taste separately), aroma, and overall acceptability. These attributes were measured separately using 30 untrained panelists. Most preferred sample out of four samples was given the ranking number 4 while ranking number 1 was given to the lowest preferred sample.

Selected lime juice sample was evaluated for its optimum dilution levels to be used for the product that made to mimics of lime to taste. For this, five-point hedonic scale was used for the selection of the most preferred dilution, in comparison with two commercial products.

3. RESULTS AND DISCUSSION

Lime is an important agricultural product and has been used as are medy for indigestion, heartburn and nausea. It also has cooling effects on fevers and can help ease coughs and various respiratory disorders (Theansuwan, et al; 2008). Further, lime used as flavor enhancer too. However, delayed bitterness of juice is a common problems associated with lime. Because when the extracted juice is heated or allowed to stand, a bitter or astringent flavor usually develops, rendering it unpalatable. The intensity of this bitterness is dependent on the maturity of the fruit, being most pronounced in juices from early-season fruit, and it may vary from season to season (Ranganna et al., 1983). Food industry has been developed a number of strategies to remove bitterness. For instance, limonin might be removed by adsorption with cellulose monoacetate gel beads (Tsen, et al; 1991), genetically modifications etc. The present study was based on the evaluation of activated carbon based process for the controlling of delayed bitterness in lime juice. Activated carbon surface properties are both hydrophobic and oleophilic. In the filtration process through activated carbon, when flow conditions are suitable, dissolved chemicals in lime juice flowing over the carbon surface and “stick” to the carbon in a thin film while the other hydrophilic compound.

Physicochemical properties

The results showed (Table 1) that the pH, TA, TSS and TPC of lime juices filtered through activated carbon were significantly different ($p<0.05$) compared with the untreated lime juice. It can be seen that the pH value of the each sample treated lime juice of activated carbon after the sedimentation process was increased than the natural lime juice sample value. Further, titratable acidity of filtered juices has been showed lower value than the natural lime juice (Table 1).

Table 1. Mean values of pH, Acidity, TSS% and total phenolic content of lime juices

Treatments	pH	Acidity (g citric acid equivalent/100mL)	TSS/Brix	Phenolic content (µg of GAE/g
Filtered through a 5cm activated carbon bed	2.17±0.1	5.03± 0.06	4.90±0.6	4.70±0.2
Filtered through a 10cm activated carbon bed	2.21±0.1	5.10±0.10	4.70±0.2	4.50±0.3
Filtered through a 15cm activated carbon bed	2.31±0.0	4.32±0.01	4.03±0.4	4.50±0.1
Untreated (control) sample	2.01±0.1	6.25±0.05	7.73±0.3	6.13±0.2

This difference may be due to removal of organic acid such as citric acid during the filtration process. Citrus fruits are classed as acid fruits, because their soluble solids are composed chiefly of organic acids and sugars. The acidity of citrus juices is due primarily to their content of citric and malic acids. Trace amounts of tartaric, benzoic, and succinic acids as well as oxalic and formic acids have also been reported (Bamise and Oziegbe, 2013). Total Soluble Solid content of the juices has been reduced with the filtration process. According to Yebuda and Anglea (2010), the liminoids may exist as aglycones, or be linked to a glucose molecule (the glucoside) the level of naturally occurring phytochemicals is usually reduced or removed during the debittering process as it is believed that these phytochemicals detract from the quality of the citrus or plant juice adsorption resin to substantially reduce the level of naturally occurring components, including flavonoids, such as narirutin, hesperidin, limonoids. Therefore, with the removing of flavones, limonin, the glucosides can also be removed. That could be the reason for reduction of TSS value of treated lime juices than the control lime juice.

Sensory analyses

The simple ranking test was done to determine the effect of active carbon treatment on four quality attributes namely appearance, aroma, taste (sourness and bitterness) and overall acceptability. Sensory results (Table 2) realized that the sour taste of 10cm height activated carbon treated sample was not significantly different ($p<0.05$) from natural lime juice, while other 2 treated lime juices significantly different ($p<0.05$) from natural lime juice. However, for bitterness, natural lime juice significantly different ($p<0.05$) from the entire treated samples that are tested. For the overall acceptability lime juice treated with 10cm height activated carbon column and natural lime juice were not showed significant difference while other two samples significantly different from the natural lime juice. Moreover, appearances of the treated sample were significantly different from natural lime juice. Based on the ranking test, lime juice subjected to de-sedimentation and filtered through the 10cm height activated carbon column was selected for further comparison with commercial lime juices.

