Comparative Analysis of Bioactive and Physiochemical Properties of *Citrus limon* Burm and *Citrus aurantifolia* Juice

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ABSTRACT: In the global scenario, citrus is widely cultivated fruit crop having strong phytochemical properties that contain volatile compounds produces essential oil or essence that used in different food, beverage and cosmetic industry. Assam lemon or *Citrus limon* Burm is unique in its characteristic aroma, flavor and taste and its uniqueness lies mainly in its bearing habits. In comparison with Assam Lemon, this study mainly focusing on the comparative analysis of physiochemical and bioactive properties of the raw juices of *Citrus limon* Burm and *Citrus aurantifolia* collected in the peak season of a year. Different behavior of *Citrus limon* Burm that contain higher amount of ascorbic acid and flavonoid content such as 55±0.1 mg/100g and 630±0.4 mg of QE/ml in comparison with *Citrus aurantifolia* juice that has ascorbic acid 38±0.2 mg/100ml to flavonoid content 400±0.4mg of QE/ml. It was also found that *Citrus aurantifolia* juice contains acidity as 8.5±0.3, total phenolic 700±0.3 mg of GAE/ml and 83±0.3% of antioxidant activity in comparison with *Citrus limon* Burm juice which shows 77±0.6 % of antioxidant activity. This study reveals that the changing bioactive properties in two different varieties of citrus juice mainly due to the genetic potential of individual polyphenol biosynthesis which shows the potential of storage and processing of raw juices maintaining consistency of volatile compound and commercialized without addition of alternate chemical additives.

Keywords: Physiochemical, Polyphenol, Biosynthesis.

Introduction:

In India, one of the popular and widely cultivated fruit crop is citrus with higher nutritional and functional properties with refreshing flavor and aroma (Baldwin *et.al.*,2014). Citrus related genera viz., *Fortunella, Poncirus, Eremocitrus*, and *Microcitrusa* are belonging to Angiosperm, belonging to the family Rutaceae citrus there have approximately 140 genes and 1300 species are present. (Wu *et al.*,2018). It is believed that citrus is originated from foothills of Himalaya, Southeast Asia under positive effect of monsoon climate and then spread into North-eastern India, China and Burma. Since ancient times, citrus fruit have been grown worldwide with its efficient harvesting and processing. According to FAO, in Brazil annual production of citrus with oranges accounting for 17.5 million tons of its total citrus production (Ollitrault *et al.*,2013). In the world India ranks sixth in the production of citrus fruit, commonly produced mandarins, sweet oranges and limes having 50, 20 and 15% of total area respectively, In NE region of India, citrus is cultivated covering 57.2 thousand hectares, with 300.7 thousand tones production (Bujarbaruah *et al.*,2019). Global production of citrus was 92,088,000 metric tons in 2017 with 51.86 % for oranges,32.60% tangerines, 7.20% contain mandarins and 8.34% for grape fruit. Researches on citrus depicts that 68.44% citrus used for consumption and 21.51% is used for processing further (Ollitrault *et al.*,2013, USDA,2018). From the Northern hemisphere half of the citrus fruits are imported (62% in 2003), and the major exporter area is the Mediterranean contain 60% of the volume. Southern hemisphere countries like South Africa, Argentina, Australia providing citrus fruit in off seasons to the Northern area and increasing their exports. (Ollitrault *et.al.*,2013).
Similarly, Lemon is the most important species of citrus fruit after oranges and mandarins. In North East, Assam Lemon or *Citrus limon* is an exotic indigenous fruit crop and commonly known which has certain uniqueness and attributable. Assam lemon is an agricultural product of Assam which recently got the GI tag registered product with Geographical Indication of Goods (Registration and protection) Act,1999, Govt. Of India. Assam lemon is considered as an indigenous principal lemon cultivar of Assam and it has certain uniqueness in terms of quality, flavour, aroma, and the uniqueness of the fruit lies in its bearing habit produces seedless fruits with 9-12 segments and the fruits having two peak seasons in a year (Piloo *et.al.*,2020). Depending on the temperature and the storage duration clinical composition of the citrus fruits may change though they are non-climacteric in nature (Baruah *et.al.*,2018). The fruit is rich in vitamin C with other bioactive compounds such as flavonoids, limonoids, phenolic acid and volatile terpenoids. Assam lemon is sour due to presence of organic compound predominantly citric acid and aroma mainly coming from peel in the form of essential oils. The fruit is cultivated on loamy and fertile soil with uniform texture up to a depth of 3 meters and abundant rainfall results in larger quantity of juice storage inside the fruit as compared to the rough lemon (Chetiya *et al.*,2018). Assam lemon widely used for culinary, ready to drink beverages, cordials, jam, jellies, pickles marmalades, industrial and medicinal uses (Singh *et al.*,2020). The fruit is rich in ascorbic acid which boosts the immune system to fight against diseases and infections and also treat scurvy (Umadevi *et al.*, 2011). There are different types of citrus varieties such as sweet oranges (*Citrus sinensis*), sour and bitter oranges (*Citrus aurantium*), pomlo (*Citrus grandis*), limon (*Citrus limetta*), sweet lime (*Citrus limettiodes*), rough lemon (*Citrus jambhiri*), rangpur (*Citrus limonia*), mandarins (*Citrus reticulata*) etc. (Kundu *et.al.*,2019).

