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Biochemical changes associated with fruit development in *Abelmoschus esculentus cv*. Arka Anamika

Sreeshma L.S.* **and Bindu R. Nair**

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. Pin: 695 581, India

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Abstract The study intends to trace the time course variations in the biochemical composition of the fruits of *Abelmoschus esculentus cv*. Arka Anamika, starting from anthesis to full maturity. Nine time points were selected for the study over a period of 33 days and a total of 13 parameters were observed. The total phenol, crude fibre, vitamin C, thiamine, riboflavin, ascorbic acid oxidase, catalase and peroxidase, showed a steady increase right from anthesis to fruit maturation. Other parameters such as total protein, carbohydrate, lipid, β carotene and mucilage content though, showed a steady increase at initial stages of development, decreased thereafter. The results of the study provide information on fruit quality at these fruit development stages.

Key words: *Abelmoschus esculentus cv*. Arka Anamika, anthesis, biochemical constituents, development, maturity

Introduction

Consumption of fruits and vegetables is vital to human health. The major benefit from such a diet is the increased intake of various nutritional components such as carbohydrates, protein, organic acids, enzymes, pectin, pigments, minerals and vitamins in addition to necessary antioxidants such as carotenoids, ascorbate, tocopherol and phenolics. Since malnutrition is a serious problem in most of the tropical countries, a lot of interest is currently being focused on the possibilities of exploiting the vast number of familiar fruits and vegetables for utilization as sources of nutrients, vitamins and antioxidants.

Biochemical changes occur in the fruit during the course of development. In most plants, early fruit development can be divided in to four phases: fruit set, a period of rapid cell division, a cell expansion phase and ripening/maturation phase. Although specific biochemical programmes

* **Corresponding author**: Sreeshma L.S.; **e-mail**: sreeshma.ls@gmail.com, sreeshmals@yahoo.com

resulting in ripening phenomena vary among genera or types of fruits, changes typically include modification of colour through the alteration of chlorophyll, carotenoid and flavonoid accumulation; textural modification *via* alteration of cell turgor, cell wall structure or metabolism and modification of sugars, acids and volatile profiles that affect nutritional quality, flavor and aroma. The structural, biochemical and physiological events occurring during cell expansion and ripening phase transform the flavor, texture and overall attractiveness of the mature fruit. Tracing these events during the course of fruit development is imperative since the fruits can be consumed at the right developmental stage.

Abelmoschus esculentus (Okra) is cultivated for its young tender fruits and is one of the most popular vegetables in India. The fruits have a high fibre and mucilage content and are rich in several nutrients. The superior fibre found in Okra is capable of stabilizing blood sugar by curbing the rate at which sugar is absorbed from intestinal tract. Since it is a good source of iodine, it is widely used for the treatment of goiter. The fruit is also useful against urinary disorders, spermatorrhoea and chronic dysentery.

Fruit quality is highly influenced by a combination of several factors such as genotype, geoclimate, and probably carbon partitioning (Lourdes *et al*., 2002). Studying the biochemical constituents in the fruits and their rates of production would shed light on fruit quality. Fruit developmental studies have focused primarily on fleshy species because of their importance in the human diet **(**Giovannoni, 2004)**.** Although Okra is considered to be an important vegetable crop in the world, detailed studies on ontogeny of fruits are rare. Hence, the present study attempts to trace the time course and partitioning of the different biochemical constituents in fruits of *Abelmoschus esculentus cv.* Arka Anamika from the time of anthesis to maturity.

Materials and methods

Abelmoschus esculentus cv. Arka Anamika was selected for the study. It is a high yielding variety tolerant to Yellow Vein Mosaic Virus, released from IIHR Bangalore. This cultivar was developed through hybridization, back crossing and selection from *A. esculentus* x *A. tetraphyllus* ssp. *tetraphyllus*. The seeds for the present study were procured from Kerala Agricultural College Vellayani, Thiruvananthapuram.

