Chapter - 5

Functional Properties of Starch

A Major Storage Component of Mango Ginger Rhizomes

You cannot depend on your eyes
When your imagination is out of focus
– Mark Twain
Introduction

Starches from cereals, tubers and roots are the major dietary source of energy for humans. Starch determines the functionality of many food applications, accordingly they are used in the food industry as stabilizers, thickening agent in snacks, meat products, fruit juices, etc [Betancur and Chel, 1997]. The enthalpy and temperature of gelatinization of starch depend on the microstructure, size, shape and composition of granules, amylose/amylopectin ratio in particular [Hizukuri, 2004]. The granule size in tubers varies from 0.5-85 μm, it is also reported to vary from 1 to 110 μm, depending on the source, while the shape varies from irregular to oval, elliptical, spherical and polygonal [Hoover, 2001; Mara, et al., 2006]. Research on characterization of potato, cassava and sweet potato starch has resulted in their extensive utilization in food industries. On the other hand, structural and biochemical properties of many starchy tubers and spicy roots like mango ginger have not yet been studied. There is a need to explore these starches, to use in food industry.

Mango ginger is composed of 6.39 % of starch by fresh weight basis and 45.64 % of starch by dry weight basis [Shetty, et al., 1963]. It is interesting to investigate the structure and biochemical properties of mango ginger starch for development of product of health benefits. A valuable, but little understood storage polysaccharide from an unconventional source like mango ginger can give a complete picture of the true diversity of natural starches in tuberous crops.
Chapter 5: Functional properties of Starch

MATERIALS AND METHODS

Plant material

Fresh [2 days old after harvest] and healthy mango ginger [Curcuma amada Roxb.] rhizomes were procured from the local market, Mysore, India. Rhizomes were washed, sliced and dried in a hot air oven at 50°C for 72 hrs and powdered to 100-120 meshes in an apex grinder [Apex Constructions, London].

Isolation of starch

Mango ginger powder [100 g] was extracted with 70 % methanol by constant stirring at 40°C for 36 h. The slurry was poured through three layers of muslin cloth and centrifuged at 8,000 rpm for 25 min. The yellowish residue settled was collected and stirred for 10 min with 200 mL of petroleum ether and acetone [1:1, v/v] followed by 500 mL of water. The procedure was repeated till the residue becomes colourless. The starch was allowed to settle. Then starch was washed with distilled water repeatedly till starch becomes completely white and free from debris. The starch was then collected and dried at room temperature [26 ± 2°C] and then stored at ambient temperature.

Moisture and ash content

Moisture and ash content of mango ginger starch were determined according to the AOAC official procedures [AOAC, 1990].

Solubility

The solubility of mango ginger starch was recorded at 25, 35, 45, 55, 65 and 75°C by using the method of Ju and Mittal [1995].

Differential scanning calorimetry [DSC]

Gelatinization of mango ginger starch was measured according to the method of Hoover, [2001] using differential scanning calorimeter [Mettler DSC 30, Switzerland]. Starch was mixed with de-ionized water in 1:3 ratio and transferred to DSC pans, which was then sealed and allowed to stand for 2 h at room temperature before analysis. The sample was heated at 10°C/min over a temperature range of 25-100°C. The thermogram
was recorded with an empty aluminium pan as the reference. The transition temperatures reported are the onset \(T_o\), peak \(T_p\) and conclusion \(T_c\).

**Water holding capacity [WHC]**

Water holding capacity of mango ginger starch at 25, 35, 45, 55, 65 and 75°C was determined by the method of Ju and Mittal [1995].

**Amylose content**

Amylose content in the isolated mango ginger starch was determined by using the method of Hoover and Ratnayake [2002].

**Light transmittance**

Light transmittance of mango ginger starch solution [1 %] in DMSO and water was measured by method of Yeh and Yeh [1993] and Craig, *et al.*, [1989]. The transmittance [%] of the solution was recorded at 1, 2, 4, 6, 12 and 24 hrs at 650 nm on a Shimadzu UV-160A instrument [Shimadzu, Singapore] at room temperature.

**X-Ray Diffraction**

The mango ginger starch was analyzed by powder X-ray diffraction method for quantitative phase identification. The X-ray powder diffraction patterns were obtained using Rigaku X-ray diffractometer [Rigaku Co., Tokyo, Japan], Cu Kα radiation operating at 40 kV and 30 mA. The diffracted intensity was measured from 4°- 50° as a function of 2θ at a scanning speed of 4°/ min. The starch was equilibrated at 100 % relative humidity for 24 h at 25°C prior to analysis.