Tree

Leaf

Flower

Fruit

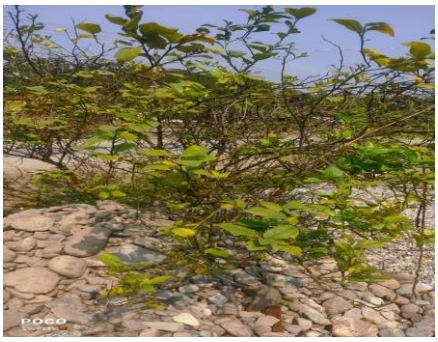
Figure1. Overview of *Citrus limon*

Citrus aurantifolia commonly known as Green lemon is another type of citrus fruit that found in Assam belonging to the family Rutaceae. It is broadly utilized due to its antibacterial, anti-inflammation,anti-diabetic, anticancer, antihypertensive, and antioxidant activities. It also has medicinal properties, for e.g. it can help in preventing diseases related to liver, heart, bone and urinary infection. Secondary metabolites of the citrus fruit are mainly carotenoids, alkaloids, coumarins, phenolics, flavonoids, and terpenoids where as hesperetin, apigenin, quercetin, kaempferol, naringenin, limonoids and rutin are the another important constituent of *Citrus aurantifolia* (Narang *et.al.*,2016). Perennial evergreen fruit *Citrus aurantifolia* grows to a height of 3-5m, have sharp thorns in size 1 cm or less and leaves are alternate, elliptical and 1-2cm long petioles (Manner *et.al.*,2006).

Blossoms of the *Citrus aurantifolia* tree are short and axillary racemes, bearing not many blossoms which are white and fragrant. The natural products or the fruits are resembling green round, 3-5 cm in breadth (diameter) and it is yellow when tear. All citrus organic product present similar physical product (Enejoh *et.al*.,2015). They incorporate flavedo is the outer part of the citrus products and has a great deal of flavonoids. Carpal layers or spectrum presenting around 8-11 glandular portions which are usually aligned and situated around (Rivera *et al*.,2010).

Fruit consumption plays an important role in human health as they are an important source of vitamin, essential micronutrient, proteins, minerals, dietary fibre and other bio functional components (Wargovich, *et.al.*,2000). Consumption of adequate amount of fruits and vegetables daily have been linked to reduction in cases of non-communicable diseases (Pem *et.al.*, 2015; Mo *et.al*., 2019, Conard *et.al.*, 2018). More recently the mounting consumer's awareness and focus towards health and has led to increased demand of products with good nutrition or healthy foods (Sheehan *et.al.*, 2007; Silva *et al.*, 2005; Rantaz *et.al.*,2018).

Based on the different physiochemical and biochemical properties this study is based on evaluation of different properties of Assam lemon and Green lemon on the basis of their storage behaviour.



Tree

Leaf

Flower

Fruit

Figure 1.1 Overview of *Citrus aurantifolia*

Considering the above mentioned aspects, this research study is based on comparative analysis of physiochemical and bioactive properties of raw *Citrus limon* and *Citrus aurantifolia* juice.