Experimental Method

In *Abelmoschus esculentus cv.* Arka Anamika, fruits develop over a period of 38 days from anthesis to full maturity. Based on the number of days taken for seed dehiscence, nine time points were selected for the study.

The first sample: One Day After Anthesis (1 D.A.A) was taken (at the time when non-essential whorls were completely detached from the thalamus). Other eight samples were taken at intervals of four days *ie*, 1 D.A.A, 5 D.A.A, 9 D.A.A, 13 D.A.A, 17 D.A.A, 21 D.A.A, 25 D.A.A, 29 D.A.A, and 33 D.A.A. After 33 days the fruits became stiff, dry and the colour changed from pale yellow to brown. For this reason, all the characters were analyzed until 33 days only.

During the present investigation, a total of 13 biochemical parameters were determined at different fruit developmental stages from anthesis to full maturity. Analysis of different biochemical parameters were done as per standard procedures (Table1). Statistical analysis (ANOVA with Duncan's multiple range tests) was done using SPSS Version 7.5 for the data obtained.

Table 1. List of procedures adopted for the analysis of biochemical parameters of *Abelmoschus esculentus cv*. Arka Anamika

Parameters	Methods followed
1.Protein	Lowry *et al* .,1951
2.Carbohydrate	Roe *et al* .,1965
3.Lipid	Bligh and Dyer (1959)
4.Thiamine	Okwu (2005)
5.Riboflavin	Okwu (2005)
6. β carotene	Roy (1984)
7. Ascorbic acid	Sadasivam and Manickam (2005)
8. Ascorbicacid oxidase	Sadasivam and Manickam (2005)
9.Peroxidase	Sadasivam and Manickam (2005)
10.Catalase	Sadasivam and Manickam (2005)
11.Crude fibre	Sadasivam and Manickam (2005)
12.Mucilage	Baveja *et al.*1988 and Wahi *et al.* 1985
13.Phenol	Sadasivam and Manickam (2005)

Results and discussions

During the present investigation, a total of 13 parameters were determined at different fruit developmental stages (from anthesis to full maturity). Brief descriptions concerning the studied characters are furnished below.

Total protein, carbohydrate and lipids

In the present study, it was observed that total protein and carbohydrate content increased until 13 D.A.A showing a leap between 5 - 9 D.A.A. and then decreasing gradually. The total lipid content was almost negligible showing slight increase up to 21 D.A.A. (Table 2). An increase in protein content may probably be associated with fruit development and is found to be at its maximum as expected at picking time (9 -13 D.A.A). The subsequent decrease may be explained as due to protein degradation. Montz *et al* (1978) demonstrated that majority of proteins are degraded during pericarp senescence in crop legumes and explained that protein loss may be due to the translocation of proteinogenous amino acids from the pericarp into the seeds. Thus it appears that under normal conditions of plant growth, the protein degradation products are transferred to the growing seeds.

Fruits play a vital role to maintain adequate carbohydrate level in the diet and also function as energy storage molecules. The total carbohydrate showed an increase until 13 D.A.A in *cv*. Arka Anamika but tend to decrease later. Comparable results were reported for fruits of strawberry (Lourdes *et al*., 2002), *Solanum habrochates* and *S. peruvianum*, (Kortstee *et al.*, 2007) and Papaya (Selvaraj *et al*., 1982).

Fruits having high levels of carbohydrates with low fats are excellent sources of energy that is useful for human metabolism. In *cv*. Arka Anamika, the carbohydrate level was higher than the lipid content at the edible stage, which suggests its importance. Statistical analysis revealed that the total protein, carbohydrates and lipid content were significantly different at different stages of fruit development (Table 2).

Table 2. Details of primary metabolites studied during different stages of fruit development in *A. esculentus cv*. Arka Anamika.

