J. bio-sci. 21: 35-41, 2013 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index

CHANGES IN CONTENTS OF SOME CHEMICAL COMPOSITIONS AND ACTIVITIES OF HYDROLYTIC AND OXIDATIVE ENZYMES OF *COCCINIA CORDIFOLIA* L. FRUITS

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Abstract

Context: *Coccinia Cordifolia* Lin. is an important tropical vegetable and it belongs to the *Cucurbitaceae* family. The fruits of *C. cordifolia* as a rich source of different nutrients were analyzed.
Objective: Studies were conducted to investigate the changes of nutritional compositions and hydrolytic and oxidative enzymes of *C. cordifolia* fruits at different maturity levels.
Materials and Methods: The pH was measured by pH meter. The moisture and ash contents were determined by the method of AOAC. Total and water-soluble proteins were determined by the micro-Kjeldhal method and spectrophotometrically respectively. Lipid contents were determined by Bligh and Dyer. Total sugar and starch content were estimated by Anthrone method. Thiamin and riboflavin were estimated by Anonymous and β-carotene was estimated by Jensen. Vitamin-C content was determined by the titrimetric method. Calcium, iron, sodium, potassium, cupper and magnesium content were determined by Atomic Absorption Spectroscopic method. Phosphorus was determined by colorimetric means. The protease and amylase activity were measured by Kunitz and Jayaraman respectively. Invertase activity was assayed by Mahadevan and Sridhar.
Results: The pH was acidic. The moisture content decreased and ash content increased with age. Protein, total sugar, reducing sugar, lipid and vitamin contents increased rapidly while starch content decreased with maturation. Mineral contents increased up to the mature stage and decreased in ripen stage. The activity of amylase and invertase increased up to mature and thereafter decreased. Polyphenol oxidase and peroxidase activity were high in immature stage but decreased in matured stage and thereafter increased in ripen stage while the activity of protease and lipase increased all the maturity stage.
Conclusion: In this study, ripen *C. cordifolia* fruits contained the highest amount of protein, total sugar, reducing sugar, β-Carotene, vitamin B_1, vitamin B_2 and vitamin C whereas matured and immatured *C. cordifolia* fruits are rich sources of minerals and starch respectively.

Key words: Chemical compositions, *C. cordifolia* fruits, mineral, vitamin, maturation.

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 principal 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 fruits are available in Bangladesh those are rich in nutrients but most of them are seasonal and expensive and people consumed as cooked food. *Coccinia cordifolia* Linn. Syn. *Coccinia indica* and *Coccinia grandis* (English-Ivy gourd) is a tropical plant of the Cucurbitaceae family. It grows well in India,

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Bangladesh, Malaysia, Indonesia and Thailand, as well as in other tropical areas. Young and tender green fruits are eaten raw in salads or cooked and added to curries. Ripe scarlet fruit 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. *C. cordifolia* is a good source of calcium, protein and fiber (Bharathi 2007, Simopoulos & Gopalan 2003). It also contains beta carotene (Sachan & Chundawat 1985). During maturation and senescence of fruits, proteolytic and hydrolytic enzymes play an important physiological role (Hashinaga et al. 1983, Desai & 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 *C. cordifolia* L. fruits grown in Bangladesh are not available. This research work is designed to obtain information on the nutritional quality of *C. cordifolia* L. fruits available in Bangladesh.

Therefore, in present investigation, *C. cordifolia* L. fruits have been selected to analyze their chemical compositions as well as the activities of some enzymes at three different maturity stages.

Materials and Methods

Coccinia cordifolia fruits were collected from Rajshahi University Campus, Bangladesh at different maturity stages for experimental purposes (Fig. 1). Days were required from the time of fruit harvesting for immature, mature and ripen stages are 8±3, 15±4, 25±5 days respectively.

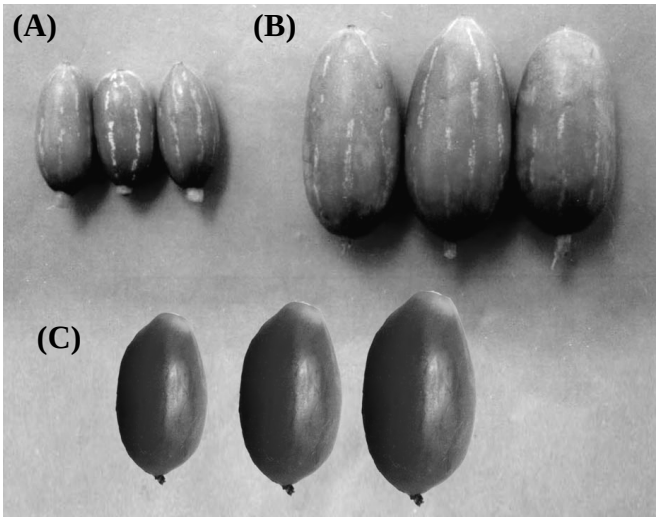

Fig. 1. *Coccinia Cordifolia* fruits at different maturity stages
(A) Immature stage (B) Mature stage and (C) Ripen stage

Freshly harvested *Coccinia cordifolia* fruits at different maturity levels were brought to the laboratory of Biochemistry, Rajshahi University for experimental purpose. The nutritional compositions and enzyme activities were studied by the following methods:

About 5g of fruits 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 pH of the filtrate was determined using pH meter (Corning 215). 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 & 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 & Narula 1995).