Table 2. Sensory (simple ranking test) analysis for bitter taste for natural lime juice, 5cm, 10cm, 15cm activated carbon column treated sample

Treatments	Mean value of bitterness	Mean value of sourness	Mean value of overall acceptability
15cm	3.100 ±0.96	3.267±1.05	3.567±0.68a
10cm	3.033±0.89	3.233 ±0.63	3.067±0.83a
5cm	2.400±0.89	1.833 ±0.83	1.867± 0.73b
fresh lime juice	1.467±0.94	1.700 ±0.83	1.500±0.73 b

Table 3. Selection of dilution factor based on the acceptability

Lime juice sample* with dilution (lime:water)	Aroma	Appearance	Sour taste	Overall acceptability
1:3	4.00[a]	4.00[a]	4.47[a]	4.13[a]
1:4	3.80[b]	3.60[a]	3.27[a]	3.40[b]
1:5	3.00[b]	3.46[a]	2.93[b]	3.20[b]
1:2	2.60[c]	2.80[a]	3.00[b]	2.80[c]
1:1	1.93[c]	2.67[a]	2.67[b]	2.60[c]

*Lime juice filtered with active carbon column of 10cm height

Five point hedonic scale sensory tests were carried out to determine whether there was a significant difference with two commercial samples which mimics lime taste. Lime juice sample which is filtered through active carbon column with 10cm height was diluted as in Table 3 and diluted series was tested for sensory properties. According to the sensory results (Table 3), lime juice diluted with three times water obtained the overall acceptability than the other types. Appearance wise no any sample showed significant difference with each other.

Physico-chemical properties of filtered and diluted juice

Figure 2 shows that the commercial products have lower pH values very much near to1.4-1.5 and it was lower than the value of control lime juice. Figure 3 shows that the commercial products have higher titratable acidity values (6.8 to 7.0g citric acid equivalent/100ml) and it was higher than the value of control lime juice. The selected lime juice with dilution has lower titrabale acidity than all the other tested lime juices and it is near to 4.5 g citric acid equivalent/100ml. Figure 4 showed that the commercial product have significantly higher TSS values (around 10°). That is higher than the value of control (fresh juice 7.8°). However, filtered product has lower value of TSS (4.5°). The higher pH, lower acidity and TSS in the active carbon filtered juice indicating some of organic acids may be removed or adsorbed to active carbon during the filtration process.

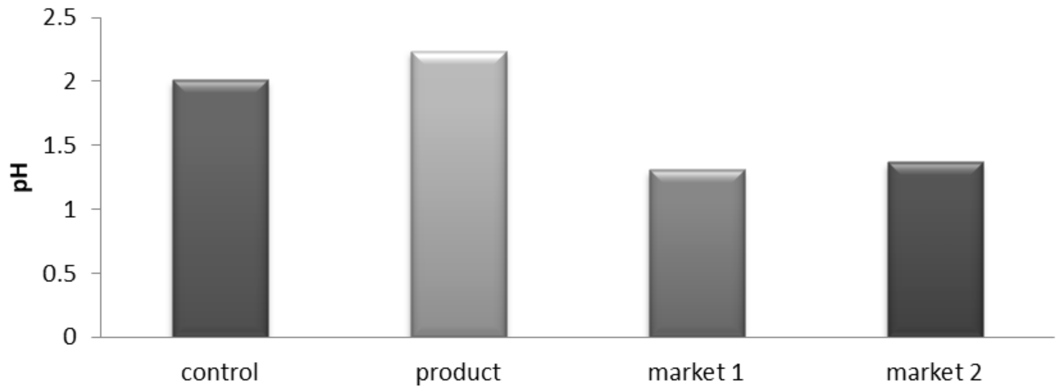

Figure 2. pH of the selected lime juice sample and commercial samples (control = fresh lime juice; product = lime juice filtered through 10 cm height active carbon column; market1 and market2- = two commercial products which made to mimics lime taste)