Days taken for experiment	Total protein (mg/gm)	Total carbohydrate (mg/gm)	Total lipid (mg/gm)
1 D.A.A	0.538±0.013 [a]	1.143±0.054 [a]	0.0015±0.00017[a]
5 D.A.A	1.791±0.140 [c]	5.063±0.033 [e]	0.010±0.0002 [b]
9 D.A.A	5.837±0.078 [c]	7.569±0.043 [f]	0.015±0.0003 [c]
13 D.A.A	6.834±0.135 [g]	7.815±0.045 [g]	0.018±0.0004 [d]
17 D.A.A	4.732±0.105 [f]	5.8743±0.039 [e]	0.024±0.001 [e]
21 D.A.A	3.468±0.0803[e]	4.614±0.123 [d]	0.031±0.0006 [g]
25 D.A.A	2.362 ± 0.034 [d]	3.541±0.058 [bc]	0.028±0.0003[f]
29 D.A.A	1.079±0.079 [b]	3.607±0.107 [b]	0.019±0.0002 [d]
33 D.A.A	0.948±0.0093 [b]	3.409±0.012 [c]	0.018±0.003 [d]

Same superscripts indicate homogenous sets

Vitamins

Vegetables, fruits and cereals are good sources of vitamins. Of the four vitamins studied, thiamine, riboflavin and ascorbic acid contents were found to increase throughout fruit development, whereas β carotene increased only up to 17 D.A.A, then it decreased gradually. Thiamine and riboflavin content in the studied samples were nearly uniform (samples - 5, 9, 13, 17 D.A.A). The β carotene and Vitamin C content were significantly different in the selected samples. The variation in the vitamin content is shown in Table 3.

Thiamine and Riboflavin are the B group vitamins. An adequate supply of B–complex vitamins is necessary for critical protein utilization. Studies conducted by Akubugo *et al* (2007) showed that thiamine and riboflavin helps release energy from foods and is needed for the proper functioning of the nervous system.

Beta carotene (precursor of vitamin A) has potential antioxidant activity (Burns *et al.,* 2003). They have important functions in photosynthesis, nutrition and protection against photo oxidative damage. They can inactivate the free radicals and singlet oxygen formation by a process termed as ‘quenching’. An increased consumption of beta carotene rich foods is considered to be preferable to the massive synthetic Vitamin A dosage approach and can be one of the strategies for improving nutritional status (Goplan, 1992; Mc Laren & Frigg, 2001).

Ascorbic acid is a monosaccharide antioxidant vitamin found in both plants and animals. As it cannot be synthesized in humans it must be obtained from the diet. A lot of literature is available on the health benefits of Vitamin C (Weisberger, 1995). It is instrumental in the formation of collagen, a protein that gives structure to bones, muscles and blood vessels. Vitamin C also aids in the absorption of iron and maintain capillaries, bones and teeth. There is evidence that large doses of Vitamin C either in multiple divided oral doses or administered intravenously have beneficial effects in cancer therapy (Donaldson, 2004). It is suggested to enhance the body’s immune system *via* the production of interferon’s, T-lymphocytes and immunoglobulins. Vitamin C was found to increase steadily in the developing fruits of okra, which continued until fruit maturity and dehiscence suggesting that okra could be used as a very good source of Vitamin C (Table 3).

Table 3. Details of the variations in the vitamin content at different fruit development stages in *A. esculentus cv*. Arka Anamika

Days taken for experiment	Thiamine (mg/gm)	Riboflavin (mg/gm)	β carotene (mg/gm)	Ascorbic acid (mg/gm)
1 D.A.A	0.016±0.005[a]	0.011±003[a]	0.105±0.052[a]	0.027±0.012[a]
5 D.A.A	0.054±0.002[b]	0.057±0.002[b]	1.557±0.116[c]	0.158±0.003[b]
9 D.A.A	0.066±0.002[bc]	0.073±0.002[bc]	2.871±0.094[e]	0.247±0.010[c]
13 D.A.A	0.085±0.002[bc]	0.085±0.002[c]	3.644±0.088[f]	0.312±0.003[d]
17 D.A.A	0.094±0.001[c]	0.094±0.001[c]	4.899±0.064[h]	0.466±0.0107[e]
21 D.A.A	0.156±0.027[d]	0.123±0.016[d]	4.106±0.056[g]	0.695±0.005[f]
25 D.A.A	0.208±0.003[e]	0.209±0.004[e]	2.572±0.029[d]	0.763±0.007[g]
29 D.A.A	0.252±005[f]	0.254±0.007[f]	0.709±0.004[b]	0.834±0.004[h]
33 D.A.A	0.285±0.005[g]	0.311±0.009[g]	0.624±0.004[b]	0.922±0.005[i]