**Fourier-transform infrared [FTIR] spectroscopy**

The IR spectrum of mango ginger starch was recorded on Nicolet 5700 [Thermo Electron Corporation, Madison, WI, US] IR Spectrometer at room temperature. The starch powder was blended with KBr powder, and pressed into tablets before measurement. A region from 400-4000 cm\(^{-1}\) was used for scanning.
**Scanning Electron Microscopy [SEM]**

The scanning electron micrographs of mango ginger starch were recorded on LEO-435 VP scanning electron microscope [Cambridge, UK]. Starch granules were sprinkled on cellophane tape attached to a stub and coated with gold. The starch granules were analyzed for surface morphology, shape and size.

**Statistical analysis**

All the biochemical analysis were carried out in triplicates. Significant differences [$P < 0.05$] were determined by Duncan’s Multiple Range Test [DMRT].
RESULTS AND DISCUSSION

Isolation of starch

The mango ginger starch granules could not be separated from colored pigments and adhered cell components by adopting the earlier methods [Lilia, et al., 1999; Ana, et al., 2004; Singh, et al., 2005; Jayakody, et al., 2005]. The starch obtained after centrifugation of filtered methanol extract was yellowish in color. The colouration may be attributed to presence of terpenoid pigments. Among different solvent gradients tried, petroleum ether and acetone at ratio of 1:1, v/v, was effective in removal of adhered pigments completely on the starch. Organic layer containing dissolved pigments was separated from starch by repeated wash with distilled water. The starch thus obtained was pure and white in colour. They were dried at room temperature [26±2°C] and stored for further characterization.

Moisture and ash content

Mango ginger starch contains 9.8 % of moisture and 1.3 % ash content [Table 1]. The moisture content of dry starch varies from 6-16 % among tuber starches, which have been reported by several researchers [Takeda, et al., 1986; Moorthy, 2002].

Solubility

Mango ginger starch [1 g/100 ml] exhibited very low solubility [1.5 %] in water at 25°C, when stirred it formed a suspension. The solubility of mango ginger starch was high [12.3 %] at 75°C. There was a linear increase in solubility of starch in water with increase in temperature [Fig. 5.1].
**Differential Scanning Colorimeter [DSC]**

Thermal analysis of mango ginger starch by DSC showed that, the gelatinization onset temperature \([T_o]\) be 69.70°C, peak temperature \([T_p]\) was 77.33°C and conclusion temperature \([T_c]\) was 90.52°C. The gelatinization temperature range \([T_c - T_o]\) for *C. amada* starch was 20.82°C, which is lower than that of *C. longa* starch [27°C], *Z. officinale* starch [30°C] and higher than that of *Curcuma malabarica* starch [7.8°C] and *Curcuma zedoaria* starch [17.4°C] [Mara, *et al.*, 2006; Jyothi, *et al.*, 2003]. According to Noda, *et al.*, [1998], \(T_o\), \(T_p\), and \(T_c\) are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains and not by the proportions of crystalline region. The low \(T_o\), \(T_p\), and \(T_c\) reflect the presence of abundant short amylopectin chains.

| Table 5.1: Composition and morphological features of mango ginger starch |
| --- | --- |
| Moisture content | 9.8 ± 0.6 % |
| Total ash content | 1.3 ± 0.1 % |
| Amylose content | 43 ± 1.8 % |
| Granule size | |
| 1) Small granules | 3-20 µm |
| 2) Large granules | 20-48 µm |
| Granule shape | round, elliptical, polygonal |
| Fissures | absent |
| Concentric rings | absent |

* Each value represents mean of three different observations ± S.D.

**Water holding capacity [WHC]**

Water holding capacity of mango ginger starch increased linearly with increase in temperature [Fig. 5.2]. The WHC has been reported to depend upon the degree of the engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains [Hoover and Sosulski, 1986]. Differences in the degree of availability of water binding sites in the starches can have role in the variation of water binding capacity [Wotton and Bamunuarachchi, 1978].

