International Food Research Journal 20(5): 2309-2316 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis

Sonu Sharma and *Ramana Rao, T. V.

BRD School of Biosciences, Sardar Patel University Vallabh Vidyanagar, Gujarat – 388 120, India

Article history

Received: 25 August 2012
Received in revised form: 9 April 2013
Accepted: 12 April 2013

Keywords

Biochemical analysis
Fruit
Nutrition
Pumpkin
Ripening

Abstract

Growing population, together with unpredictable climatic changes and dwindling fertile land, has forced to explore the alternate underutilized flora from the rich biome. There is a great opportunity for economic growth in agriculture system by introducing underutilized commodity in market place. Therefore, the present study was undertaken to elucidate the nutritional potential of an important underutilized fruit of pumpkin (*Cucurbita maxima* D.) at its sequential stages of development. Changes in various physico-biochemical properties such as pH, titratable acidity (TA), carbohydrates, free amino acids, total proteins, total phenols, carotenoids, ascorbic acid and the specific activities of softening enzymes (β-galactosidase, cellulase, pectin methylesterase and polygalacturonase), hydrolytic enzymes (amylase and invertase) and antioxidant enzymes (peroxidase, polyphenoloxidase, superoxide dismutase, catalase and ascorbic acid oxidase) were investigated. The results revealed that pumpkin fruits on ripening accumulated considerable amount of carotenoids, vitamin C and proteins along with carbohydrates. Due to enhanced activities of amylase and invertase during ripening of pumpkin fruit, starch degradation occurred concomitantly with the increasing pattern of reducing sugars and total sugars. β-galactosidase and cellulase enzymes actively involved in cell wall degradation than that of pectin methylesterase (PME) and polygalacturonase (PG), while the activities of antioxidant enzymes exhibited declining pattern towards its maturation and ripening. The physico-biochemical study of particular commodity would be useful to assist in the selection of fruit at appropriate stage of development and its utilization in acceptable manner.

Introduction

Consumption of fruits and vegetables has been increased rapidly by people due to awareness regarding their health benefits. However, the perishable nature of fruits and vegetables and over-dependency of human on fewer plant species created extreme pressure on the fresh produce industries to supply bulk of fresh fruits and vegetables to the burgeoning population. Such increased demand can only be fulfilled by either using the technology to prevent the deterioration of commodity after harvest and/or to introduce underutilized fruits or vegetables for their commercial utilization. "Underutilized species" are plants whose nutritional values are either unknown or unexplored by researchers. These less important underutilized fruits remained uncared for and remained confined mainly to natural wild, semi-wild and semi domesticated conditions albeit with large ever increasing variability. Besides their importance as potential horticulture species these plants are incidentally store houses of genes for adaptation to hot and hardy climates, salt tolerance, diseases tolerance and several essential nutritional values. Further, efforts to cultivate these plants have not been explored as their economic potential has either been not completely explored or such products are confined mostly limited to traditional/local usage (Patel, 2009). Many of the indigenous tropical and temperate fruits and vegetables have still remained underexploited due to the lack of awareness of their potential, market demand and low and erratic bearing in many cases. These species have multipurpose uses as fruits, vegetables and also have therapeutic and medicinal properties (Malik *et al.*, 2010). According to Padulosi (1998), there is an urgent need not only to acknowledge underutilized commodity, but also employed and explored these commodities to meet needs of present and future generations.

The genus *Cucurbita*, indigenous to the western hemisphere, is comprised of five domesticated species. Three of these, *Cucurbita pepo* L., *Cucurbita maxima* D. and *Cucurbita moschata* D. represent economically important species cultivated worldwide for human consumption (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). According to

*Corresponding author.
Email: *tadapanenirao@yahoo.com*

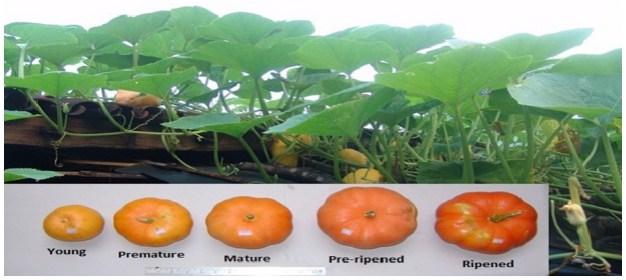

Figure 1. *Cucurbita maxima* fruit at its sequential stages of growth and ripening

Food and Agriculture Organization of United Nation (FAO), the world production of pumpkins, squashes, and gourds in 2011 was estimated over 24.3 million tons harvested from 1.7 million hectares (FAOSTAT, 2013). Cultivation of *Cucurbita cultigens* as a food source on an international scale over the world attributed to their adaptability in range of climatic conditions provide great opportunities for increased diversity and market growth by introducing underutilized forms of existing species (Rai *et al.*, 2008).