Same superscripts indicate homogenous sets

Enzymes

The enzymes included in the study such as peroxidase, catalase and ascorbic acid oxidase have antioxidant properties. The activity of the three enzymes increased throughout fruit development. Though the quantities of ascorbic acid oxidase and catalase were less at the initial stages of development they increased later on and the values differed significantly in all the samples. However peroxidase activity was significantly high at all stages. The variation in the enzyme activity is shown in Table 4.

Table 4. Details of the variations in the enzyme activity at different fruit development stages in *A. esculentus cv*. Arka Anamika

Days taken for experiment	Ascorbic acid oxidase (units /gm tissue)	Catalase (units/ml extract)	Peroxidase (units/l)
1 D.A.A	0.029±0.002 [a]	5847.3±124.57 [a]	2.213±0.047 [a]
5 D.A.A	0.056±0.005 [a]	5925.6±30.80 [a]	2.637±0.037 [b]
9 D.A.A	0.139±0.005 [b]	6403.3±22.0002 [b]	3.415±0.046 [c]
13 D.A.A	0.183±0.008 [c]	7609±14.29 [c]	3.863±0.045 [d]
17 D.A.A	0.269±0.013 [d]	8583.3±21.85 [d]	4.1303±0.070 [e]
21 D.A.A	0.351±0.021 [e]	17005.3±29.56[e]	5.091±0.015 [f]
25 D.A.A	0.459±0.015 [f]	17996.6±16.25 [f]	5.305±0.048 [g]
29 D.A.A	0.528±0.017 [g]	24043±174.24 [g]	5.561±0.059 [h]
33 D.A.A	0.807±0.008 [h]	34032±140.63 [h]	6.878±0.024 [i]

Same superscripts indicate homogenous sets

Peroxidases catalyses the dehydrogenation of a large number of organic compounds such as phenols, aromatic amines, and hydroquinone. Peroxidases have been implicated in several primary and secondary metabolic reactions including the regulation of cell elongation, cross-linking of cell wall polysaccharide, lignifications, wound healing, systemic resistance and phenol oxidation (Hammerschmi & Kuc, 1987; Lagrimini, 1991; Alcazar *et al*., 1995).

Catalase is the most potent catalyst present in plants, animals and microbes and appears to play an important role in several aspects of protection of cell organelles. It catalyses the conversion of H_2O_2 to H_2O and molecular O_2. By preventing excess H_2O_2 build up, catalase allows important cellular processes, which produce H_2O_2 as a by-product, to take place safely.

Ascorbic acid oxidase is widespread in plant tissues including fruits and vegetables. The role of this enzyme is to regulate the levels of oxidized and reduced glutathione and NADPH.

Crude fibre, mucilage and phenol

The variations in the crude fibre, mucilage and phenol contents in *cv*. Arka Anamika are presented in Table 5.

