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CHANGES IN PHYSICO-CHEMICAL COMPOSITIONS AND ACTIVITIES OF SOME HYDROLYTIC AND OXIDATIVE ENZYMES IN THE TWO TYPES OF SAJNA (*MORINGA OLEIFERA* LAM.) LEAVES AT DIFFERENT MATURITY LEVELS

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SUMMARY

Two different types of sajna 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 sajna leaves was acidic at all the maturity stages and continued to increase gradually with the advances of maturity. The moisture content decreased gradually while ash content increased remarkably with age. Chlorophyll, protein and lipid contents increased rapidly upto mature stage and thereafter decreased rapidly at the ripen stage. Total sugar content increased gradually with the advances of maturity while starch content decreased sharply after mature stage. The vitamins such as thiamine and riboflavin were found to be present in low amount in sajna leaves, while it contained good amount of ascorbic acid at mature stage and carotene at ripen stage. The activities of protease and peroxidase increased rapidly with the maturity, while amylase, cellulase, invertase, ascorbic acid oxidase and polyphenol oxidase increased upto mature stage and then decreased drastically at ripen stage. Fresh sajna leaves contained significantly higher amount of oxidative enzymes as compared to the hydrolytic enzymes at all maturity stages.

***Key words*: Hydrolytic and oxidative enzymes, maturity levels, physico-chemical composition, sajna leaves.**

INTRODUCTION

Sajna (*Moringa oleifera* Lam.) leaf is one of the most familiar and widely distributed vegetables of Bangladesh. Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human ailments and are also eaten as vegetables (Chopra *et al.* 1956, Sastri 1962, Nadkarni 1976). Sajna leaves are not affected by diseases but there is a transformation in the leaves. The young green leaves are transformed into yellowish green with age, which may be due to loss of chlorophyll and synthesis of caroténoid in the leaves. Proteolytic and hydrolytic enzymes play important physiological roles during maturation and senescence of fruit (Hashinaga *et al.* 1983, Desai and Deshpande 1978). Dilley (1970) suggested that the dramatic physical and chemical changes during ripening occur as a result of catabolic and metabolic processes, which might be enzyme directed processes.

In this study the physico-chemical compositions as well as the activities of some oxidative and hydrolytic enzymes of Sajna leaves were analysed at different maturity stages.

MATERIALS AND METHODS

Fresh Sajna leaves were collected from Kazla of Rajshahi District, Bangladesh. Two locally available sajna leaves indentified as "large size" and "small size" were harvested at different maturity stages for

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experimental purposes (Fig. 1). Days required from the time of leaf harvesting for immature, mature and ripen stages were upto 10 ± 4, 30 ± 10 and 50 ± 10 days respectively.

About 2g of leaves were crushed thoroughly in a mortar with a pestle and homogenized well with 10 ml of distilled water and then filtered through two layers of muslin cloth. The filtrate was further clarified by centrifugation at 6000 g for 10 min and used for the experimental purposes.

The pH of the filtrate was determined using a Corning 215 pH- meter. The ash content of leaves was determined following the method of A.O.A.C. (1980). Chlorophyll content of the leaves was estimated as per Mahadevan and Sridhar (1982). The total protein and water soluble protein were determined by the micro-Kjeldhal method (Jayaraman 1981) and spectrophotometrically (Lowry *et al.* 1951) respectively. Total phenol and lipid contents were determined colorimetrically as per Bray and Thorpe (1954) and Bligh and Dyer (1959) respectively. Total sugar and starch contents were estimated colorimetrically by Anthrone method (Dubois *et al.* 1951) and (Jayaraman 1981) respectively. The vitamins such as thiamine, riboflavin were estimated following the procedure as described by Anonymous (1965) and ascorbic acid by Bessey and King (1933), while β-carotene was estimated following the method described by Jensen (1978). The minerals such as calcium content was determined as described by Basset *et al.* (1978), while iron and phosphorus contents were determined following the method described by Ranganna (1986).

For preparation of crude enzyme extract about 10 g of leaves were cut into small pieces and grinded in a mortar with pestle and then homogenized well with cold 0.1 M phosphate buffer of respective pH (amylase, pH 6.7, invertase and protease, pH 7.0, polyphenol oxidase, peroxidase and ascorbic acid oxidase, pH 6.0), while for the measurement of cellulase 0.1 M sodium-acetate buffer, pH 5.2 was used. After centrifugation at 8000g, 4°C for 10 min. the supernatant was used as crude enzyme extract.