In the present investigation, the fruits of *Cucurbita maxima* D. subspecies growing in Jambughoda region of South Gujarat, India were collected in order to follow the changes in their biochemical properties during maturation and ripening. It is characterized by yellow to orange colored skin, slightly globosely shaped, flattened at both stem and blossom end with pronounced ribs on its surface. This important horticultural commodity has not received its commercial cultivation, but local people grow it on the roofs of their houses (Figure 1) or in their kitchen gardens and they use mature fruits as a vegetable.

Since, India contains a high proportion of low income population that requires balanced food and nutrition at affordable prices (Pareek *et al*., 1998). Such less known potential commodity would serve as staple food for weaker sections of society during grain scarcity and also as profitable opportunity for many farmers because of their long storage life, good nutritive value, wide use in cooking, and low price. In addition, it has potentiality for introducing as minimally processed product in fresh produce market due to its attractive orange colored flesh indication of high carotenoid content and better postharvest shelf-life. Carotenoids are among the phytochemicals, believed to reduce the risk of developing degenerative diseases.

Such commodity are needed to be evaluated for understanding their nutritive value, potential uses for other purposes, for possibilities of commercial cultivation in suitable areas and change in marketing. A cross section of literature revealed that various aspects on *Curcurbita* sps. have been explored in detail which included their morphological and anatomical features (Agbadwa and Ndukwu, 2004), morpho-physiological characteristics associated with high yield and better quality (Loy, 2004), production and marketing profile (Radovich, 2010), nutritive and mineral composition (Aliu *et al*., 2012), composition and functional properties of dietary fibers (de Escalada Pla *et al*., 2007) etc. Habibunnisa *et al.* (2001) studied the performance of minimally processed pumpkin fruits under modified atmosphere package (MAP) conditions. Pandya and Rao (2010) studied physiological and biochemical changes in the fruit of *Cucurbita moschata* during its growth and ripening in relation to its seed development. However, a perusal of literature indicated that there is scare information regarding the influence of maturity on nutritional properties of *Cucurbita maxima.* Therefore, the present study has been undertaken to elucidate the physico-chemical characteristics during its growth and ripening, which would provide better scope to enhance its utilization through assisted selection of fruit at appropriate stage of development.

Materials and Methods

Plant material

The fruits of pumpkin were collected at their five sequential stages of development i.e. young, pre-mature, mature, pre-ripened and ripened from the roof tops of the houses located in the regions of Jambughoda, South Gujarat, India, during the September month of calendar year 2010 (Figure 1). The selection of three fruit samples from each stage of the development was done on the basis of their morphological attributes such as size, weight and colour. After harvest the fruits were brought to the laboratory, washed thoroughly in tap water and air dried. After recording the physical parameters i.e., length, diameter and fresh weight, the fruits were stored in refrigerator at 7°C - 10°C until further use for their biochemical analyses.

pH and titratable acidity (TA)

5 g of fruit tissue was homogenized with 25 ml of distilled water. The pH of fruit was measured by using a digital pH meter (Model Li 120, Elico). The mixture was titrated with 0.1 M NaOH to pH 8.3 and the results were expressed in % citric acid (Rangana, 1977).

Biochemical analyses

The quantitative analysis of carotenoids was carried out as per the method described by Tomes (1968). The concentrations of reducing sugars and non-reducing sugars were determined by the

dinitrosalicylic acid method (Miller, 1959), while the anthrone method was followed for the estimation of total sugars and starch (Thimmaiah, 1999). The amount of free amino acids was determined as per the method described by Moore and Stein (1948), while total proteins and total phenolics were quantified according to the methods suggested by Lowry *et al.* (1954) and Bray and Thorpe (1954), respectively. Vitamin C content was estimated by following the method of Roe and Oesterling (1944).

Enzymes assay

The specific activities of amylase and invertase were evaluated following the methods described by Bernfeld (1955) and Mazumdar and Majumder (2003) respectively, while softening enzymes such as polygalacturonase (PG), pectin methyl esterase (PME, EC 3.1.1.11), β-galactosidase (EC 3.2.1.23) and cellulase (EC 3.2.1.4) were assayed as per the methods described by Zainon and Brady (1982), Hagerman and Austin (1986), Biswas (1985) and Mazumdar and Majumder (2003), respectively. The antioxidant enzymes such as peroxidase (POX; EC 1.11.1.7), and polyphenol oxidase (PPO; EC 1.14.18.1) were also assayed as described by Civello *et al.* (1995) and Rocha and Morais (2001) respectively. For ascorbic acid oxidase (AAO; EC 1.10.3.3) assay, the methodology suggested by Mazumdar and Majumder (2003) was followed. Determination of superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) activity was performed by the method of Wang *et al.* (2005).

