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Changes in contents of some chemical compositions and activities *of* some hydrolytic and oxidative enzymes of *Aegle Marmelos* L. Leaves

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Abstract

Aegle marmelos L. leaves were analyzed at different maturity levels for their physico-chemical compositions and change in contents of some hydrolytic and oxidative enzymes. The pH of the *Aegle marmelos* L. leaves was in the acidic ranges at all the maturity stages and the acidity of the leaves increased gradually with the advancement of maturity. The moisture content decreased gradually while ash content increased remarkably with age. Protein, total sugar, reducing sugar, sucrose and vitamin contents increased rapidly with the advancement of maturity while lipid and starch content decreased with maturation. The activity of amylase and invertase increased up to mature stage and thereafter decreased rapidly at the ripen stage. Mineral contents increased up to the mature stage and then decreased in ripen stage. Polyphenol oxidase and peroxidase activity high in immature stage but decreased dramatically in mature stage and thereafter increased in ripen stage while the activity of protease and lipase increased all the maturity stage. In this study, ripen *Aegle marmelos* L. leaves contained the highest amount of protein, carbohydrate, β-Carotene, vitamin B_1, vitamin B_2 and vitamin C whereas mature and immature *Aegle marmelos* L. leaves are rich sources of minerals and starch respectively.

Keywords: Physico-chemical; *Aegle marmelos* L. Leaves; Mineral; Vitamin; Maturation

1. Introduction

Nutritional status is the great factor of the health profile of a community. Good health is directly related to better education level. According to the UNDP estimates, people of Bangladesh living under the poverty line in 2009 accounted for 40.0% (UNDP, 2009).In this country, earlier nutritional survey carried out and indicated that the principle impediment to better nutrition is the inadequate calorie intake. Reis et al (1987) reported that the density of the nutrient of cooked food is lower compared to uncooked food. Different kinds of vegetables and leaves are available in Bangladesh those are rich in nutrients but most of them are seasonal and expensive and people consumed as cooked food.

Aegle marmelos L. Correa, a tree species of the Rotaceae family. It grows well in India, Bangladesh, Malaysia, Indonesia and Thailand, as well as in tropical areas. Young and tender green leaves are eaten raw in salads or cooked and added to curries. Ripe scarlet leaves is eaten raw (Gardenbed.com, 2001). Different parts of this plant are used in the indigenous system of medicine for the treatment of a variety of human disease such as diabetes, gonorrhea, cataracts, skin eruption. *Aegle marmelos* is a good source of calcium, protein and fiber (Bharathi 2007, Simopoulos and Gopalan 2003). It also contains beta carotene (Sachan and Chundawat 1985). During maturation and senescence of leaves, proteolytic and hydrolytic enzymes play an important physiological role (Hashinaga et al. 1983, Desai and Deshpande 1978). As a result of catabolic and metabolic processes, dramatic chemical and physical changes occur during ripening, which might be enzyme directed processes (Dilley, 1970). Data on the physico-chemical compositions of different maturity stages of *Aegle marmelos* L. leaves grown in Bangladesh are not available. This research work is designed to obtain information on the nutritional quality of *Aegle marmelos* L. leaves available in Bangladesh.

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Therefore, in present investigation, *Aegle marmelos* L. leaves have been selected to analyze their physico-chemical compositions as well as the activities of some enzymes at three different maturity stages.

2. Materials and methods

2.1. Analysis of *Aegle marmelos* L. leaves

About 5 g of leaves were crushed thoroughly in a mortar with pestle and homogenized well with 10-20 ml of distilled water and then filtered through two layers of muslin cloth.The filtrate was then clarified by centrifugation at 3000 g for 10 min and used for the experimental purposes.

The p^H of the filtrate was determined using Corning 215 p^H meter. The moisture and ash contents were determined by the method of AOAC, 1990. The total protein and water-soluble protein were determined by the micro-Kjeldhal method by Jayaraman (1981) and spectrophotometrically (Lowry *et al.,* 1951) respectively. Lipid contents were determined colorimetrically as per Bligh and Dyer, 1959. Total sugar content was estimated colorimetrically by Anthrone method (Dubois *et al.,* 1951). Starch content was estimated colorimetrically by Anthrone method as described by Jayaraman, 1981.

