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EFFECT OF PROCESSING ON GRANULAR AND THERMAL PROPERTIES OF STARCH AND RESISTANT STARCH FROM KODO AND KUTKI

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Present study investigated the effect of roasting, cooking without pressure and storage post cooking (-20 ºC, 30 days) on the molecular and thermal characteristics of starch and α-amylase resistant starch from Kodo and Kutki. SEM images showed the native starch possessed predominantly polygonal morphology. The size of Kodo starch granules ranged between 5.9-10.7 µm and Kutki starch in the range of 1.68-8.9 µm. Both the starch granules exhibited deep indentations on the surface. SEM images also revealed several pinholes on the surface of starch granules indicative of α-amylase attack. DSC thermograms of native Kodo and Kutki starch exhibited an endothermic transition over temperature range of 40 ºC- 140 ºC. Resistant starch exhibited multiple endothermic transitions at different temperatures. The retrograded α-amylase resistant starch recorded increased onset, peak and conclusion temperatures and decreased enthalpy.

Keywords: Starch, Processing, Kodo, Kutki, SEM, DSC

INTRODUCTION

Minor millets such as Kodo (*Paspalum scrobiculatum*) and Kutki (*Panicum sumatrance*) are staple food for millions of tribal in India. Millets are nutritionally superior, rich source of calcium, minerals and contain high amount of fiber than wheat and rice (Wen *et al.*, 2014; and Nazni and Bhuvaneswari, 2016). Minor millets are unique due to their short growing season, drought resistance, long storability and nutritional security as they contain all the required nutrients for humans (wwwswarajorg/shikshantar/milletspdf). This uniqueness of millets makes them suitable for large scale utilization in manufacture of variety of food products. Starch present in minor millets is reported to be resistant to hydrolysis and thus provide millets with hypoglycaemic property (Sarita Singh, 2016). Starch is the important glycaemic carbohydrate and its digestibility is of nutritional interest in relation to diet related diseases (Jaspreet *et al.*, 2010; and Shujun Wang and Copeland, 2013).

Starch has been processed or cooked before being consumed by humans; hence extent of disruption of starch structure determines its susceptibility to enzymatic digestion (Trevor Wang *et al.*, 1998; and Tester *et al.*, 2006). The structural changes of starch during processing are the major determinants of starch functional properties for food processing, during digestion and in industrial applications (Klaus Englyst *et al.*, 2000; and Wang *et al.*, 2015). Roasting and cooking without pressure are some of the major processes used in household. Boiling Starch in water results in loss of crystalline structure, swelling of the granule and leaching of amylose from the granule (Klaus Englyst *et al.*, 2000; Zhou *et al.*, 2010; and Dhital *et al.*, 2017). Gelatinized starch upon cooling and storage form an ordered structure that could be highly resistant to hydrolysis by α-amylase and is referred to as Resistant Starch (RS), a physiologically important indigestible starch fraction (Annison and Topping, 1994; Eerlingen and Delcour, 1995; and Sandhu

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and Singh, 2007). Resistant starch has received much attention because of its physiological health benefits and applications in variety of food products (Annison and Topping, 1994; Haralampu, 2000; Nugent, 2005; Sajilata *et al.*, 2006; Fuentes-Zaragoza *et al.*, 2010; and Birt *et al.*, 2013). The relationship between the resistant starch structure and its physicochemical and molecular properties is of importance for understanding and application of RS as potential ingredient in food products (Zhen and Boye, 2016). This study is aimed at investigating the molecular properties and thermal behaviour of native starch and enzyme resistant starch from Kodo and Kutki after processing such as roasting, cooking without pressure and cooking and storage at -20 ºC for 30 days.

MATERIALS AND METHODS

Seeds of Kodo (*Paspalum scrobiculatum)* and Kutki *(Panicum Sumatrance)* were obtained from local market in M.P., India. Seeds were authenticated from the Department of Botany; RTM Nagpur University, Nagpur (MH) India.

Sample Preparation and Storage

Native-Raw millet seeds were washed, dried and milled to fine flour (less than 0.250 mm) and taken for further analysis.

Roasting: Millet seeds were roasted on a low flame with occasional stirring until the colour changed to light brown. The seeds were then ground in a blender to fine flour as described above.