The activities of invertase, cellulase, ascorbic acid oxidase, polyphenol oxidase and peroxidase were estimated following the procedures described by Mahadevan and Sridhar (1982). The protease activity was measured by the method of Kunitz (1947), while the activity of amylase was determined as per Jayaraman (1981).

RESULTS AND DISCUSSION

As shown in Table 1 the pH of the juices of both the varieties of sajna leaves was in acidic ranges at all the maturity stages and the pH increased with the advancement of maturity. The small sized leaf was found to be slightly more acidic than the large sized leaf. It may be suggested from the results that pH value of 5.95 or above is an indication of ripening of sajna leaves. The moisture and ash content were also higher in small sized leaves as compared to large sized leaves. Moisture content decreased gradually, while ash content increased in the leaves with the advancement of maturity. The decrease in moisture content with the advancement of maturity might be due to accumulation of solid materials which also showed good

Table 1. pH, moisture and ash contents of sajna leaves at different maturity levels (on the basis of fresh weight).

	Type of leaves	Stages of maturation		
		Immature	Mature	Ripen
pH	SSL	4.00 ± 0.05	5.30 ± 0.06	5.95 ± 0.05
	LSL	4.35 ± 0.04	5.50 ± 0.06	6.02 ± 0.03
Moisture (%)	SSL	83.3 ± 0.02	82.5 ± 0.03	75.9 ± 0.01
	LSL	75.01 ± 0.04	72.04 ± 0.05	67.06 ± 0.06
Ash (%)	SSL	2.47 ± 0.01	3.78 ± 0.01	5.28 ± 0.02
	LSL	2.0 ± 0.02	3.25 ± 0.03	4.8 ± 0.03

SSL = Small sized leaves.
LSL = Large sized leaves.

correlation as the mineral contents increased with the advancement of maturity. Further ash content increased significantly more from mature to ripen stage, although the contents of most of the other solid materials decreased from mature to ripen stage. The ash content obtained in the present study was comparable with that of sajna leaves produced in India (Sastri 1962).

It was found that chlorophyll contents of sajna leaves increased remarkably upto mature stage and, thereafter decreased drastically at ripen stage. This is also supported from the physical observation that the color of leaves became light yellowish green at ripen stage (Fig. 1). As shown in the Table 2 sajna leaves are good sources of β-carotene and the amount of β-carotene in sajna leaves was found to vary between 0.0906-0.1289%. Further, the β carotene content increased remarkably with the advancement of maturity. Total protein and water soluble protein contents of both the types of sajna leaves were found to be very similar. Total poretin content of leaves