Statistical analysis

Experiment was performed according to a completely randomized design with triplicates. Data was subjected to statistical analysis by using one way Analysis of variance (ANOVA) and means were compared by Duncan's multiple range test (DMRT) using IRRISTAT software (Version 3) (Bliss, 1967) at a significance level of 0.05.

Results and Discussion

Changes in physical characteristics

The developmental process is characterized by irreversible increase in volume as consequences of cell divisions and cell elongation accompanied by differentiation up to certain limit. In the present study, the fruits of pumpkin were approximately 8-9 cm long, 11-12 cm transverse diameter and weighing 400 gm to 500 gm at its young stage, reached to the their maximum values with 9-10 cm long, 19-20 cm transverse diameter and weighing 1500 - 2000 gm at its ripened stage. The results indicated that fresh weight gain of fruits is mainly achieved by an increase in its diameter rather than its length (Table 1).

Changes in pH and titratable acidity

Flavor quality of the commodity which determines their value to consumers depends on the content of sugars, organic acids, phenolic compounds, volatiles etc. During the growth and ripening of *C. maxima* fruit, the pH of its mesocarp fluctuated up to maturation but it attained increasing pattern from mature stage (5.66) to ripened stage (6.72) (Table 1). However, titratable acidity declined from 0.64% citric acid equivalent at mature stage to 0.38% citric acid equivalent at ripened stage. The changes in the pH of the fruit flesh influences the activities of ripening related enzymes and antioxidant system, ultimately affects the sensory quality (McCollum *et al.*, 1988).

Changes in Carbohydrates

Starch is the main storage carbohydrate in early stages of fruit development, which degrades with the onset of ripening. In present study, pumpkin fruits accumulated starch approximately by two-fold up to its premature stage, and thereafter declined towards the ripening. Similarly, the amount of total soluble sugars presented increasing trend from young stage (91.03 mg g^{-1}) to pre-mature stage (106.58 mg g^{-1}), but showed declining pattern in the subsequent stages (Table 2). An increase in the content of reducing sugars from young stage (31.51 mg g^{-1}) to ripened stage (77.30 mg g^{-1}) exhibited, which was concomitant with decreasing patterns of starch content and non-reducing sugars during ripening (Table 2). Paliyath and Murr (2008) reviewed the pattern of sugar metabolism, and they opined that catabolic process leads to degradation of starch into glucose and fructose, which enters the metabolic pool where they are used as respiratory substrates or further converted to other metabolites. Similar interpretation in sugar metabolism has also been reported during fruit development of *Cucurbita maxima* D. 'Delica' (Harvey *et al*., 1997).

Changes in total proteins and total free amino acids

The quantitative analysis of total free amino acids in the presently studied pumpkin fruit showed a six fold increase from young to mature stage, but eventually declined as the ripening progresses. The total proteins content enhanced from its young stage (4.85 mg.g^{-1}) to pre-mature stage (7.57 mg.g^{-1}), however it remained more or less unchanged in subsequent stages (Table 2). Similar results were reported in Arnonia pepper (Martínez *et al.*, 2007). Patel and Rao (2011) opined that the reduction in the total free amino acids content may be due to their

Table1. Measurement of fresh weight, diameter, length, pH and titratable acidity of *Cucurbita maxima* fruit at successive stages of growth and ripening

Stage no.	Stages of development	Fresh weight (gm)	Diameter (cm)	Length (cm)	pH	Titratable acidity (% citric acid)
1.	Young	454.17±6.78^{a}	11.37±0.15^{a}	8.03±0.06^{b}	5.46±0.12^{a}	0.63±0.01^{c}
2.	Premature	750.17±5.75^{b}	14.53±0.31^{b}	8.24±0.12^{a}	6.60±0.02^{c}	0.39±0.00^{b}
3.	Mature	1006.00±14.00^{c}	16.03±0.25^{c}	8.67±0.67^{bc}	5.66±0.16^{a}	0.64±0.00^{c}
4.	Pre-ripened	1570.00±10.26^{d}	18.87±0.06^{d}	9.73±0.12^{d}	6.24±0.22^{b}	0.39±0.00^{b}
5.	Ripened	1693.50±9.58^{e}	19.60±0.17^{e}	9.13±0.12^{cd}	6.72±0.01^{c}	0.38±0.00^{a}

*Means in the same column followed by the different letter are statistically significant ($P < 0.05$) according to Duncun's multiple range test (DMRT).