Table 5. Details of the variations in crude fibre, mucilage and total phenol at different fruit development stages in *A. esculentus cv*. Arka Anamika

Days taken for experiment	**Crude fibre (mg/gm)**	**Mucilage content (mg/gm)**	**Total phenol (mg/gm)**
1 D.A.A	000±000 [a]	0.363±0.023 [c]	0.024±0.002 [a]
5 D.A.A	0.004±0005 [ab]	0.444±0.012 [d]	0.380±0.009 [b]
9 D.A.A	0.026±0029 [ab]	0.540±0.010 [e]	0.525±0.058 [c]
13 D.A.A	0.057±0026 [b]	0.748±0.022 [f]	0.872±0.010 [d]
17 D.A.A	0.196±0300 [c]	0.924±0.006 [h]	1.158±0.019 [e]
21 D.A.A	0.867±028 [d]	0.852±0.011 [g]	1.360±0.022 [f]
25 D.A.A	0.981±003 [e]	0.414±0.007 [d]	1.414±0.013 [fg]
29 D.A.A	1.057±025 f	0.227±0.009 [b]	1.474±0.006 [g]
33 D.A.A	1.177±019 [g]	0.147±0.002 [a]	1.905±0.010 [h]

Same superscripts indicate homogenous sets

Crude fibre consists largely of cellulose, lignin and some mineral matter. The non-starch polysaccharides act as food fibres / dietary fibres (DF), which has an important role in the absorption of sugar (Indira Gopalan & Mohanram, 1996). Dietary fibre plays an important role in decreasing the risk of many disorders such as constipation, diabetes, cardiovascular diseases, diverticulosis and obesity (Spiller, 2001). Plant foods are the only sources of DF. The polysaccharides comprising a major part of DF in fruits and vegetables are beneficial to humans. Consumption of fibres lowers blood cholesterol levels. It was also reported that, DF of fruits and vegetables offers protection against development of stroke in men (Gillman *et al*., 1995; Holland *et al*., 1998).

Present study revealed that the crude fibre was completely absent in the first sample (1D.A.A). The fibre content started to show a steady increase with fruit age thereafter (Table 5).

Mucilage is the pectinous matrix of cell layers (Western, Skinner and Haughn, 2000), which swells considerably upon wetting. This capacity of cell matrix to swell markedly indicates that such cell walls consist of mucilage with considerable amounts of unesterified galacturonic acid with a large capacity for hydration. It is a high molecular weight water soluble polysaccharide containing more than 30,000 different sugars commonly present in higher plants. According to Distelbarth & Kullu (1985) mucilage may serve as reserve substance or may contribute to the moisture balance or resistance to drying out. The mucilage contains usable form of soluble fibre. Okra fruits are good source of mucilage. Mucilage content increased from 1D.A.A to 17 D.A.A, and then decreased gradually from the fruit tissues (Table 5). The mucilage content was significantly different in the samples and was least in the last two samples (29 and 33 D.A.A).

Phenolics are the aromatic compounds with hydroxyl groups and are widespread in plant kingdom. They occur in all parts of the plants. They are said to offer resistance to diseases and pests in plants. Phenols include an array of compounds including tannins and flavanols. Phenolic compounds may help to protect the gastrointestinal tract against damage by reactive species present in foods or generated within the stomach and intestine. Phenolic compounds have been reported to be associated with antioxidant activities in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice Evans *et al*., 1997).

The total phenol content in the fruits of *cv.* Arka Anamika increased throughout fruit development. The increase in total phenol content was more apparent after fruit maturity. All the samples showed a significant variation in the total phenol content (Table 5).

On the basis of the study, it can be suggested that, fresh fruits, 9-13 days after anthesis (the harvesting stage) are best for consumption, when the nutritional constituents such as proteins, carbohydrates, lipids and vitamins are at their maximum. The increase in total protein, carbohydrate and lipid is suggestive of an increase in nutritional quality of the fruits at harvest time and the subsequent decrease reflects the loss in nutritive value. Vitamin C, thiamine and riboflavin were found to increase steadily in the developing fruits of okra, which continued until dehiscence suggesting that okra could be used as a very good source of these Vitamins. Okra fruits are a good source of mucilage and can be used for mucilage extraction. The increase in crude fibre, antioxidant enzymes and phenol until fruit maturation suggests the protective role of these constituents throughout seed development until dehiscence.

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