Materials and Methods:

2.1. Chemicals:

Chemicals used were analytical grade supplied by Merck India, Hi-media laboratories and Sigma chemicals India. The glassware's used were obtained from Borosil Glass Works Ltd.

2.2. Materials:

The fresh fruit samples of *Citrus limon* and *Citrus aurantifolia* were purchased from local market from local sources, Assam, India. The fruits were stored in a refrigerator at 4^{o}C until further use.

2.3. Extraction of *Citrus limon and Citrus aurantifolia* juice:

2.3.1. Juice extraction from Citrus limon and Citrus aurantifolia:

The fruits were cleaned and washed thoroughly with running tap water,further rinsed with distilled water. The pulp was removed with a sharp stainless steel knife and the fruit was again washed with distilled water. The juice was extracted using a screw press extractor and made it a concentrated extract. This extract was filtered using a muslin cloth to remove any insoluble materials and transferred to a screw capped glass bottles and stored in a refrigerated condition at 4^{0}C for further storage study.

2.4. Determination of Physiochemical Properties:

2.4.1. Determination of fruit weight: Fruit weight was measured using an electronic balance (digital) and expressed in gram (g).

2.4.2. Determination of total juice yield: Juice content was expressed in percentage (%), with respect to the fruit weight. Fruit juice was strained and volume was measured using a measuring cylinder.

2.4.3. Determination of pH:

The pH of the extract was determined using a hand held pH meter **(**Eutech, Singapore), which was calibrated using buffer solutions at pH 7, pH 4 and pH 9. The pH of the juice samples was measured and recorded by dipping the hand help pH meter.

3.4.4. Determination of total titratable acidity:

Total titratable acidity of the juice samples was determined using the titration method mentioned as by Ranganna, (2017). Briefly, 10 ml of the juice samples was titrated with standardized 0.1 N sodium hydroxide (NaOH) solution with 2-3 drops of phenolphthalein as an indicator, till there is appearance of a pink colour which persists for 30 seconds. This was expressed using the following formula in terms of citric acid.

$$\text{Acidity (\%)} = \frac{\text{Volume of the titre} \times \text{Normality of NaOH} \times \text{Equivalent weight } 0.064}{\text{Volume of the sample}} \times 100$$

2.4.5. Ascorbic acid:

Ascorbic acid was determined by the titration method mentioned by Ranganna, (2017). The dye solution was prepared by exactly weighing 50 mg of 2,6-dichlorophenolindophenol in 150 ml of distilled water with 42 mg sodium bicarbonate, the solution was then made upto 200 ml with distilled water. The dye was later standardized by titrating with a 5 ml standard ascorbic acid solution (0.1 mg/ml) added with 5 ml 3% HPO_3 and titrating till pink colour appears. The dye factor (mg of ascorbic acid per ml of the dye) is determined by using the formula:

$$\text{Dye Factor} = \frac{5}{\text{titre}}$$

For estimation 20 ml of the juice was made to 100 ml with 3% HPO_3, 3 ml aliquot of the sample was taken and titrated with the standard dye to a pink end point which persisted for 15s. The ascorbic acid content of the sample was determined using the following equation:

$$\text{mg of ascorbic acid per 100ml} = \frac{\text{Titre x Dye factor x Volume made up x 100}}{\text{Aliquot of extract taken for estimation x Volume of sample taken for estimation}}$$

2.5. Determination of Bioactive Properties:

2.5.1. Total Phenolic Content Estimation (TPC):

Total phenolic content of the juice was estimated using the Folin-Ciocalteau assay with slight modification. 100 to 500 µl of the standard and 200 µl of the sample were taken in different test tubes and 2 ml water was added to it followed by 0.3 ml of Folin-Ciocalteau's phenol reagent and 0.8 ml 20 % sodium carbonate was added after 5 min and the final volume was made to 5 ml. After incubating the samples for 30 min the absorbance was measured at 765 nm in a UV-Vis spectrophotometer. The results were expressed in mg GAE/ml.