Table 2. Changes in the biochemical composition of *Cucurbita maxima* fruit at successive stages of growth and ripening

	Stages of Growth and Ripening				
Parameters	Young	Pre-mature	Mature	Pre-ripened	Ripened
Reducing sugars (mg/g FW)	31.51±0.40^{a}	45.32±0.42^{b}	64.16±0.34^{c}	65.82±0.60^{d}	77.30±1.10^{e}
Non-reducing sugars (mg/g FW)	59.52±0.70^{a}	43.38±0.75^{b}	42.42±0.82^{c}	26.19±0.76^{d}	13.73±1.20^{e}
Total sugars (mg/g FW)	91.03±0.20^{b}	88.69±0.37^{a}	106.58±0.60^{c}	92.01±0.17^{b}	90.13±0.38^{a}
Starch (mg/g FW)	75.86±0.24^{b}	138.47±0.70^{d}	117.45±0.50^{b}	75.29±0.50^{c}	68.55±0.70^{a}
Total free amino acids (mg/g FW)	4.32±0.14^{b}	5.37±0.06^{c}	10.77±0.06^{d}	2.3±0.24^{a}	2.07±0.43^{a}
Total proteins (mg/g FW)	4.85±0.90^{a}	7.57±1.31^{b}	7.25±1.19^{ab}	7.26±1.34^{ab}	7.25±1.43^{ab}
Total phenols (mg/g FW)	0.56±0.00^{c}	0.52±0.01^{bc}	0.48±0.08^{ab}	0.42±0.00^{a}	0.47±0.01^{ab}
Vitamin C (mg/100 g FW)	4.30±0.31^{a}	7.40±0.04^{ab}	11.35±0.01^{bc}	12.81±0.04^{bc}	15.00±0.02^{c}
Carotenoids (mg/100 g FW)	0.67±0.03^{a}	1.92±0.03^{b}	3.16±0.04^{c}	4.10±0.25^{d}	7.47±0.10^{e}

*Means in the same row followed by the different letter are statistically significant ($P < 0.05$) according to Duncun's multiple range test (DMRT).

incorporation into proteins required for the synthesis of various enzymes.

Changes in total phenols and Vitamin C

Polyphenols are among major determinant factors for the quality traits like color, aroma, bitterness, astringency of fresh fruits as well as fruit products such as jam, juices, jellies etc. The total phenols in pumpkin fruit were found highest (0.56 mg g^{-1}) at its young stage and such high amount of phenolic compounds in young stage might be responsible for their possible role in several protection mechanisms in order to prevent their early senescence. Similar findings had reported in the fruit of *C. moschata* (Pandya and Rao, 2010). Moreover, a gradual increase in the amount of vitamin C occurred i.e., 4.3 mg.100 g^{-1} FW in young fruit to 15 mg.100 g^{-1} FW in ripe fruit (Table 2). Lim *et al.* (2006) attributed similar finding in guava fruit, who suggested that the significant ($P < 0.05$) increase in the biosynthesis of ascorbic acid are mainly due to the breakdown of starch into glucose.

Changes in carotenoids

It is well documented that carotenoids biosynthesis increases during maturing or ripening of carotenogenic fruits and fruit vegetables (Gross, 1987). The biosynthesis and metabolism of carotenoids in vegetables can significantly affected by the differences in growing environment, such as temperature, nutrient availability, soil, intensity of sunlight, ripening stage, post harvesting (Rodriguez-Amaya, 1999; Cazzonelli and Pogson, 2010). In the pumpkin fruit, carotenoids dramatically increased by eleven fold in its ripened stage, responsible for development of color from yellow at young stage to orange on ripening (Table 2). Similar results for carotenogenesis during ripening were also reported in *C. moschata* 'Menina Verde' (Arima and Rodriguez-Amaya, 1988) and *C. moschata* 'Menina Brasileira' (Arima and Rodriguez-Amaya, 1990). Provesi *et al.* (2011) reported that α-carotene, β-carotene and lutein are the primary carotenoids found in *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposição'.

Changes in the activities of softening, hydrolytic and antioxidant enzymes

Fruit ripening is associated with the softening, characterized by alterations in cell wall and middle lamella structure (Tucker and Grierson, 1987) through the action of enzymes such as PG, PME, β-galactosidase and cellulase (Huber, 1983). Goncalaves *et al.* (2005) reported that the tissue softening during ripening and storage in the cultivars of *C. maxima* and *C. moschata* is due to the solublization of total pectin, consequently decrease the cell-cell adhesion, cell wall thickness and ultimately affect textural properties. In many fruits, PG is responsible for the major cell wall disassembly during fruit ripening and is known to be induced by ethylene (Hadfield and Bennett, 1998). In contrast for PME activity, ethylene is not necessary and developmentally controlled. During the course of pumpkin growth and ripening, PME showed a gradual increase in its activity from young to mature