Cooking: Seeds were cooked in water for 25-30 min without pressure, drained and dried at 60 ºC to constant weight, milled to fine flour and stored at ambient temperature in airtight container until use.

Cooking and storage at -20 ºC for 30 days-Part of the cooked samples from the above operation was stored at -20 ºC for 30 days. The samples were then thawed to ambient temperature, dried and milled as described above.

Isolation of Starch

Starch was isolated by alkaline steeping method described by Ackar *et al.* (2010) with slight modification. Briefly, defatted millet flour (100 g) was steeped in 0.5% sodium hydroxide solution (NaOH). 0.5% NaOH was added with continuous stirring for 1 hr. The alkaline slurry was centrifuged at 5000 rpm for 20 min and precipitate was repeatedly washed with distilled water until white coloured starch was obtained. The precipitated starch was suspended in 100 ml distilled water, stirred for 10 min, neutralized with 1 M HCl and centrifuged again. Upper brown layer was removed and white starch was suspended in 100 mL distilled water and passed through 300 mesh sieve. The starch suspension was centrifuged again and resultant starch was air dried and passed through 250 mesh sieve. The resultant starch is referred to as Total Starch (TS) in the present study.

Isolation of ~-Amylase Resistant Starch (RS)

RS was isolated by a method of Champ, M. with minor changes (Champ, 1992). 100 mg starch was suspended in 0.1 M tris maleate buffer (calcium chloride 4 mM, pH 6.8) to which pancreatic α amylase (500 U) was added. The mixture was incubated at 37 °C for 16 hrs. 40 ml ethanol was added and allowed to stand for 1 hr and then centrifuged. The precipitate was washed twice with 80% ethanol, and then vacuum dried. The Resistant Starch obtained was stored in air tight container and used for further characterization.

Scanning Electron Microscopy

The starch was characterized with respect to its shape, size and surface by SEM. Finely ground samples were coated with thin film of palladium and examined under SEM (model: JEOL JSM-6380 A) analytical scanning electron Microscope. The samples were examined at different angles by rotation device provided with the instrument and captures the images at magnification range between 1000 X and 8000 X.

Differential Scanning Calorimetry

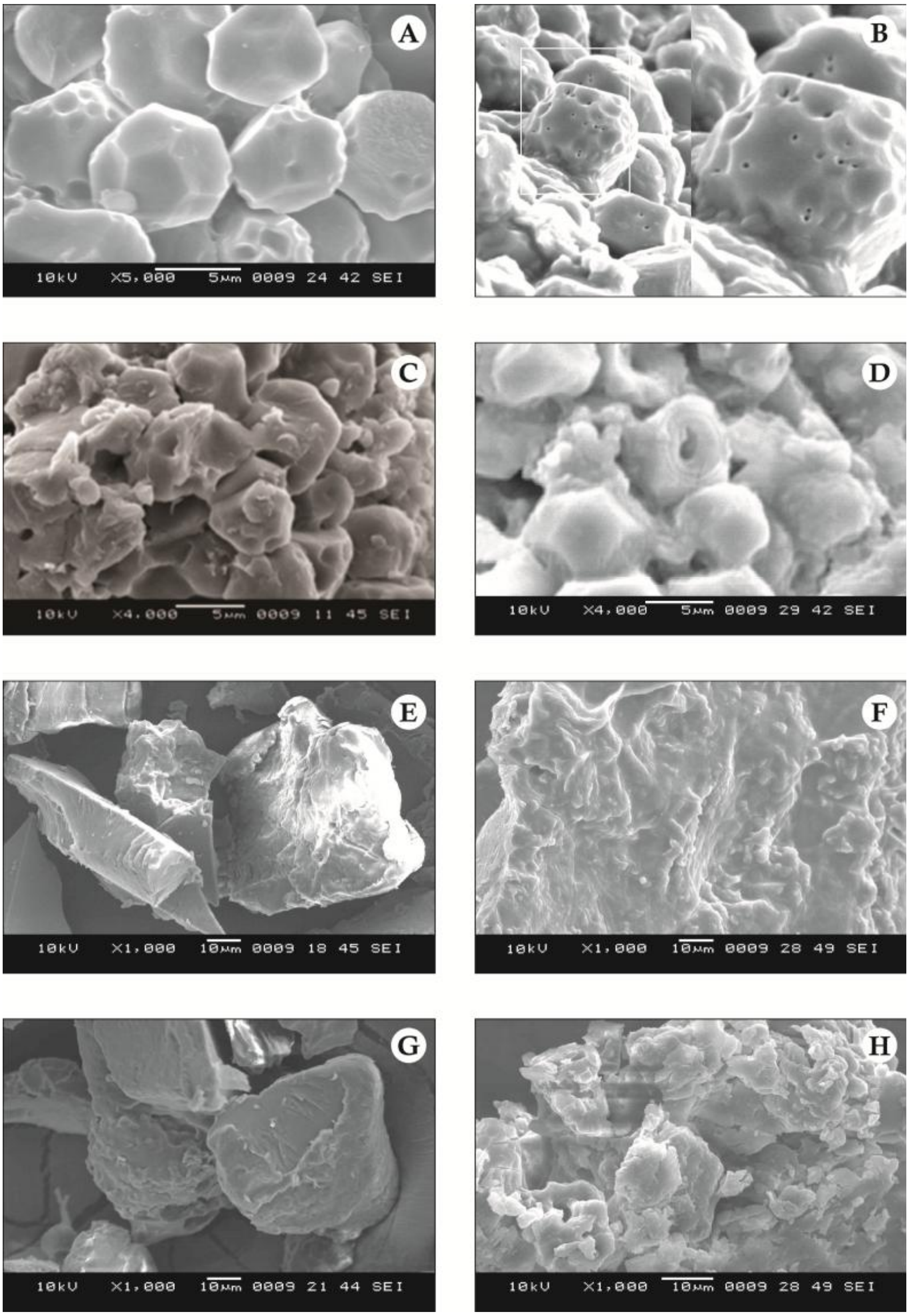
Thermal behaviour of starch and RS residues were evaluated using a DSC (821^{e}Mettler Toledo instrument). 10 mg sample was weighed in aluminium pans. Measured amount of deionised water was added and pans were sealed hermetically and allowed to equilibrate overnight. The DSC run was performed from 20 ºC to 180 ºC at the rate of 5 ºC/ min. A sealed empty pan was used as a reference. Onset (To), peak (Tp), and endset (Te) temperatures and enthalpy change of starch were calculated using STARe software.