For preparation of crude enzyme extract about 10 g of fruits were ground in a mortar with pestle and then homogenized well with cold 0.1 M phosphate buffer of respective pH (amylase, pH 6.7, protease, pH 7.0, invertase, pH 7.0,), while for the measurement of lipase 50 mM acetate buffer, pH 5.6 was used. After centrifugation at 8000g, 4^{o}C 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 & Sridhar 1982).

All data were expressed on the mean and ± standard deviation (SD) of three experiments and were subjected to one way analysis of variance (ANOVA). The mean values were compared at $P < 0.05$ significance level by Duncan's multiple range tests using SPSS 11.5 software package.

Results and Discussion

Table1 shows the pH, moisture and ash contents of *C. cordifolia* fruits at different maturity stages. The pH of *C. cordifolia* fruits is in acidic ranges at all the maturity stages. The moisture contents of *C. cordifolia* fruits were found 91.98%, 89.88% and 87.14% in mature, immature and ripen stages respectively. The results also revealed that the moisture of *C. cordifolia* fruits decreased gradually, while ash content increased in the fruits with the advancement of maturity. The decreased in moisture content with the advancement of maturity might be due to accumulation of solid materials.

Table 2 shows the total protein, water soluble protein and total lipid content of fruits at different maturity levels. The total protein content of *C. cordifolia* fruits were found 2.58% in immature, 7.37 % in mature and 11.29 % in ripen stages. Present study also indicated that like total protein, the amount of the water soluble protein is also increased significantly in all maturity stages but the total protein content determined by Micro-Kjeldhal method showed considerably higher value than water soluble protein by the Lowry method. Bhatnagar and Tewari (1971) reported that *C. cordifolia* fruits are rich sources of protein which is similar to this study. In this study, in ripen stage (11.29 %), *C. cordifolia* fruits contain highest amount of protein than immature and mature stage. *C. cordifolia* fruits contents low amount of lipid. It may be concluded from the result that the lipid content increased with the advancement of maturity but the difference is not significant ($P < 0.05$).

Table 1. pH, Moisture and ash contents of *C. cordifolia* fruits at different maturity levels (on the basis of fresh weight).

Parameters	Stages of Maturation		
	Immature	Mature	Ripen
pH	6.20±0.04^{c}	5.40±0.01^{b}	4.10±0.02^{a}
Moisture (gm %)	91.98±0.2^{c}	89.88±0.34^{b}	87.14±0.02^{a}
Ash (gm %)	1.00±0.03^{a}	1.68±0.04^{b}	2.12±0.01^{c}

Values are mean ± S.D. of triplicate analyses. Mean in the same row with different superscripts are significantly ($P < 0.05$) different.

Table 2. Total protein, water soluble protein and total lipid content of *C. cordifolia* fruits at different maturity levels (on the basis of fresh weight).

Parameters	Stages of Maturation		
	Immature	Mature	Ripen
Total protein (gm %)	2.58±0.001^{a}	7.37±0.003^{b}	11.29±0.002^{c}
Water soluble protein (gm %)	1.06±0.006^{a}	2.55±0.001^{b}	4.32±0.001^{c}
Total lipid (gm %)	0.20±0.004^{a}	0.22±0.005^{a}	0.25±0.002^{a}

Values are mean ± S.D. of triplicate analyses. Mean in the same row with different superscripts are significantly ($P < 0.05$) different.

Table 3. Total sugar, reducing sugar and starch contents of *C. cordifolia* fruits at different maturity levels. (on the basis of fresh weight).

Parameters	Stages of Maturation		
	Immature	Mature	Ripen
Total sugar (gm %)	0.89±0.003a	1.55±0.001b	6.50±0.005c
Reducing sugar (gm %)	0.39±0.003a	0.52±0.004b	2.06±0.003c
Starch (gm %)	5.55±0.002c	3.09±0.007b	1.88±0.001a

Values are mean ± S.D. of triplicate analyses. Mean in the same row with different superscripts are significantly ($P < 0.05$) different.

Total sugar, reducing sugar and starch contents of *C. cordifolia* fruit were shown in Table 3. The result indicated that the total sugar content of *C. cordifolia* fruits increased significantly with the change of maturity. These results were similar to the finding of Abdullah *et al.* (1985) who reported that total sugar content of banana increased with the change of maturity. The reducing sugar contents of *C. cordifolia* fruits also increased significantly with the change of maturity, while the starch content of the fruit decreased significantly with maturation. The total sugar and reducing sugar contents were found to vary between 0.89- 6.50% and 0.39- 2.06% respectively. The starch contents of *C. cordifolia* fruits was found to vary from 1.88 to 5.55% 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 4. Vitamins and minerals content of *C. cordifolia* fruits at different maturity levels (on the basis of fresh weight).