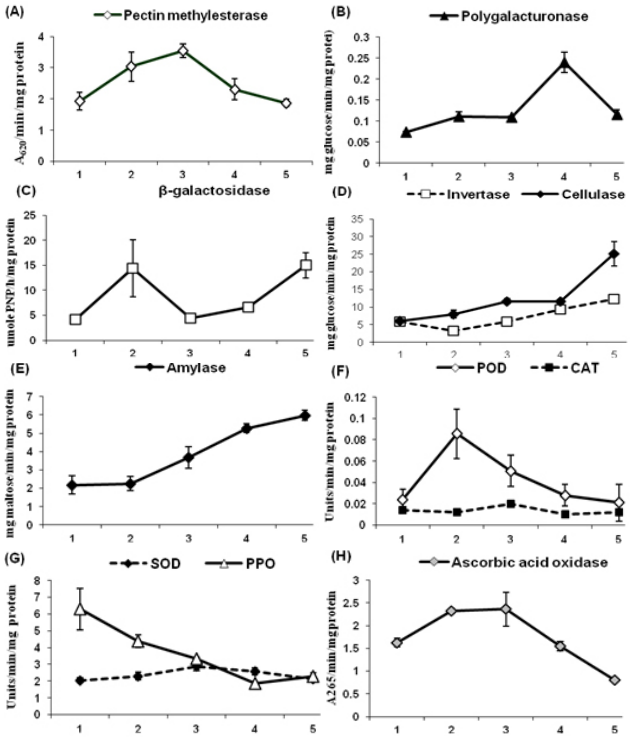

Figure 2. Specific activities of enzymes in fruit of *Cucurbita maxima* during its successive stages of growth and ripening. Numbers on the X-axis indicate stages of fruit development: 1- Young, 2- Pre-mature, 3- Mature, 4- Pre-ripened, 5- Ripened. Vertical bars represent ± standard deviation of means for three replicates.

stage while PG activity got enhanced by two fold in pre-ripened stage. Such pattern supported the view of Prasanna *et al*. (2007), who opined that PME action is pre-requisite for the action of PG during ripening. However, the activities of both enzymes declined in ripened stage (Figure 2 A and B) suggesting the non-climacteric nature of pumpkin fruit and in such fruits cell wall degradation may be catalyzed by other enzymes (Ranwala *et al*., 1992). A progressively increasing pattern of β-galactosidase and cellulase activities towards ripening of pumpkin fruit suggested their important role in cell wall modification (Figure 2 C and D). Payasi and Sanwal (2005) reported increasing β-galactosidase activity in papaya fruits turning from 50% to 75% yellow stage of ripeness due to pectin and hemicellulose modification. Earlier reports in guava (Abu-Goukh and Bashir, 2003), durian (Ketsa and Daengkanit, 1999) and melon (Ranwala *et al*., 1992) exhibited similar trend of softening during maturation and ripening.

The solublization of carbohydrate is one of the important factors influencing fruit ripening because their proportion with other compounds is most crucial from an economic point of view. In the presently studied pumpkin, amylase activity increased consistently from its young stage to ripened stage (Figure 2 E), while the activity of invertase declined until its pre-mature stage, but increased in the subsequent stages of ripening (Figure 2 D). The hydrolytic enzymes profile during the growth and the ripening suggested that starch, total soluble sugars, reducing and non-reducing sugars all are present at its ripened stage, although reducing sugars are predominant. Similar trend has been reported in the fruit of sunberry (Patel *et al*., 2011) and cherry laurel (Var and Ayaz, 2004).

Ripening has been described as an oxidative process in climacteric fruits (Rogiers *et al.*, 1998). During the oxidative phosphorylation, molecular oxygen is mainly utilized for metabolism of fats, proteins and carbohydrates for energy at the cost of generating partially reduced oxygen species including free radicals. These are being highly reactive, damages proteins and membranes by initiating oxidation of fatty acids in membrane lipids. Lipid peroxidation (LPO) is oxidative deterioration of polyunsaturated lipids and leads to toxicity. To prevent negative impact on cellular metabolism, plants counter harmful effects of reactive oxygen species (ROS) with antioxidant metabolites and enzymes.