RESULTS AND DISCUSSION

SEM

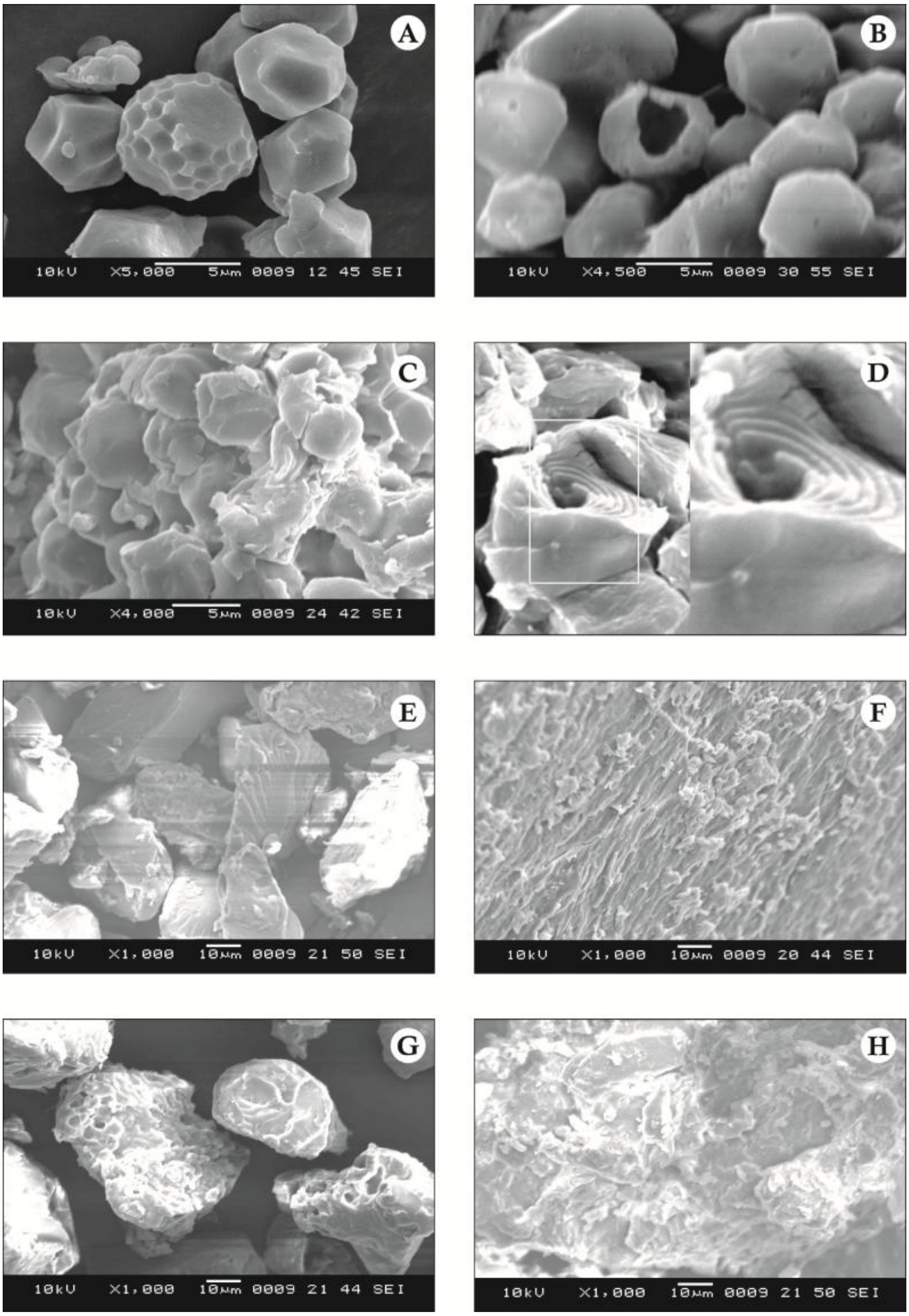
Effect of processing on kodo and kutki starch (TS) and Resistant Starch (RS) were studied by scanning electron microscopy (Figures 1 and 2). Morphology of native starch granules of kodo and kutki exhibited mainly polygonal shape and very few spherical granules. Kodo starch granules ranged between 5.97 µm to 10.7 µm whereas Kutki between 1.68 µm to 8.90 µm. Surface of both, native kodo

Figure 1: SEM Images of Kodo Millet Starch



Note: (A) TS Native, (B) RS Native, (C) TS Roasted, (D) RS Roasted, (E) TS Cooked, (F) RS Cooked, (G) TS Cooked and Stored at -20 °C for 30 days, (H) RS Cooked and Stored at -20 °C for 30 days. TS: Total starch; and RS: Resistant starch.

Figure 2: SEM Images of Kutki Millet Starch



Note: (A) TS Native, (B) RS Native, (C) TS Roasted, (D) RS Roasted, (E) TS Cooked, (F) RS Cooked, (G) TS Cooked and Stored at -20 °C for 30 days, (H) RS Cooked and Stored at -20 °C for 30 days. TS: Total starch, RS: Resistant starch.

and kutki starch granules exhibited characteristic deep indentations indicating dense packing of endosperms with protein bodies. This is in agreement with the observations of Robutti *et al.* (1974) who suggests that the grains having hard endosperm shows polygonal starch granules, whereas grains having soft endosperm exhibit round shaped granules.

Roasting of seeds resulted in aggregation of starch granules with slight gelatinization (Figure 1). The starch was isolated after cooking without pressure for 25-30 min, starch granules swell but did not collapse. This indicates that cooking with pressure is required to assure complete gelatinization of starch. Xueju *et al.* (2006) have reported that the shape of chemically modified corn starch citrate granules remained unchanged after heating at 100 ºC for 30 min. The cooked starch granules of Kutki showed characteristic striated structures on the surface (Figure 1E). Appearance of such striated surface may be the result of degradation and subsequent removal of outer layers of granules due to cooking process.

SEM images of RS granules of kodo and kutki revealed numerous pinholes. Pinholes are the indication of α amylase attack which starts from the small pinhole and increases in size penetrating in the inner portions of the granules towards centre (Fuwa *et al.*, 1979). The attack was more pronounced on the surface of Kodo starch granules (Figure 2B). It was possible to observe inner growth rings in some starch granules. The SEM images of RS of kutki (roasted) at higher magnification (5000 X) showed the layered internal structures or growth rings (Figure 1D). The layered structure was also observed in RS of roasted kodo millet.