The vitamins such as thiamin and riboflavin were estimated following the procedure as described by Anonymous (1965) while β-carotene were estimated following the method described by Jensen (1978). Vitamin-C content was determined by the titrimetric method (Bessey and King, 1933). The minerals such as calcium, iron, sodium, potassium, cupper and magnesium content were determined by Atomic Absorption Spectroscopic method of Issac and Johnson (1975).Phosphorus was determined by colorimetric means (Virmani and Narula, 1995).

For preparation of crude enzyme extract about 10 g of seed were ground in a mortar with pestle and then homogenized well with cold 0.1 M phosphate buffer of respective p^H (amylase, p^H 6.7, protease, p^H 7.0, invertase, p^H 7.0,), while for the measurement of lipase 50 mM acetate buffer, p^H 5.6 was used. After centrifugation at 8000g, 4 ℃ for 10 min. the clear supernatant was used as crude enzyme extract. The protease activity was measured by the method of Kunitz (1947) while the activity of amylase was determined as per Jayaraman (1981). Invertase activity was assayed following the modified method as described in methods in physiological Plant Pathology (Mahadevan and Sridhar, 1982).

2.2. Statistical analysis

All data were expressed on fresh weight basis as the mean and standard deviation (SD) of three experiments.

3. Results and discussion

Table-1 shows the pH, moisture and ash contents of *Aegle marmelos* L. leaves at different maturity stages. The pH of *Aegle marmelos* L. leaves is in acidic ranges at all the maturity stages. It may suggest that the acidity of the leaves increased gradually with the advancement of maturity. Mature leaves is more acidic than ripen and immature leaves. The moisture contents of *Aegle marmelos* L. leaves were found 37.8%, 40.01% and 32.50% in immature, mature and ripen stages respectively. The results also revealed that the moisture of *Aegle marmelos* L. leaves decreased at ripen stage, while ash content increased in the leaves with the advancement of maturity. The decreased in moisture content with the advancement of maturity might be due to accumulation of solid materials.

Table 1 pH, Moisture and ash contents of *Aegle marmelos* L. leaves at different maturity levels

Parameters	**Stages of Maturation**		
	Immature	**Mature**	**Ripen**
pH	6.15±0.04	6.00±0.01	6.30±0.02
Moisture (gm%)	37.8±0.21	40.01±0.34	32.50±0.02
Ash (gm %)	0.012±0.03	0.82±0.04	0.75±0.01

Table-2 shows the water soluble protein, lipid, crude fibers total phenol content of leaves at different maturity levels. The water-soluble protein content of *Aegle marmelos* L. leaves were found 0.15% in immature, 0.26% in mature and 0.21 % in ripen stages. The result also revealed that the water soluble protein content of *Aegle marmelos* L. leaves

slightly decreases with the changes of maturity. Lipid content of three different stages of *Aegle marmelos* L. were found 0.001%, 0.05% and 0.02% in immature, mature and ripen stages respectively. The present data clearly indicate that *Aegle marmelos* L. leaves contained very little amount of lipid at three maturity stages. So it cannot be considered as a good source of lipid. It may be concluded from the result that the lipid content increased with the advancement of maturity. The crude fiber content of three different stages of *Aegle marmelos* L. leaves were found 8.1% in immature, 11.0% in mature and 9.5% in ripen stages. The highest amount of crude fibers was at mature stage. The phenol content of three stages of *Aegle marmelos* L. leaves were 1.9 in immature, 5.01 in mature and 3.99 in ripen stage. The highest amount of phenol was present at the mature stage compared with the ripen stage.

