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Proximate Analysis and Physico-Chemical Properties of Flour from the Seeds of the China Chestnut, *Sterculia monosperma* Ventenat

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Abstract: The aim of this study was to evaluate nutritional composition of china chestnut seeds, *Sterculia monosperma* Vent. and analyze the physico-chemical properties of flour from the seeds. The results obtained on proximate analysis of china chestnut seeds, *S. monosperma*, revealed that they contained mostly carbohydrate (73.7% dm), followed by fat (12.0% dm), protein (7.8% dm), fiber (5.5% dm) and ash (1.0% dm). They have a relatively high content of potassium (12.3 mg g^{-1} dm) following by phosphorus (2.30 mg g^{-1} dm), magnesium (1.87 mg g^{-1} dm), sulfur (0.88 mg g^{-1} dm) and calcium (0.14 mg g^{-1} dm). The fatty acids profile was found to be composed of mainly palmitic (42%) and oleic acids (34%), with general long-chain fatty acids the other significant component by mass (13%). Glutamic acid (17.4%), aspartic acid (12.5%) and arginine (12.5%) were the three major amino acid constituents. The purified seed starch was investigated for its morphological, starch content and physico-chemical properties, such as amylose content, swelling power, solubility and pasting properties. The starch granules were quite round, about 10-15 micron diameter and composed of more than 35% (w/w) of amylose. The pasting properties of flour from the seeds of *S. monosperma* revealed that gelatinization began at 72.6-73.2°C and the maximum viscosity in the holding period at 95°C was 633 BU. Interestingly and potentially of use, was that the viscosity at the cooling period was more than two-fold higher than that in the holding period.

Key words: *Sterculia monosperma*, China chestnut, physico-chemical properties, proximate analysis, flour

INTRODUCTION

All ecosystems and human societies depend on a healthy and productive natural environment that contains diverse plant and animal species. Approximately 60% of the world's food supply comes from rice, wheat and corn, as much as 20,000 other plant species have been used by humans as food (Pimentel *et al*., 1997). *Sterculia* is a genus colloquially termed the tropical chestnuts. Some of *Sterculia* was used as traditional drug such as *S. lychnophora* (Wang *et al*., 2003; Wu *et al*., 2007a), *S. urens* (Singh and Pal, 2008; Eastwood *et al*., 1983). Sometimes its raw or cooked seeds are consumed by man and also by several species of fauna such as seeds of *S. striata* (Oliveira *et al*., 2000), *S. setigera* (Idu *et al*., 2008), *S. lychnophora* (Wu *et al*., 2007b).

The small evergreen china chestnut tree, *S. monosperma* Ventenat (Sterculiaceae), also commonly known as Seven Sister's fruit, Ping pong and Pheng Phoh, is a member of the tropical chestnut trees and is found throughout the tropical and subtropical areas. In Thailand, this plant is known as "Kao-lat Thai" because these trees are found abundantly in the northern part of Thailand, especially in the Nan province. Seeds of *S. monosperma* have long been used in cooking and one of its distinctive characteristics is that when cooked, they have the color of an egg yolk. Vegetarians lavish them in their cooking. Chinese gourmets use them to enrich the taste and flavors of dumplings and in brewing pots of herbal chicken, duck or pork. However, little is known about their nutritional composition. Only one report, from the Food and Agriculture Organization of the United Nations (FAO, 1972), has reported the food composition of china chestnut seeds.

In this study the biochemical composition and nutritional values of cultivated china chestnut seeds, *S. monosperma*, are reported. Moreover, starch granules derived from the seeds were characterized by scanning

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electron microscopy (SEM) and their physico-chemical properties, such as amylose/amylopectin composition, swelling power, solubility and pasting properties, were also determined.

MATERIALS AND METHODS

This research project was conducted during years 2005 to 2007 A.D. Seeds of *S. monosperma* were obtained from Nan Province in the northern part of Thailand (Fig. 1). Fresh seeds were used for proximate analysis. For starch isolation, they were washed, packed under vacuum and kept at 4°C until use.