2.5.2. Total Flavonoid Content Estimation (TFC):

Total flavonoid content of the juices were estimated using 100 µl of the sample was with 900 µL of methanol was mixed with it, to this mixture 10 ml distilled water was added to dilute the samples followed by 0.3 ml 5% $NaNO_2$, after 5 min 0.3 ml of 10% NH_4Cl was added followed 2 ml of 1M NaOH 6 min later. The samples were further mixed with 2.4ml of distilled water and then incubated for 1 h. A standard curve was prepared using quercetin hydrate standard (0 - 250 µg/ml). The reading was measured at 415 nm in a UV-Vis spectrophotometer. Results were expressed as quercetin equivalent (mg QE/ml) of sample.

2.5.3. Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity:

Radical scavenging activity of the juice samples were measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. Methanolic solution of 0.1 mM DPPH radical was added to 200 µl of juice samples. The mixture was then incubated at dark for 30 min at room temperature. 1 mM DPPH solution with added 200 µl was used as blank. The absorbance was measured at 517 nm in a UV-Vis Spectrophotometer. The results were expressed in terms of radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \frac{A_0 - A_S}{A_0} \text{ x } 100$$

Where, A_o is absorbance of control blank, and As is absorbance of samples.

Results and Discussion:

3.1. Physiochemical properties of *Citrus limon* and *Citrus aurantifolia* juice:

The physicochemical property of any food product is important to understand as it gives a good idea about the intended use and processing of the product. The physicochemical properties for the fresh juice are reported as in Table 4.1.

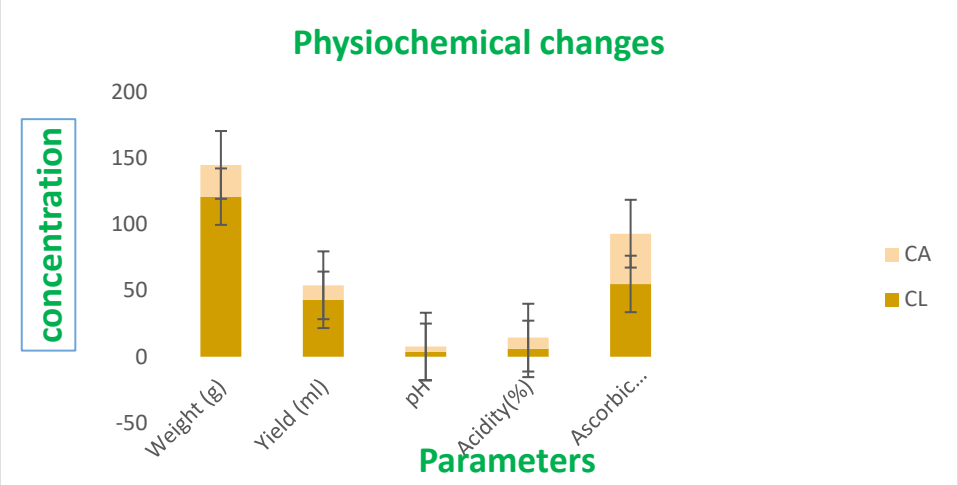
Table 3.1 Physiochemical properties of *Citrus limon and Citrus aurantifolia* juice:

Sl. No.	Parameter	*Citrus limon*	*Citrus aurantifolia*
1	Fruit weight	122.11± 0.1	24.83±0.5
2	Juice yield	43± 0.2	11±0.8
3	pH	3.5±0.2	3.9±0.5
4	Total titratable acidity (%)	5.98±0.2	8.5±0.3
5	Ascorbic acid (mg/100ml)	55±0.1	38±0.2
6	Total Phenolic content(mg/ml)	460±0.3	700±0.3
7	Total flavanoid content (mg/ml)	630±0.4	400±0.4
8	Total Antioxidant Activity (%)	77±0.6	83±0.3

3.4. Comparative changes in physicochemical and bioactive properties of the *Citrus lemon* and *Citrus aurantifolia* juice:
3.4.1. Changes in physicochemical properties
3.4.1.1. Determination of fruit weight and juice yield: Fruit weight was measured 122.11± 0.1 gram in *Citrus limon* and 24.83±0.5 gram in *Citrus aurantifolia*. Total juice yield in *Citrus lemon* 43± 0.2 ml and 11±0.8 ml in *Citrus aurantifolia*. Juice yield parameter might differ with size, shape, and weight of the fruit.