Table 2 Water soluble protein, lipid, crude fibers and total phenol contents of *Aegle marmelos* L. leaves at different maturity levels

Parameters	**Stages of Maturation**		
	Immature	**Mature**	**Ripen**
Water soluble protein (gm%)	0.15±0.001	0.26±0.003	0.21±0.002
Lipid (gm%)	0.001±0.002	0.05±0.005	0.02±0.004
Crude fibers (gm%)	8.1±0.003	11.0±0.004	9.5±0.005
Total phenol (gm%)	1.9±0.005	5.01±0.008	3.99±0.001

Total sugar, reducing sugar, non reducing sugar and starch contents of *Aegle marmelos* L. leaves are shown in Table 3. The result indicated that the total sugar content of *Aegle marmelos* L. leaves increased with the change of maturity. The reducing sugar and starch contents of *Aegle marmelos* L. leaves also increased with the change of maturity. The increase in reducing sugar were due to enzymatic conversion of starch to reducing sugar and also conversion of some non-reducing sugar. The total sugar and reducing sugar contents were found to vary between 0.90-1.7% and 0.30-0.90% respectively. The non-reducing sugar and starch contents of *Aegle marmelos* L. leaves were found to vary between 0.57-0.95% and 0.8-2.5% respectively. The reduction of starch with the change of maturity might be due to the hydrolysis of starch, which shows good correlation with the increase in the contents of total soluble sugar.

Table 3 Total sugar, reducing sugar, non-reducing sugar and starch contents of *Aegle marmelos* L. leaves at different maturity levels

Parameters	**Stages of Maturation**		
	Immature	**Mature**	**Ripen**
Total sugar (gm%)	0.9±0.003	1.9±0.001	1.7±0.005
Reducing sugar (gm%)	0.30±0.003	0.90±0.004	0.75±0.003
Non-reducing sugar (gm%)	0.57±0.004	0.95±0.003	0.90±0.009
Starch (gm%)	0.8±0.002	2.5±0.007	2.0±0.001

The vitamins and minerals content were shown in Table-4. It was found that *Aegle marmelos* L. leaves are good sources of vitamin C and minerals. Vitamin C (3.5-7.1 mg %) contents increased with the advancement of maturity. The major minerals analyzed in *Aegle marmelos* L. leaves are potassium (1.52-5.001 mg %), calcium (0.001-0.25 mg %), sodium (2.5-7.0 mg %), phosphorus (3.5-7.82 mg %). All minerals content increased up to the mature stage and then decreased in ripen stage.

Table 4 Vitamin and minerals content of *Aegle marmelos* L. leaves at different maturity levels

Parameters	Stages of Maturation		
	Immature	Mature	Ripen
Vitamin-C (mg%)	3.5±0.001	7.1±0.002	6.0±0.005
Potassium (%)	1.52	5.001	3.99
Calcium (%)	0.001	0.25	0.21
Sodium (%)	2.5	7.0	6.5
Phosphorus (%)	3.5	7.82	7.11

Activities of some hydrolytic and oxidative enzymes in *Aegle marmelos* L. leaves are shown in table 5. The activities of amylase, protease, invertase, cellulose and polyphenol oxidase in different maturity stages of *Aegle marmelos* L. leaves were found between 0.17 to 0.22 mg %, 0.051 to 0.069 mg %, 0.74 to 0.92 mg %, 0.13 to 0.19 mg % and 0.41 to 0.53 mg % respectively. Activities of all these enzyme increase up to mature stage and thereafter decreased significantly. Among these enzymes, invertase shows the highest activity at mature stages.

Table 5 Activities of amylase, protease, invertase, cellulose and polyphenol oxidase enzymes of *Aegle marmelos* L. leaves at different maturity levels

Name of the enzymes	Stages of maturation		
	Immature	Mature	Ripen
Amylase (unit mg min leaves).	0.17±0.05	0.22±0.05	0.20±0.05
Protease (unit mg min^{-1} leaves).	0.051±0.001	0.069±0.001	0.60±0.001
Invertase (unit gm^{-1} leaves).	0.74±0.001	0.92±0.001	0.80±0.001
Cellulase (unit gm^{-1} leaves)	0.13±0.002	0.19±0.002	0.15±0.002
Polyphenol oxidase (unit $min^{-1}gm^{-1}$ leaves)	0.41±0.001	0.53±0.001	0.45±0.001

4. Conclusion

In conclusion, ripen *Aegle marmelos* L. leaves might be considered as nutritionally rich source since it contained the highest amount of protein, carbohydrate, β-Carotene, vitamin B_1,vitamin B_2 and vitamin C whereas mature and immature *Aegle marmelos* L. leaves are rich sources of minerals and starch respectively.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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