Proximate analysis: The moisture content of fresh china chestnut seeds was determined in triplicate by drying at 120°C to constant dry weight in a hot-air oven. The total nitrogen content was determined by Kjeldahl nitrogen analysis, according to AOAC, method-991.20 (AOAC, 1995a). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25. The total fat content was determined by extraction of 2.0-2.5 g of dry ground sample for 12 h in a Soxhlet with petroleum ether and removed the solvent by rotary evaporator then dried the sample in hot air oven at 100°C for about 1 h to allow the ether evaporate (AOAC, 1995b). The crude fiber and ash contents were determined according to AOAC methods (AOAC, 1995c). Finally, the total carbohydrate content was calculated by subtracting the total percentage of all the other above components from 100. Each sample was analyzed in triplicate.

Mineral analysis: The qualitative analysis of mineral compositions was analyzed by X-ray fluorescence using a Wavelength Dispersive X-ray fluorescence spectrometer (Model PW2400, Netherland) and quantitative analysis was carried out by atomic absorption spectrophotometry (AA) (Varian Model Spectra A-300, Australia) and ICP atomic emission spectrometry (Perkin-Elmer Model PLASMA-1000, USA).

Fig. 1: Fruits and seeds of *S. monosperma*

Fatty acids analysis: The fatty acid composition was analyzed by GC (Model Shimadzu 15A, Shimadzu Corporation, Japan), after methylation with boron trifluoride for fatty acid methyl esters (FAMEs) (Morrison and Smith, 1964). The FAMEs were suspended in heptane and analyzed by Shimadzu 15A gas chromatography with a 30 m x 0.32 mm SE-30 column and using nitrogen as the carrier gas. The injection and flame ionization detector temperatures were set at 250°C and the oven temperature was 220°C.

Amino acid analysis: The dry ground seed sample (200 mg) was hydrolyzed with 6 N HCl containing 1% phenol in a heating block at 110°C for 22 h in sealed glass tubes under a N_2 atmosphere. The HCl and phenol were then driven off by evaporation. An internal standard was then added into the cooled hydrolysate which was diluted with deionized water and then 10 µL of this filtrate was mixed with 70 µL of AccQ fluor derivatization buffer and 20 µL of AccQ fluor reagent. Samples were then heated at 55°C for 10 min in a heating block, before cooling and being analyzed by using high-performance liquid chromatography (HPLC) with a WATERS Alliance 2659 and a WATERS 2475 Multi λ Fluorescence detector set at an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Separation was achieved in an AccQ Wag column (150×3.9 mm, particle size 4 µm) (Liu *et al*., 1995). This was analyzed by Ajinomoto Co., (Thailand) Ltd.

Vitamin analysis: Water soluble vitamins such as vitamin C, niacin and niacinamide, vitamin B1, B2, B6, B12 and pantothenic acid were analyzed by IQA Laboratory Co., Ltd.

Starch isolation: Vacuum packed china chestnut seeds were peeled and sliced into small pieces. An equal volume of water was added to each sample which was then milled in a blender. The resultant suspension was filtered and the flour was collected and dried at 40°C. The dried flour samples were passed through 100 µm mesh size sieves and then defatted to obtain the starch by soxhlet extraction method (AOAC, 1995b).

Morphological properties: Fresh seeds were peeled and dehydrated in an increasing ethanol gradient and then the dried samples were cut in cross section with thickness 3 mm. The samples were mounted with double sticky tape and coated with gold under 15 mA working distance 5 cm for 3 min before analysis by scanning electron microscopy (SEM) (JEOL model JSM-5410LV, Japan).

Total starch content: The total starch content of each flour sample was assessed by a polarimeter (JASCO model DIP-370, Japan) using the International Standard (ISO 10520, 1997).

Amylose content: The amylose content of the isolated seed starch was determined by the method of Williams *et al*. (1970), with the quantity of amylose being determined from a standard curve developed by using known concentration and ratio blends of amylose and amylopectin.

Swelling power and solubility: The swelling power and the solubility of the flour were determined using a 3.33 (w/v) aqueous suspension of flour at 85°C by the method of Leach *et al*. (1959).

Pasting properties: The pasting properties of each flour sample were measured using a Brabender viscograph (BRABENDER Model PT 100, Germany). Viscosity profiles of the flour samples were recorded using 6% (w/v) flour suspensions (30 g total weight). The temperature-time conditions included a heating step from 35 to 95°C at 1.5°C min^{-1}, a holding phase at 95°C for 30 min, a cooling step from 95 to 35°C at 1.5 C min^{-1} and a holding phase at 35°C for 1 min.