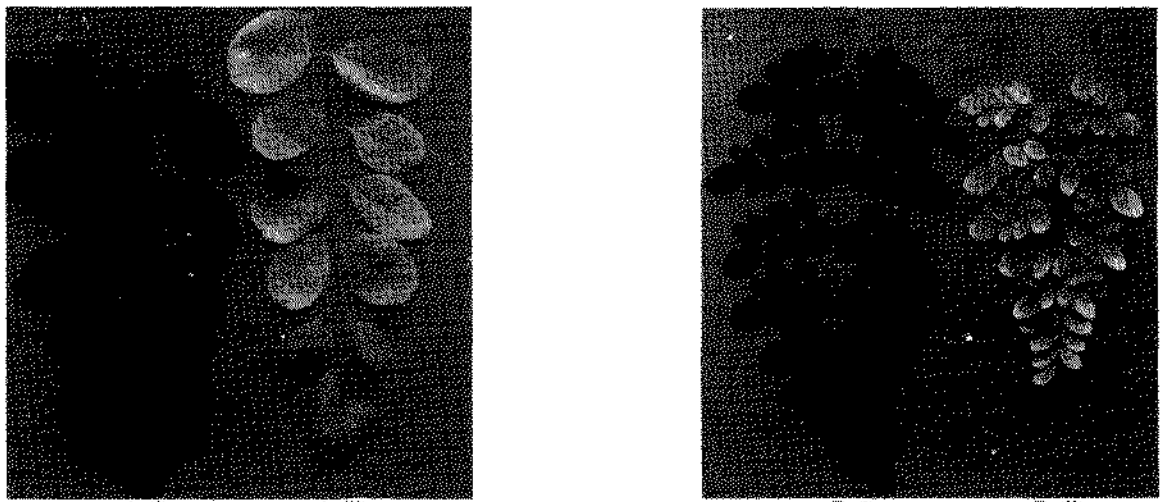

Fig. 1. Photographs of the locally available sajna leaves at mature and ripe stages. A. Large size leaves: i) mature stage ii) ripe stage; B. Small sized leaves: i) mature stage ii) ripe stage

Table 2. Total chlorophyll, carotene, total protein, water soluble protein and lipid contents of sajna leaves at different maturity levels (on the basis of fresh weight).

	Type of leaves	Stages of maturation		
		Immature	Mature	Ripen
Total chlorophyll (mg g^{-1})	SSL	0.728±0.01	1.66±0.02	0.35±0.03
	LSL	1.69±0.02	2.58±0.01	0.32±0.01
β-Carotene (%)	SSL	0.09888±0.001	0.11184±0.001	0.1289±0.002
	LSL	0.0906±0.001	0.102±0.002	0.1195±0.003
Total protein (%)	SSL	5.88±0.03	7.65±0.01	4.10±0.01
	LSL	5.58±0.01	7.2±0.01	4.0±0.01
Water soluble protein (%)	SSL	1.02±0.01	1.50±0.02	1.04±0.03
	LSL	1.08±0.01	1.57±0.01	1.09±0.01
Total lipid (%)	SSL	0.5±0.01	1.0±0.01	1.9±0.01
	LSL	0.7±0.02	1.12±0.02	1.95±0.01

increased significantly until the end of growth i.e. upto mature stage and thereafter decreased remarkably. At mature stage, the small sized leaves contained 7.65% and 1.5% while large sized leaves contained 7.2% and 1.57% of total protein and water soluble protein respectively. Leaves of sajna contained low amount of lipid and the content increased gradually with the advancement of maturity. Large sized leaves contained slightly higher amount of lipid as compared to that in small sized leaves.

Total sugar contents of sajna leaves increased gardually while starch content decreased remarkably with the advancement of maturity. The large sized leaves contained higher amounts of total sugar and starch at all the maturity stages (Table 3). The total sugar and starch contents were found to vary between 1.2-3.23% and 3.2-6.8% in small sized leaves and 2.3-4.5% and 3.9-7.8% in large sized leaves respectively at different maturity stages. The reduction of starch with the changes of maturity might be due to hydrolysis of starch, which shows good correlation with the increase in the contents of total soluble sugars. Mature sajna leaves produced in Bangladesh contained about 8-10% total carbohydrate, compared to those produced in India which contained about 13.4% carbohydrate (Sastri 1962). The small sized leaves contained slightly higher amounts of phenol than the large sized leaves and the phenol content varied between 0.093-0.114%. Phenol content increased gradually upto mature stage and thereafter decreased at ripen stage.

The amounts of thiamin, riboflavin, ascorbic acid, calcium, iron and phosphorus in sajna leaves are shown in Table 4. The results indicated that sajna leaves are good sources of ascorbic acid and phosphorus. The phosphorus content increased gradually with the advancement of maturity while ascorbic acid decreased slightly after mature stage. The amounts of thiamine, riboflavin, ascorbic acid, calcium, iron and phosphorus in sajna leaves were found to vary between 0.0021 - 0.0055%, 0.00006 - 0.00017%, 0.2 - 0.28% 0.0052 - 0.0084%, 0.0055 - 0.007% and 0.035 - 0.065% respectively at different maturity levels. Phosphorus, carotene and ascorbic acid content of mature sajna leaves were at par with those the leaves produced in India (Sastri 1962).