Parameters	Stages of Maturation		
	Immature	Mature	Ripen
Vitamin B_1 (mg %)	0.50±0.12[a]	0.60±0.32[b]	0.63±0.23[c]
Vitamin B_2 (mg %)	0.21±0.04[a]	0.25±0.05[b]	0.29±0.05[c]
ß-Carotene (µg %)	3405±0.24[a]	3900±0.12[b]	4123±0.14[c]
Vitamin-C (mg %)	12.00±0.001[a]	13.50±0.002[b]	14.00±0.005[c]
Potassium (mg %)	137.40±0.03[a]	148.40±0.04[b]	137.00±0.05[a]
Calcium (mg %)	48.84±0.05[a]	56.35±0.14[c]	50.80±0.24[b]
Sodium (mg %)	0.72±0.40[a]	0.95±0.05[b]	0.72±0.03[a]
Phosphorus (mg %)	10.11±0.52[a]	12.02±0.15[b]	10.00±0.03[a]
Iron (mg %)	1.00±0.23[a]	1.99±0.13[b]	1.10±0.15[a]
Cupper (mg %)	46.00±0.55[a]	56.0±0.34[b]	46.00±0.24[a]
Magnesium (mg %)	69.20±0.03[a]	79.45±0.05[c]	69.00±0.04[a]

Values are mean ± S.D. of triplicate analyses. Mean in the same row with different superscripts are significantly ($P < 0.05$) different.

The vitamins and minerals content were shown in Table 4. It was found that *C. cordifolia* fruits are good sources of vitamins. Vitamin B_1 (0.50-0.63 mg %), Vitamin B_2 (0.21-0.29 mg %), ß-Carotene (3405-4123 µg %) and vitamin C (12.00-14.00 mg %) contents increased with the advancement of maturity but the change was not significant ($p<0.05$). The major minerals analyzed in *C. cordifolia* fruits are potassium (137.00-148.40 mg %), calcium (48.84-56.35 mg %), sodium (0.72-0.95 mg %), phosphorus (10.00-12.02 mg %), iron (1.00-1.99 mg %), copper (46.00-56.00 mg %) and magnesium (69.00-79.45 mg %). All minerals content significantly increased up to the mature stage and then decreased in ripen stage.

Table 5. Activities of amylase, protease, invertase, lipase, polyphenol oxidase and peroxidase enzymes of *C. cordifolia* fruits at different maturity levels (On the basis of fresh weight).

Name of the enzymes	Stages of maturation		
	Immature	Mature	Ripen
Amylase (unit gm^{-1} fruit).	16.55±0.01[b]	22.25±0.08[c]	14.15±0.01[a]
Protease (unit gm^{-1} fruit).	1.55±0.03[a]	4.05±0.06[b]	8.35±0.02[c]
Invertase (unit gm^{-1} fruit).	1.89±0.04[a]	4.77±0.02[b]	1.14±0.05[a]
Lipase (unit gm^{-1} fruit)	10.20±0.06[a]	26.30±0.08[b]	33.10±0.01[c]
Polyphenol oxidase (unit $min^{-1}gm^{-1}$ fruit)	34.80±0.09[c]	14.10±0.01[a]	29.40±0.05[b]
Peroxidase (unit $min^{-1}gm^{-1}$ fruit)	66.10±0.02[c]	38.40±0.15[a]	44.30±0.09[b]

Values are mean ± S.D. of triplicate analyses. Mean in the same row with different superscripts are significantly ($P < 0.05$) different.

Activities of some hydrolytic and oxidative enzymes in *C. cordifolia* fruits were shown in Table 5. The activities of amylase, protease invertase and lipase in different maturity stages of *C. cordifolia* fruits were found between 14.15 to 22.25 mg %, 1.55 to 8.35 mg %, 1.14 to 2.77 mg % and 10.20 to 33.10 mg **%** respectively. Activities of amylase and invertase increase significantly up to mature stage and thereafter decreased significantly, while the activity of protease and lipase increased with the advancement of maturity (Table 5). Among the hydrolytic enzymes, lipase shows the highest activity at ripen stages. Polyphenol oxidase and peroxidase activity increased greatly in immature stage and then decreased dramatically in mature stage and thereafter increased in ripen stage.

Conclusion

In conclusion, ripen *C. cordifolia* fruits might be considered as nutritionally rich source since it contained the highest amount of protein, total sugar, reducing sugar, β-Carotene, vitamin B_1, vitamin B_2 and vitamin C whereas mature and immature *C. cordifolia* fruits are rich sources of minerals and starch respectively.

Acknowledgement

The authors are grateful to the Chairman of the Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh to give the lab facility to complete this research work.

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