RESULTS AND DISCUSSION

Proximate analysis: The seeds of *S. monosperma,* which are approximately 1.5 cm wide and 2 cm long, are composed of three layers of tissues, in which the high starch content tissue (approximately 50% by volume) is located in the center. China chestnut seeds have a very high moisture content, typically over 50% (w/w) and an average (±1 SD) of 62.8±0.74%, which from a human consumption point of view is essential for their storage and supply to fresh markets. However, the high moisture content also presents a mould problem during storage and delivery. Indeed, the average moisture content of *S. monosperma* seeds is slightly higher than that for *C. sativa* (European chesnut) but significantly higher than *S. setigera*, *S. striata* and the comparatively very dry *Irvingia malayana* and *Pachira aquatica* (Table 1).

The total protein content in *S. monosperma* seeds is similar to that for *C. sativa*, having an average value of 7.77±1.05% dm (2.89% wet weight), but apart from *S. setigera* and *S. striata*, whilst the total fat content in *S. monosperma* seeds is 12.04±1.44% dm (4.48% wet weight) (Table 1).

Finally, the average carbohydrate content, as deduced indirectly as being the remainder of the seed mass, was 73.70% dm, which is higher than that for *S. setigera*, *S. striata* and *P. aquatica* but broadly comparable to that for *C. sativa*.

Mineral and vitamin analysis: The main macrominerals of *S. monosperma* seeds are, in order of prevalence, calcium, potassium, phosphorus, magnesium and sodium and compared to the other representative seeds, they are low in calcium but high in potassium (Table 2). For the microelements, only sulfur was detectable, with no detectable levels of iron, copper, manganese and zinc in *S. monosperma* seeds. The water soluble vitamins of *S. monosperma* seeds, only vitamin C, niacin, pantothenic acid and vitamin B2 were detected, but niacinamide, vitamin B12, B1 and B6 are lower than LOD values (Table 3).

Fatty and amino acid analysis: The fatty acid compositions of *S. monosperma* seeds were mainly composed of palmitic acid (42.2%) and then oleic acid (34.2%) with a smaller but significant level of long-chain fatty acids (>C18) (13.2%) and smaller levels of stearic (5.5%) and linoleic acid (4.9%). However, there was no detectable level of palmitoleic acid. These results differ slightly from those of Berry (1982) who analyzed a Malaysian population of *S. monosperma*, where although palmitic and oleic acids were the main components too, a significant level of linoleic acid was found in contrast to this study.

Table 1: A proximate analysis of the *Sterculia monosperma* seed composition and its comparison with other representative food seeds, *Irvingia malayana*, *Castanea sativa*, *Sterculia setigera*, *Pachira aquatica* and *Sterculia striata*

Composition	*S. monosperma*[a]	*S. setigera*[b]	*S. striata*[c]	*C. sativa*[d]	*I. malayana*[e]	*P. aquatica*[c]
Ash (% dm)	1.03±0.10	na	3.04±0.08	na	5.0±0.1	3.5±0.09
Protein (% dm)	7.77±1.05	21.40±0.4	22.50±0.68	6-8.6	17.1	12.9±0.45
Fat (% dm)	12.04±1.44	11.58±0.2	28.64±1.51	1.3-3	70.3±1.1	53.9±2.86
Fiber (% dm)	5.46±0.70	7.73±0.5	na	2-3	na	na
Carbohydrate (% dm)	73.7	21.03±0.4	45.82	56-81	na	29.7
Mineral (% dm)	1.75	na	na	1.8-3	na	na
Moisture (%)	62.8±0.74	16.42±0.2	11.45±0.26	48-59	7.5±0.2	6.0±0.12

na = Not available. [a]This report, [b](Idu *et al*., 2008), [c](Oliveira *et al*., 2000), [d](Borges *et al*., 2008), [e](Bandelier *et al*., 2002)

