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

Keywords
Biochemical analysis  
Fruit  
Nutrition  
Pumpkin  
Ripening

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
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 *Cucurbita* 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. Habibunnsa 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
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
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 solubilization 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
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.

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Pumpkin is a delicious type of winter squash, native to North America and particularly popular around Thanksgiving and Halloween. While commonly viewed as a vegetable, pumpkin is scientifically a fruit, as it contains seeds. That said, it’s nutritionally more similar to vegetables than fruits. Beyond its delicious taste, pumpkin is nutritious and linked to many health benefits. Here are 9 impressive nutrition and health benefits of pumpkin.

1. Highly Nutritious and Particularly Rich in Vitamin A. Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. Article. Full-text available. 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. ß-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.