The antioxidant system plays significant role in both senescence and fruit ripening, includes enzymes such as peroxidase, polyphenol oxidase, ascorbic acid oxidase, superoxide dismutase and catalase. Many earlier studies had reported the involvement of POX and PPO in plant cells during ripening receive oxidative stresses from byproducts such as superoxide radicals, hydrogen peroxide and lipid peroxides (Richard-Forget and Gauillard, 1997; Robb, 1984). In pumpkin fruit, POX activity increased from young to pre-mature stage but it declined in the subsequent stages (Figure 2 F). Patel and Rao (2011) reported similar results in Indian cherry. However, a decreasing pattern in PPO activity (Figure 2 G) was observed during pumpkin fruit development, as reported in medlar fruit (Aydin and Kadioglu, 2001). The specific activity of AAO significantly ($P < 0.05$) increased by 73% from the young stage to mature stage, but it declined on ripening (Figure 2 H). These findings are in accordance with that of previous studies on AAO activity in *Cucumis melo* (Sanmartin *et al.*, 2007). Also, the result indicated that no major quantitative change in SOD and CAT activity occur during the growth and ripening of pumpkin fruit. López *et al.* (2010) demonstrated similar findings regarding SOD and CAT profile during strawberry fruit ripening.

Seymour *et al.* (1993) considered the fruit ripening as a specialized form of senescence. Increased level of ROS have been reported during banana, pear, pepper and tomato ripening (Thompson

et al., 1987; Rogiers *et al.*, 1998), possibly due to a decline in the activity of certain antioxidant enzymes (Procházková and Wilhelmová, 2007). During the course of pumpkin fruit development, the declining activity towards ripening indicated an insignificant role of antioxidant enzymes against ROS. On the other hand, the increasing pattern of vitamin C and carotenoids toward ripening of pumpkin fruit suggested their definite involvement as antioxidants in combating stressed situation leading to deteriorative consequences.

Conclusion

The study demonstrated the influence of maturity stages on the physico-biochemical attributes with emphasis on the nutritional potentiality of pumpkin fruit during its development. The results indicated that the presently studied fruit is a rich source of carotenoids and vitamin C, besides higher amounts of sugars, starch and total proteins. A significant correlation was established between the activity of hydrolytic enzymes with the carbohydrate metabolism during pumpkin growth and ripening. A comparatively lower activity of softening enzymes is an indication of better postharvest self-life as a whole or minimally processed form. Thus, the present study enables us for better utilization of such a nutritionally rich underutilized commodity. These results also support the concept that non-climacteric fruit, like pumpkin, can be harvested when the fruit attain the commercial maturation stage.

Acknowledgement

The authors are thankful to the Head, B. R. Doshi School of Biosciences, Sardar Patel University, Gujarat, India for providing necessary facilities for carrying out this work.

References

Abu-Goukh, A. A. and Bashir, H. A. 2003. Changes in pectic enzymes and cellulase activity during guava fruit ripening. Food Chemistry 83: 213–218.

Agbadwa, I. O. and Ndukwu, B. C. 2004. The value of morpho-anatomical features in the systematic of *Cucurbita* L. (*Cucurbitaceae*) species in Nigeria. African Journal of Biotechnology 3(10): 541-546.

Aliu, S., Rusinovci, I., Fetahu, S., Salihu, S. and Zogaj, R. 2012. Nutritive and mineral composition in a collection of *Cucurbita pepo* L. grown in Kosova. Food and Nutrition Science 3: 634-638.

Arima, H. K. and Rodriguez-Amaya, D. B. 1988. Carotenoid composition and vitamin A value of commercial Brazilian squashes and pumpkins. Journal of Micronutrient Analysis 4: 177–191.

Arima, H. K. and Rodriguez-Amaya, D. B. 1990. Carotenoid composition and vitamin A value of a squash and a pumpkin from Northeastern Brazil. Archivos Latinoamericanos de Nutricio´n 40: 284-292.

Aydin, N. and Kadioglu, A. 2001. Changes in the chemical composition, polyphenoloxidase and peroxide activities during development and ripening of medlar fruits (*Mespilus germanica* L.). Bulgarian Journal of Plant Physiology 27(3-4): 85-92.

Bernfeld, P. 1955. Amylases α and β. In Colowick, S. P. and Kaplan, N. O. (Eds). Methods in Enzymology, p. 149-158. New York: Academic Press.

Biswas, T. P. 1985. β-galactosidase activity in the germinating seeds of *Vigna sinensis* L. Phytochemistry 24(12): 2831-2833.

Bliss, C. I. 1967. Statistics in Biology: Statistical methods for research in natural sciences. New York: McGraw Hill Book Company.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. Methods of Biochemical Analysis 1: 27-52.

Cazzonelli, C. I. and Pogson, B. J. 2010. Source to sink: Regulation of carotenoid biosynthesis in plants. Trends in Plant Science 15: 266–274.

Civello, P. M., Martinez, G. A., Chaves, A. R. and Anon, M. C. 1995. Peroxidase from strawberry fruit (*Fragaria ananassa* Duch.): Partial purification and determination of some properties. Journal of Agricultural and Food Chemistry 43: 2596-2601.

de Escalada Pla, M. F., Ponceb, N. M., Stortzb, C. A., Gerschensona, L. N. and Rojasa, A. M. 2007. Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata* Duch. ex Poiret). Lebensmittel-Wissenschaft and Technologie 40: 1176–1185.