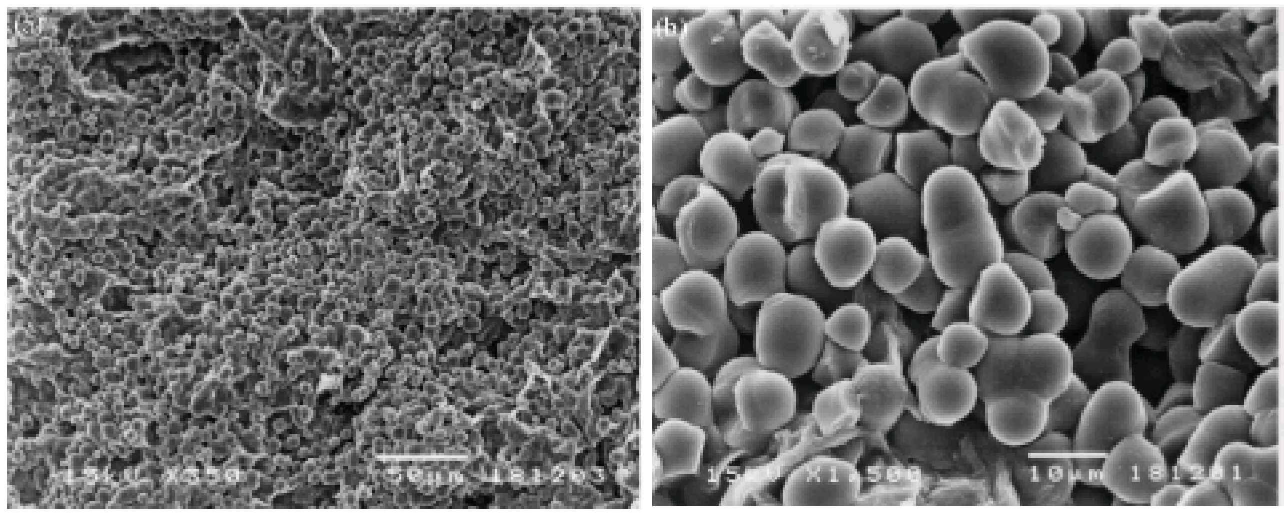

Fig. 2: Representative SEM micrographs of *S. monosperma* seed starch granules: (a) X 350 and (b) X 1500

Table 2: Mineral composition of *S. monosperma* seeds (mg g^{-1} dm) and its comparison with three other representative food seeds, *I. malayana*, *Grenoble walnut* and *S. setigera*

Minerals	*S. monosperma*[a]	*S. setigera*[b]	*I. malayana*[c]	*Grenoblewalnut*[c]
Calcium	0.14	1.08	1.7	0.9
Potassium	12.34	1.05	5.6	5
Sodium	nd	0.28	0.12	0.03
Magnesium	1.87	0.59	2.2	1.28
Phosphorus	2.3	na	2.7	5
Sulfer	0.88	na	na	na
Iron	nd	nd	0.11	0.05
Copper	nd	nd	0.02	nd
Manganese	nd	nd	0.3	nd
Zinc	nd	nd	0.04	nd

nd = Not detectable. na = Not available. [a]This report, [b](Idu *et al*., 2008), [c](Bandelier *et al*., 2002)

Table 3: Vitamins composition of seeds from *S. monosperma*

Vitamin (units)	Result (s)	Method
Vitamin C (mg/100 g)	6.11	BDMS (1998)
Niacin (mg/100 g)	2.30	JAOAC (1993)
Niacinamide (mg/100 g)	<0.10	JAOAC (1993)
Vitamin B12 (µg/100 g)	<0.10	AOAC (2005)
Pantothenic acid (mg/100 g)	0.63	AOAC (2005)
Vitamin B1 (mg/100 g)	<0.01	JAFC (1984)
Vitamin B2 (mg/100 g)	0.03	JAFC (1984)
Vitamin B6 (mg/100 g)	<0.05	JAFC (1984)

For amino acids, the composition of *S. monosperma* seeds was mainly glutamic acid, arginine and aspartic acid, with smaller amounts of 12 other amino acids. Of note is that they were deficient in the essential amino acids methionine and cysteine, these being undetected, which is common for most legumes (Table 4).

Morphological properties: The structure of *S. monosperma* seed derived starch granules showed significant variations in both their size and shape, when viewed by SEM using operating voltage 15 kV with magnification of 350 to 1,500 times (Fig. 2). The starch granules were, however, mostly round and ranged in size between 10-15 µm for the outer diameter.