Activities of amylase, cellulase and invertase increased rapidly upto mature stage and thereafter decreased significantly, while the activity of protease increased gradually with the advancement of maturity leavels (Table 5). Activities of ascorbic acid oxidase and polyphenol oxidase increased rapidly upto mature stage and thereafter decreased drastically while the activity of peroxidase increased significantly throughout the maturity stages. At mature stage, the activities of ascorbic acid oxidase, polyphenol oxidase and peroxidase present were 34.0 - 35.0, 50.0 - 80.0 and 31.0 - 33.0 units min^{-1} g^{-1} fw respectively. The results also indicated that the large sized leaves contained higher amount of oxidative enzymes as compared to those present in small sized leaves.

In conclusion, mature sajna leaves might be considered as nutritionally rich vegetable since, it contained significant amount of protein, starch, β-carotene, ascorbic acid and ash.

Table 3. Total sugar, starch and total phenol contents of sajna leaves at different maturity levels (on the basis of fresh weight).

	Type of leaves	Stages of maturation		
		Immature	Mature	Ripen
Total sugar (%)	SSL	1.2±0.02	2.3±0.03	3.23±0.04
	LSL	2.3±0.01	3.3±0.05	4.5±0.05
Starch (%)	SSL	6.8±0.03	5.2±0.02	3.2±0.04
	LSL	7.8±0.05	6.5±0.03	3.9±0.05
Total phenol (%)	SSL	0.105±0.03	0.114±0.01	0.096±0.04
	LSL	0.102±0.05	0.112±0.02	0.093±0.04

Table 4. Thiamine, riboflavin, ascorbic acid, calcium, iron and phosphorus contents of sajna leaves at different maturity levels (on the basis of fresh weight).

	Type of leaves	Stages of maturation		
		Immature	Mature	Ripen
Thiamine %	SSL	0.003±0.0002	0.0042±0.0001	0.0021±0.0001
	LSL	0.0029±0.0002	0.0055±0.0001	0.0026±0.0001
Riboflavin (%)	SSL	0.00006±0.0001	0.000102±0.0002	0.00014±0.0003
	LSL	0.00008±0.0001	0.00012±0.0002	0.00017±0.0002
Ascorbic acid (%)	SSL	0.2±0.008	0.25±0.003	0.23±0.005
	LSL	0.22±0.004	0.28±0.005	0.25±0.003
Calcium %	SSL	0.0084±0.0001	0.0075±0.0002	0.0063±0.0003
	LSL	0.0071±0.0001	0.0063±0.0002	0.0052±0.0005
Iron (%)	SSL	0.0067±0.0001	0.0062±0.0001	0.0055±0.0001
	LSL	0.007±0.0001	0.0065±0.0002	0.0059±0.0002
Phosphorus %	SSL	0.046±0.002	0.053±0.003	0.065±0.006
	LSL	0.035±0.002	0.041±0.002	0.057±0.001

Table 5. Activities of amylase, protease, cellulase, invertase, ascorbic acid oxidase, polyphenol oxidase and peroxidase in sajna leaves at different maturity stages.

	Type of leaves	Stages of maturation		
		Immature	Mature	Ripen
Amylase (unit g^{-1} fr. wt.)	SSL	10.0±0.001	19.55±0.001	12.18±0.002
	LSL	11.33±0.003	24.3±0.003	14.6±0.002
Protease (unit g^{-1} fr. wt.)	SSL	1.2±0.002	3.01±0.001	4.10±0.001
	LSL	1.89±0.001	3.10±0.001	4.05±0.002
Cellulase (unit g^{-1} fr. wt.)	SSL	7.0±0.002	18.4±0.001	3.1±0.003
	LSL	6.5±0.001	17.2±0.001	2.95±0.002
Invertase (unit g^{-1} fr. wt.)	SSL	0.09±0.002	0.13±0.003	0.043±0.003
	LSL	0.06±0.001	0.10±0.001	0.03±0.001
Ascorbic acid oxidase (unit min^{-1} g^{-1} fr. wt.)	SSL	28.5±0.01	34.0±0.011	17.0±0.01
	LSL	30.1±0.02	35.0±0.02	20.0±0.03
Polyphenol oxidase (unit min^{-1} g^{-1} fr. wt.)	SSL	25±0.02	50±0.03	20±0.03
	LSL	40±0.02	80±0.02	30±0.03
Peroxidase (unit min^{-1} g^{-1} fr. wt.)	SSL	15.2±0.01	31.0±0.02	36.5±0.02
	LSL	16.0±0.02	33.0±0.01	37.6±0.01

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