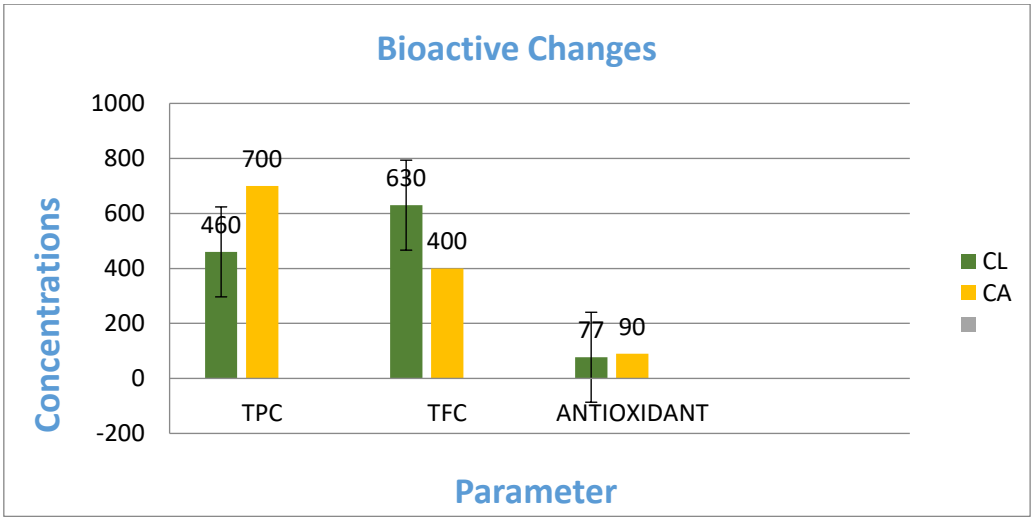
3.4.1.2 Changes in pH, total titratable acidity: pH was measured using hand held pH meter that shows different pH. Total acidity was found higher in *Citrus aurantifolia* juice than *Citrus lemon*

3.4.1.3 Ascorbic acid: Ascorbic acid content in *Citrus lemon* found high than *Citrus aurantifolia* as mentioned in table 4.1. *Citrus lemon* contain ascorbic acid 55±0.1 mg/100ml and *Citrus aurantifolia* contain ascorbic acid 38±0.2 mg/100 ml.



CL: *Citrus lemon*, CA: *Citrus aurantifolia*
Figure 3.1 Changes in physiochemical properties

3.4.6 Changes in Bioactive properties in *Citrus aurantifolia* and *Citrus lemon* juice:
TPC and TFC properties were analysed based on the absorbance values of the juice that reacted with folin–ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, total phenolic content are given in table. 1. TPC of juices varied between species and ranged from 460±0.3 mg/ml in *citrus limon* and 700±0.3mg/ml in *Citrus aurantifolia*. Our study shows *Citrus aurantifolia* juice had a higher phenolic content than *Citrus limon*. However, the TFC were analysed with the standard solutions of qurcetion equivalents. TFC are given in table 1. that reported that in *Citrus aurantifolia* juice was ranged from400±0.4 mg/ml and *Citrus limon* ranges from 630±0.4 mg/ml. Moreover, antioxidant activity of *Citrus aurantifolia* ranges 83%and*Citrus limon* ranges 77%. These variations in TPC and TFC could be due to the factors involved in polyphenol biosynthesis. These findings were consistent with previous work done by Tousni *et.al*.,2010. This study showed that juice of *Citrus aurantifolia* exhibited the most significant levels of total phenols and antioxidant activities when compared to *Citrus limon*. This study is supported with Maksoud *et.al*.,2021.



CL: *Citrus limom*, CA: *Citrus aurantifolia*
Figure 4.2 Comparative changes in bioactive properties

CONCLUSION:

In this study the physiochemical and bioactive properties of the raw *Citrus limon* than *Citrus aurantifolia* juice were analysed. From this study it was found that *Citrus limon* contain higher amount of ascorbic acid and flavonoid content in comparison of *Citrus aurantifolia* juice. It was also found that *Citrus aurantifolia* juice contains higher acidity, total phenolics content and higher amount of antioxidant activity in comparison with *Citrus limon* juice. This study reveals that the changing bioactive properties in two different verities of citrus juice might be due to genetic potential of individual polyphenol biosynthesis. There need to do further work to see the changing properties of juices on refrigerated storage.

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