Total starch and amylose content: The level of starch and amylose content of the purified starch granules, evaluated after the isolation process, is summarized in Table 5. The purity of the isolated *S. monosperma* seed starch was deemed to be satisfactory, having a mean starch content of 78.8±5.81% dm, which is slightly lower than that found in the starch-rich maize flour but higher than that found in wheat and rice flours. The amylose content was broadly similar to the other three representative flours, as expected, having relatively high amylose content. However, it should be born in mind that the amylose content of starches from different and similar plant sources have been reported to be affected by the location, culture soil type and conditions, the starch isolation procedure and analytical methods used (Singh *et al*., 2006b).

Table 4: Amino acid composition (g per 100 g protein) of seeds from *S. monosperma*, *I. malayana*, *P. aquatica* and *S. striata*

Amino acid	*S. monosperma*[a]	*S. striata*[b]	*P. aquatica*[b]	*I. malayana*[c]
Essential				
Histidine	2.1	1.6	1.6	1.9
Threonine	4.4	3.7	5.5	5.3
Tyrosine	2.1	1.7	3	2.8
Valine	5.6	5.8	6.4	5.4
Methionine	nd	1.8	0.8	1.4
Cysteine	nd	1.0	0.7	1.7
Isoleucine	4	3.6	4.1	4.4
Leucine	6.5	7.2	8	7.2
Phenylalanine	4	3.8	3.9	3.2
Lysine	4.4	6.0	4.9	4.9
Tryptophan	na	1.5	2.5	na
Non-essential				
Aspartic acid	12.5	11.9	13.6	8.5
Glutamic acid	17.4	17.9	12.3	19.4
Serine	5.4	5.6	7.4	6.8
Glycine	5.4	7.7	8.8	8.4
Arginine	12.5	9.7	6.6	6.9
Alanine	8.1	7.1	6.6	6.9
Proline	5.8	2.7	3.4	4.9

nd = Not detectable. na = Not available. [a]This report, [b](Oliveira *et al*., 2000), [c](Bandelier *et al*., 2002)

Physico-chemical properties: The remaining evaluated physico-chemical parameters, those are the swelling power and solubility, of the seed flour from *S. monosperma* and the three other representative seeds (maize, wheat and rice), are summarized in Table 5. The swelling power indicates the ability of the starch to