FAOSTAT (Food and Agriculture Organization of the United Nation) 2013. *http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.*

Gonclaves, E. M., Brazao, R., Pinheiro, J., Silva, C. L. M. and Moldao-Martin, M. 2005. Influence of maturity stage on texture, pectin composition and microstructure of pumpkin. Proceedings of the 9th ENPROMER, Rio de Janeiro. Downloaded from *http://www.researchgate.net/publication/216554144_Influence_of_maturity_stage_on_texture_pectin_composition_and_microstructure_of_pumpkin.*

Gross, J. 1987. Pigments in Fruits. London: Academic Press.

Habibunnisa, Baskaran, R., Prasad, R. and Shivaiah, K. M. 2001. Storage behaviour of minimally processed pumpkin (*Cucurbita maxima*) under modified atmosphere packaging conditions. European Food Research Technology 212: 165-169.

Hadfield, K. A. and Bennett, A. B. 1998. Polygalacturonases: Many genes in search of function. Plant Physiology 117: 337-343.

Hagerman, A. E. and Austin, P. J. 1986. Continuous spectrophotometric assay of plant pectin methyl

esterase. Journal of Agricultural and Food Chemistry 34: 440-444.

Harvey, W. J., Grant, D. G. and Lammerink, J. P. 1997. Physical and sensory changes during the development and storage of buttercup squash. New Zealand Journal of Crop and Horticultural Science 25(4): 341-351.

Huber, D. J. 1983. The role of cell wall hydrolases in fruit softening. Horticultural Reviews 5: 169-219.

Ketsa, S. and Daengkanit, T. 1999. Firmness and activities of polygalacturonase, pectinesterase, β-galactosidase and cellulase in ripening durian harvested at different stages of maturity. Scientia Horticulturae 80: 181-188.

Lim, Y. Y., Lim, T. T. and Tee, J. J. 2006. Antioxidant properties of guava fruit: Comparison with some local fruits. Sunway Academic Journal 3: 9–20.

López, A. P., Gochicoa, M. T. T. and Franco, A. R. 2010. Activities of antioxidant enzymes during strawberry fruit development and ripening. Biologia Plantarum 54(2): 349-352.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1954. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 27-52.

Loy, J. B. 2004. Morpho-physiological aspects of productivity and quality in Squash and Pumpkins (*Cucurbita* spp.). Critical Review in Plant Science 23(4): 337-363.

Malik, S. K., Chaudhury, R., Dhariwal, O. P. and Bhandari, D. C. 2010. Genetic resources of tropical underutilized fruits in India. New Delhi: NBPGR.

Martínez, S., Curros, A., Bermúdez, J., Carballo, J. and Franco, I. 2007. The composition of Arnonia peppers (*Capsicum annuum* L.) at different stages of maturity. International Journal of Food Science and Nutrition 58(2): 150-161.

Mazumdar, B. C. and Majumder, K. 2003. Methods on physico-chemical analysis of fruits. Delhi: Daya Publishing House.

McCollum, T. G., Huber, D. J. and Cantliffe, D. J. 1988. Soluble sugar accumulation and activity of related enzymes during muskmelon fruit development. Journal of the American Society for Horticultural Science 113(3): 399-403.

Miller, G.S. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry 31: 426-428.

Moore, S. and Stein, W. H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry 176: 367-388.

Padulosi, S. 1998. Priority setting for Underutilized and neglected plant species of Mediterranean region. Report of the IPGRI conference. Aleppo, Syria: ICARDA.

Pandya, J. B. and Rao, T. V. R. 2010. Analysis of certain biochemical changes associated with growth and ripening of *Cucurbita moschata* Duch. fruit in relation to its seed development. Prajna-Journal of Pure and Applied Science 18: 34-39.

Paliyath, G. and Murr, D. P. 2008. Biochemistry of fruits. In Paliyath, G., Murr, D.P., Handa, A.K. and Lurie, S. (Eds.). Postharvest biology and technology of fruits, vegetables and flowers, p. 19-50. Iowa, U.S.A: Wiley-Blackwell Publishing.

Pareek A, Singla S. L and Grover A. 1998. Protein alterations associated with salinity, desiccation, high and low temperature stresses and abscisic acid application in Lalnakanda a drought tolerant rice cultivar. Current Science 75: 1170-1174.

Patel, P. R. 2009. Study of certain physiological and histo-architectural changes associated with growth and ripening of some underutilized fruits. Gujarat, India: Sardar Patel University, Ph. D. Thesis.