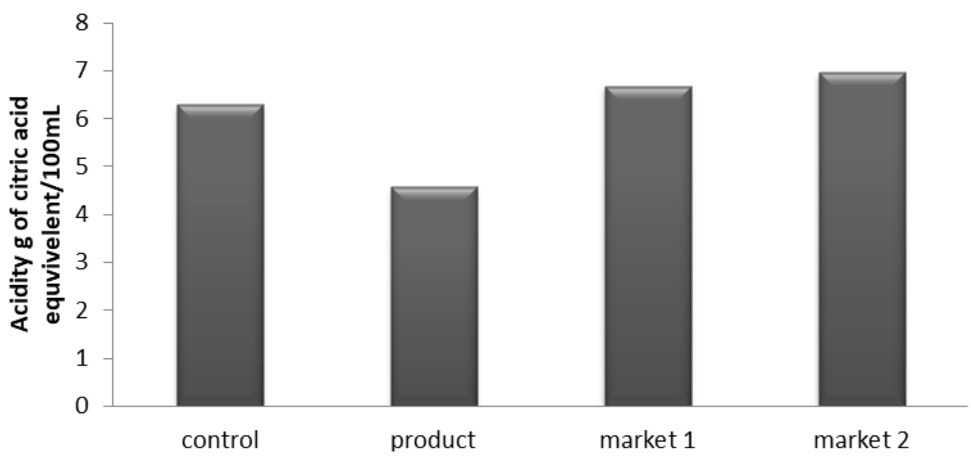

Figure 3. Titrabale acidity of the selected lime juice sample and commercial samples (control = fresh lime juice; product = lime juice filtered through 10 cm height active carbon column; market1 and market2- =two commercial products which made to mimics lime taste)

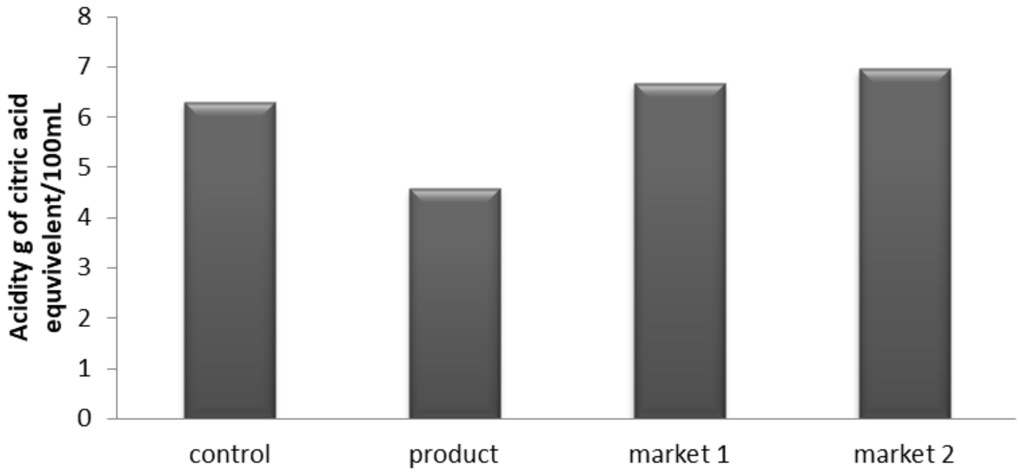

Figure 4. TSS of the selected lime juice sample and commercial samples (control = fresh lime juice; product = lime juice filtered through 10 cm hei*g*ht active carbon column; market1 and market2- =two commercial products which made to mimics lime taste)

Antioxidant activity

Figure 5 shows the total phenolic content of the selected filtered and diluted juice and two commercial products.