![Fig. 5.2: Water holding capacity of mango ginger starch at different temperatures](image-url)
Amylose content

The amylose content in mango ginger starch was 43 % [Table 1]. This is 5 % less than that of Curcuma longa starch [Mara, et al., 2006] while, 18 % and 16 % more than C. malabarica and C. zedoaria starches respectively [Jyothi, et al., 2003]. However the amylose content of C. amada was significantly higher than the Z. officinale, which has 34 % amylose. Studies indicated that high amylose content was accompanied with lowered blood glucose and insulin responses [Granfeldt, et al., 1995]. This lowers the digestibility of starch due to positive correlation between amylose content and formation of resistant starch [Berry, 1986; Sievert and Pomeranz, 1989]. Such foods, with a low glycaemic index, are considered to produce metabolic advantages [Jarvi, et al., 1999; Bjorck, et al., 2000]. The advantage of high amylose content in mango ginger and its associated functional attributes are yet to be explored.

Light Transmittance

Light transmittance of mango ginger starch dissolved in water decreased progressively as the time progress. On contrary, light transmittance of starch dissolved in DMSO showed significant increase from 1-2 h [Fig. 5.3]. Being a hydrogen bond acceptor DMSO breaks associative hydrogen bonding in the starch molecules through surface erosion thus increasing its solubility and light transmittance [French, 1984; Cooreman, et al., 1995]. Light transmittance can be used to indicate the clarity of starch paste which varies considerably with its source, solubility and amylose content [Kittiwut, et al., 2003].
X-ray crystallography

X-ray diffraction has been used to reveal the presence and characteristics of the starch crystalline structure [Singh, et al., 2003]. The X-ray diffractogram of mango ginger starch [Fig. 5.4] showed five peaks, the strongest diffraction peak at around 17° at 2θ and small peaks around 5°, 15°, 22° and 24° indicated that the mango ginger has a characteristic B-type starch. This pattern is typical to tuber and root starches [Hoover, 2001], which is characterized by a small peak at 5.6°, strong peak at 17° and a doublet at 22° and 24°. The X-ray diffractogram of C. amada was found to be similar with that of C. longa [turmeric] rather than Z. officinale [ginger] and Mangifera indica [mango] that have C-type starch [Bello-Perez, et al., 2005; Mara, et al., 2006].

Fourier Transform Infrared [FTIR] spectroscopy

The FTIR spectrum of the mango ginger starch is given in Fig. 5.5. The characteristic peak of starch between 1019 and 1156 cm⁻¹, attributed to C-O bond stretching [Fang, et al., 2002]. We observed peaks around 1021 cm⁻¹ was ascribed to
the C-O stretch of C-O-C in starch, and the peaks near 1081 and 1154 cm⁻¹ were mainly attributed to C-O stretch of C-O-H in starch. The wide band observed at 3389 cm⁻¹ can be attributed to the O-H stretching. The bands at 2930 cm⁻¹ may be attributed to the asymmetric stretching of C-H, while the band at 1644 cm⁻¹ was ascribed to adsorbed water and the bands at 1416 and at 1368 cm⁻¹ to the angular deformation of C-H. Thus, the IR spectra revealed the purity of the starch isolated.

**Scanning Electron Microscopy (SEM)**

As shown in **Fig. 5.6**, SEM revealed significant variations in shape and size of mango ginger starch. The granule shape appeared to be oval, elliptic, irregular or cuboidal and polygonal. The elliptic-shaped, large-size granules were more in number. The shape of the mango ginger starch appears to be a combination of turmeric [elliptical] and ginger [oval] starch. Absence of fissure on the surface of mango ginger starch revealed that it is similar to ginger, than turmeric starch that showed fissures [Mara, et al., 2006]. The granule diameter ranges between 3-20 μm for small and 20-48 μm for large size. Granule
structure, size and their distribution are important because they can affect the functional properties of starch [Rasper, 1971]. The difference in the granule structure may be attributed to their biological origin [Svegmark and Hermansson, 1993], mainly depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant [Badenhuizen, 1969].

**Conclusion**

Starch from an unconventional source like mango ginger was isolated and characterized for the first time. It exhibited distinct structural and biochemical features of its own. With B type X-ray diffractogram and high amylose content, it showed similarity with turmeric starch. Though morphologically rhizome resembles ginger, starch granules differ by the absence of fissures on the surface and its X-ray diffractogram pattern. Thus it occupies a position between turmeric and ginger starch. High amylose content and low solubility of mango ginger starch, preferred characters that need to be explored for preparation of products of metabolic advantages.