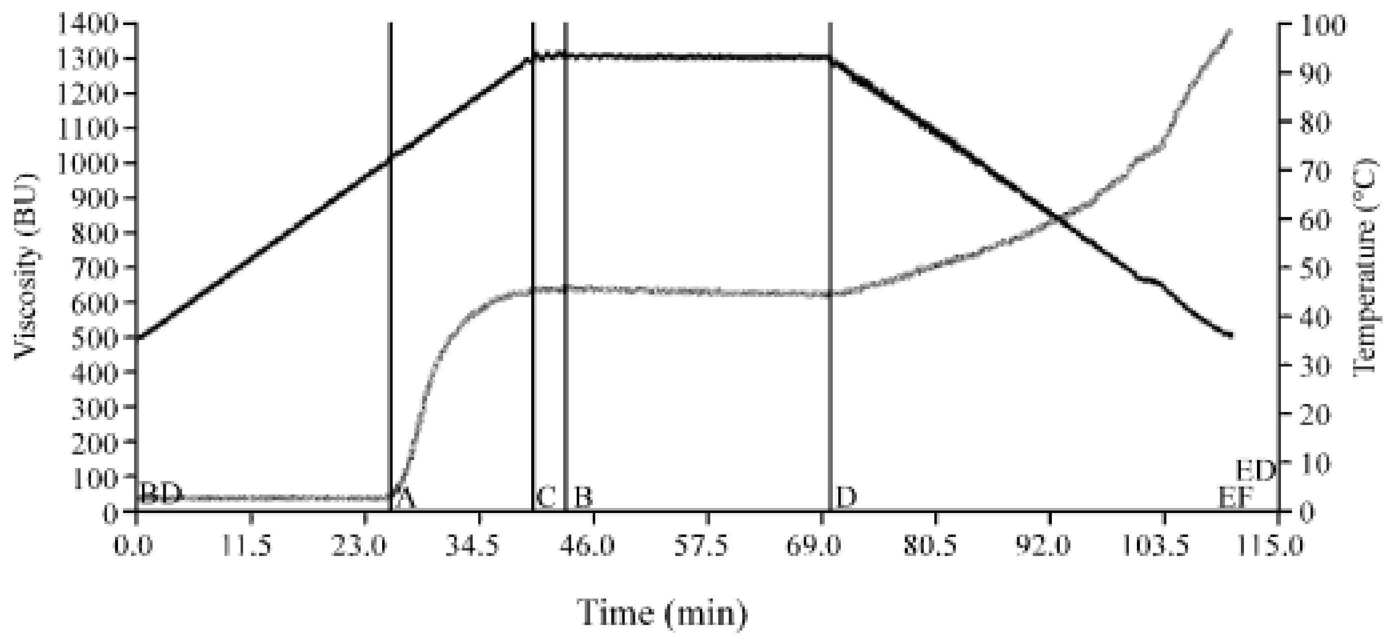

Fig. 3: Pasting property of flour isolated from the seeds of *S. monosperma*

Table 5: Total starch content and physico-chemical properties of flour derived from *S. monosperma* seeds

Flour source	Total starch content (% dm)	Amylose content (%)	Swelling power (g g^{-1})	Solubility (%)
S. monosperma flour	78.80±5.81	36.27±1.10	14.89±0.13	7.87±0.18
Wheat flour	70.55±1.21	30.04±0.87	8.11±0.12	9.33±0.46
Rice flour	63.98±4.40	28.44±0.43	7.77±0.08	3.80±0.19
Maize flour	81.06±2.09	37.32±0.14	9.90±0.05	6.27±0.23

Wheat, rice and maize flours are commercial grade. Data are shown as the Mean±SD of at least three independent experiments

Table 6: Pasting properties of *S. monosperma* seed flour

Point	Evaluation point	Viscosity (BU)	Temperature (°C)
A	Beginning of gelatinization	33±0.0	72.90±0.4
B	Maximum viscosity	633±7.8	94.40±0.0
C	Start of holding period	608±35.4	93.90±0.8
D	Start of cooling period	626±2.1	94.40±0.0
E	End of cooling period	1386±25.5	36.80±0.6
F	End of final holding period	1420±27.6	36.05±0.6
B-D	Breakdown	7.5±10.6	
E-D	Setback	757±24.8	

hydrate under a specific cooking condition, in this case at 85°C for 30 min. Rice flour showed numerically the lowest swelling capacity but this was not that dissimilar from wheat or maize flour, whereas *S. monosperma* flour showed the highest swelling capacity, being nearly two-fold higher than that for rice flour (Table 5). However, note that Singh *et al*. (2006a) reported a much higher swelling capacity for rice starch in the range of 17.2-38.0 g g^{-1}. These differences may be attributed to the differences in the amylose content, viscosity patterns and weak internal organization resulting from negatively charged phosphate groups within the rice starch granules. The swelling behavior of cereal starches has primarily been reported as a property of their amylopectin content, because amylose acts as an inhibitor of swelling, especially in the presence of lipids.

Sterculia monosperma flour showed an intermediate solubility, being slightly less soluble than wheat flour but slightly and significantly more soluble than maize and rice flours, respectively (Table 5). Swelling power and solubility together provide evidence of the magnitude of the interaction between the starch chains within the amorphous and crystalline domains. The extent of this interaction has been reported to be influenced by the amylose/amylopectin ratio and by the characteristics of amylose and amylopectin in terms of the molecular weight distribution, the degree and length of branching and their conformation (Shujun *et al*., 2006).

Pasting properties: The pasting properties of *S. monosperma* seed flour at a concentration of 6% (w/w) are shown in Fig. 3, with the values at discrete stages, marked as A-E in Fig. 3, are summarized in Table 6. The beginning of flour gelatinization ranged from 72.6-73.2°C with a viscosity of 33 BU and the maximum viscosity thereafter rose to 633 BU as the temperature increased to 95°C. At the start of holding period (95°C) the viscosity was 608 BU and remained somewhat steady at this level throughout the holding period. The increase in viscosity with temperature at this initial post-gelatinization stage may be attributed to the removal of water from the exuded amylose by the granules as they swell (Singh *et al*., 2006b). During the subsequent cooling period the maximum viscosity increased such that at the end of cooling period (35°C), the viscosity was 1386 BU. A gradual increase in viscosity at the end of the final holding period to 1420 BU, was then offset by setback to 757 BU. The setback value represents the recovery of the viscosity during cooling of the heated flour suspension. This pasting profile of *S. monosperma* seed flour is broadly similar to the pasting properties of commercial mung bean starch isolated with lactic acid fermentation solution (LFS) (Chang *et al*., 2006).

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