Results revealed that TPC in natural lime juice was significantly higher ($p<0.05$) than the treated samples indicating that the "retenant" is said to contain some residues such as hydrocolloids, sugar, proteins, phenolic compounds, and bioflavonoids (esterified by glycosides and non-esterified) compositions. The naturally occurring components that were reduced, includes limonoids, flavonoids, carotenoids polyphenolic compounds and flavones due to the adsorption process (Cheremisinoff and Morresi, 1978). Activated carbon adsorb hydrophobic and oleophilic compounds. Limonin and or isolimonin are soluble in alcohol, acetone, and benzene, but relatively insoluble in petroleum ether and extremely insoluble in water (Dreyer, 1965). Figure 5 showed that the phenolic content of active carbon filtered juice was significantly different compared with fresh juice (control) and two commercial samples.

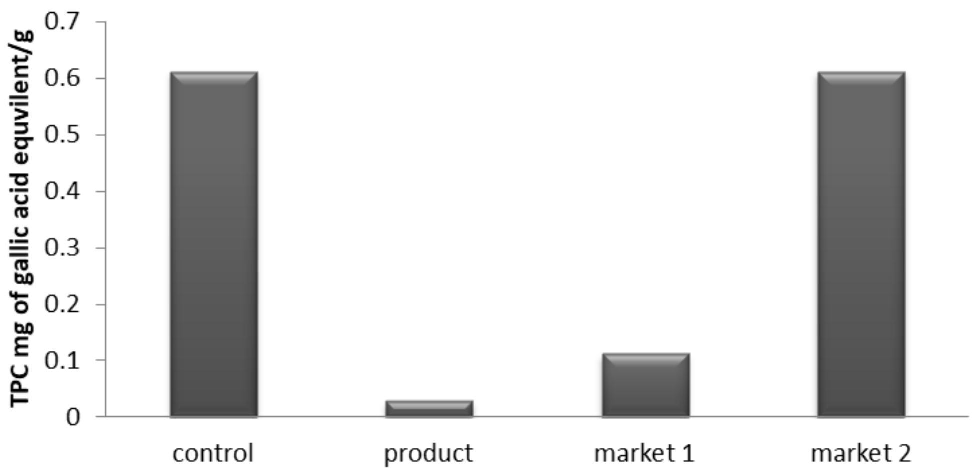

Figure 5. Total phenol content of the selected lime juice sample and commercial samples (control = fresh lime juice; product = lime juice filtered through 10 cm height active carbon column; market1 and market2- =two commercial products which made to mimics lime taste)

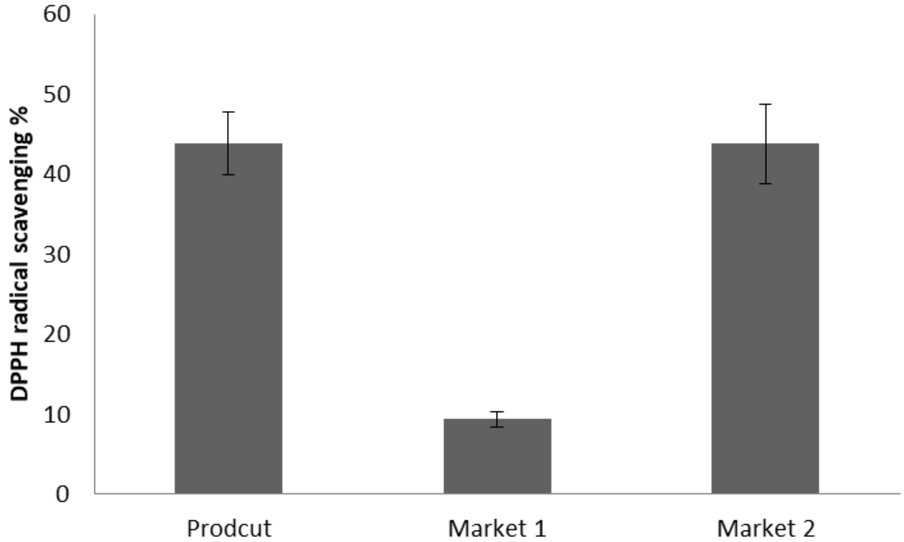

Figure 6. DPPH of the selected lime juice sample and commercial samples (control = fresh lime juice; product = lime juice filtered through 10cm height active carbon column; market1 and market2- =two commercial products which made to mimics lime taste)

Figure 6 shows the antioxidant activity of filtered and diluted lime juice in comparison with that of two commercial products. DPPH radical is a commercial oxidizing radical which can be reduced by antioxidants. High reduction of DPPH is related to the high scavenging activity performed by particular sample. According to Ghafar, et al. (2010), lime juice has free radical scavenging activity near to 80%. But the results obtained after treatment it has been reduced up to 43.86% indicating some of antioxidant in lime juice has removed or adsorbed to active carbon during filtration process.