Patel, P. R. and Rao, T. V. R. 2011. Biochemical changes in relation to growth and ripening of Indian cherry (*Cordia dichotoma* F.): An Underutilized fruit. International Journal Fruit Science 11: 1-11.

Patel, P. R., Gol, N. B. and Rao, T. V. R. 2011. Physicochemical changes in sunberry (*Physalis minima* L.) fruits during growth and ripening. Fruits 66(1): 37-46.

Payasi, A. and Sanwal, G.G. 2003. Pectate lyase activity during ripening of banana fruit. Phytochemistry 63: 243–248.

Prasanna, V., Prabha, T. N. and Tharanathan, R. N. 2007. Fruit Ripening Phenomena – An Overview. Critical Reviews in Food Science and Nutrition 47(1): 1-19.

Procházková, D. and Wilhelmová, N. 2007. Leaf senescence and activities of the antioxidant enzymes. Biologia Plantarum 51: 401- 406.

Provesi, J. G., Dias, C. O. and Amante, E. R. 2011. Changes in carotenoids during processing and storage of pumpkin puree. Food Chemistry 128: 195–202.

Radovich, T. 2010. Farm and Forestry Production and Marketing Profile for Pumpkin and Squash (*Cucurbita* spp.). In Elevitch, C. R. (Eds.). Specialty Crops for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Holualoa, Hawaii. downloaded from *http://agroforestry.net/scps*.

Rai, M., Pandey, S. and Kumar, S. 2008. Cucurbit research in India: a retrospect. In Pitrat, M. (Eds). Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, INRA. Avignon: France.

Rangana, S. 1977. Manual of analysis of fruit and vegetable products. New Delhi: Tata McGraw-Hill.

Ranwala, A. P., Suematsu, C. and Masuda, H. 1992. The role of β-Galactosidases in the modification of cell wall components during muskmelon fruit ripening. Plant Physiology 100(3): 1318-1325.

Richard-Forget, F. C. and Gauillard, F. A. 1997. Oxidation of chlorogenic acid, catechins and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenoloxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. Journal of Agricultural and Food Chemistry 45: 2472–2476.

Robb, D. A. 1984. Tyrosinase in Copper proteins and Copper enzymes. 2nd edn. Boca Raton, FL: CRC Press.

Rocha, A. M. C. N. and Morais, A. M. M. B. 2001. Polyphenol oxidase activity and total phenolic

content as related to browning of minimally processed 'Jonagored' apple. Journal of the Science of Food and Agriculture 82: 120-126.

Roe, J. H. and Oesterling, M. J. 1944. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2, 4-dinitrophenylhydrazine method. Journal of Biological Chemistry 152: 511-517.

Robinson, R.W. and Decker-Walters, D. S. 1997. *Cucurbits*. New York: CAB International.

Rodriguez-Amaya, D. B. 1999. A guide to carotenoid analysis in foods. Washington: International Life Sciences Institute (ILSI) Press.

Rogiers, S. Y., Kumar, G. N. M. and Knowles, N. R. 1998. Maturation and ripening of fruit of Amelanchier alnifolia Nutt. are accompanied by increasing oxidative stress. Annals of Botany 81: 203–211.

Sanmartin, M., Pateraki, I., Chatzopoulou, F. and Kanellis, A. K. 2007. Differential expression of the ascorbate oxidase multigene family during fruit development and in response to stress. Planta 225: 873–885.

Seymour, G. B., Taylor, J. E. and Tucker, G. A. 1993. Biochemistry of Fruit Ripening. London: Chapman and Hall.

Thimmaiah, S. K. 1999. Standard methods of biochemical analysis. New Delhi: Kalayani Publishers.

Thompson, J. E., Ledge, R. L., Barber, R. F. 1987.The role of free radicals in senescence and wounding. New Phytologist 105: 317-344.

Tomes, M. S. 1968. Temperature inhibition of carotene synthesis in tomato. Botanical Gazette 124: 180 – 185.

Tucker, G. A and Grierson, D. 1987. Fruit ripening. In Davies, D. (12th Eds). Biochemistry of Plants, p. 265-319. New York: Academic Press Inc.

Wang, Y.S., Tian, S.P. and Xu, Y., 2005. Effects of high oxygen concentration on pro- and anti oxidant enzymes in peach fruit during postharvest periods. Food Chemistry 91: 99–104.

Whitaker, T. W. and Davis, G. N. 1962. *Cucurbits*. New York: Interscience Publishers

Var, M. and Ayaz, F. A., 2004. Changes in sugar composition in cherry laurel cv. Oxygemmis fruit during development and ripening. Pakistan Journal of Botany 36(2): 389-394.

Zainon, M. A. and Brady, C. J. 1982. Purification and characterization of polygalacturonase of tomato fruits. Australian Journal of Plant Physiology 9: 155-169.