4. CONCLUSION

It can be concluded that filtration of lime juice using activated carbon minimize the incidence of delayed bitterness. That could be improved by the de-sedimentation process. However, active carbon filtration together with de-sedimentation can affect on the flavonoids, phenols, sugars, acids contents in the juice.

5. REFERENCES

[1]. Bamise, C.T. and Oziegbe, E.O. (2013). Laboratory analysis of pH and neutralizable acidity of commercial citrus fruits in Nigeria. Advances in Biological Research, 7, 72-6.

[2]. Bermejo, A. and Cano, A. (2012). Analysis of nutritional constituents in twenty citrus cultivars from the Mediterranean area at different stages of ripening. Food and Nutrition Sciences, 3(05), 639.

[3]. Carbonell, L., Izquierdo, L. and Carbonell, I. (2007). Sensory analysis of Spanish mandarin juices. Selection of attributes and panel performance. Food quality and preference, 18(2), 329-341.

[4]. Cheremishinoff, N.P. and A.C. Moressi. (1978). Carbon adsorption applications, in N.P. Cheremisinoff and F. Ellerbusch (eds.) Carbon Adsorption Handbook: 1-53. Ann Arbor: Ann Arbor Science.

[5]. Dreyer, D.L. (1965). Citrus bitter principles. III. Isolation of deacetylnomilin and deoxylimonin. The Journal of Organic Chemistry, 30(3). 749-751.

[6]. Ghafar, M.F.A., Prasad, K.N., Weng, K.K., and Ismail, A. (2010). Flavonoid, hesperidine, total phenolic contents and antioxidant activities from citrus species: African journal of biotechnology 9(3), 326-330.

[7]. Gunathilake, K.D.P.P. (2012). A fruit-based functional beverage designed to reduce the risk of cardiovascular disease. M.Sc. Thesis, Dalhousie University, Canada.

[8]. Gunathilake, K.D.P.P., Ranaweera, K.K.D.S., Rupasinghe, H.P.V. (2018). Change of phenolics, carotenoids, and antioxidant capacity following simulated gastrointestinal digestion and dialysis of selected edible green leaves. Food Chemistry 245(15), 371-379.

[9]. Gunathilake, K.D.P.P., Ranaweera, K.K.D.S. (2016). Antioxidative properties of 34 green leafy vegetables. Journal of Functional Foods 26, 176-186.
[10]. Hasegawa, S., Suhayda, C., Omura, M., and Berhow, M. (1996). Creation of transgenic citrus free from limonin bitterness: biotechnology for improved foods and flavours. 79-87.
[11]. Ranganna, S., Govindarajan, V.S., Ramana, K.V.R. and Kefford, J.F. (1983). Citrus fruits—varieties, chemistry, technology, and quality evaluation. Part II. Chemistry, technology, and quality evaluation. A. Chemistry. Critical Reviews in Food Science & Nutrition, 18(4), 313-386.
[12]. Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of FC reagent. Methods in Enzymology, 29, 152–178.
[13]. Theansuwan, W., Triratanasirichai, K. and Tangchaichit, K. (2008). Continuous production of lime juice by vacuum freeze drying. American Journal of Applied Sciences, 5(8), 959-962.
[14]. Tsen, H.Y., and Yu, G.K.(1991) Limonin andnaringin removal from grape fruit juice with naringinase entrapped in cellulose triacetate fibers: journal of food science, 56, 31-34.
[15]. Yehuda, N.B. and Anglea, T.A., TICOSOURCE, 2010. Flavonoid-rich citrus extract process. U.S. Patent Application 12/340,215.