The RS of cooked kutki showed irregular network possess the uneven surface zones induced by amylase attack. In retrograded RS of both kodo and kutki, compact and dense formations were observed.

DSC

The transition temperature parameters are summarised in Table 1. Notable changes in the DSC thermograms of RS and TS for both Kodo and Kutki starches were observed. The transition onset (To), peak (Tp) and conclusion (Tc) temperature of native starch ranged between 40 ºC-140 ºC. The broad nature of endotherm for the native starches and the higher conclusion temperature suggests that the crystalline associations within the starch granules are of higher orders. The peak temperature for native Kodo was 84.15 ºC and for kutki was 71.07 ºC. George Amponsah Annor studied the gelatinization and melting parameters of pearl, foxtail, proso and finger millets (George Amponsah Annor *et al.*, 2014). In their study they have reported the peak temperatures between 67.9 ºC and 72.2 ºC. Yu Wen *et al.*, have reported the transition temperatures for different varieties of proso millet starches between 66.81 ºC to 82.44 ºC. Differences in thermal properties of starches have been reported to depend on botanical origin, conditions of processing, moisture content and heating rate as well as the molecular architecture of crystalline granule. The transition temperature values are reported to be influenced

Table 1: DSC Endothermic Transition Temperatures of Kodo Millet Starch

S. No.	KODO Treatments	To	Tp	Tc	Enthalpy (J/g)	To	Tp	Tc	Enthalpy (J/g)	To	Tp	Tc	Enthalpy (J/g)
1	NTS	42.26	84.15	140.6	15.6	--	--	--	--	--	--	--	--
2	RTS	105.44	121.13	141.98	5.6	--	--	--	--	--	--	--	--
3	CTS	112.82	128.65	156.33	5.5	--	--	--	--	--	--	--	--
4	STS	111.58	135.5	166.17	7.3	--	--	--	--	--	--	--	--
5	NRS	93.86	116.48	132.06	5.4	--	--	--	--	--	--	--	--
6	RRS	65.62	85.85	118.04	4.6	--	--	--	--	--	--	--	--
7	CRS	99.98	111.6	119.71	6.07	134.62	136.01	139.73	2.7	146.92	156.96	161.21	2.3
8	SRS	127.59	130.83	136.77	4.66	145.68	157.92	175.69	3.2	--	--	--	--

Note: To (Onset), Tp (Peak), Tc (conclusion) as observed during DSC scanning. (Temperature is in °C; NTS: Native TS, RTS: Roasted TS, CTS: Cooked TS, STS: cooked/stored at -20 ºC/30d TS, NRS: Native RS, RRS: Roasted RS, CRS: Cooked RS, SRS: cooked/stored at -20 ºC/30d RS)

Table 2: DSC Endothermic Transition Temperatures of Kutki Millet Starch

S. No.	KUTKI Treatments	To	Tp	Tc	Enthalpy (J/g)	To	Tp	Tc	Enthalpy (J/g)	To	Tp	Tc	Enthalpy (J/g)
1	NTS	40.19	71.07	140	14.3	--	--	--	--	--	--	--	--
2	RTS	89.81	111.43	129.94	2.4	--	--	--	--	--	--	--	--
3	CTS	124.15	139.99	163.71	1.5	--	--	--	--	--	--	--	--
4	STS	133.79	134.75	143.83	14.6	--	--	--	--	--	--	--	--
5	NRS	64.03	95.49	136.28	9.1	--	--	--	--	--	--	--	--
6	RRS	62.52	84.09	110.62	6.3	--	--	--	--	--	--	--	--
7	CRS	98.03	106.85	111.34	2.78	114.34	117.03	121.06	-3.11	133.73	145.34	167.67	1
8	SRS	50.99	61.11	64.45	1.78	101.62	109.55	14.62	1.33	125.58	140.44	160.82	9.4

Note: To (Onset), Tp (Peak), Tc (conclusion) as observed during DSC scanning. (Temperature is in °C; NTS: Native TS, RTS: Roasted TS, CTS: Cooked TS, STS: cooked/stored at -20 ºC/30d TS, NRS: Native RS, RRS: Roasted RS, CRS: Cooked RS, SRS: cooked/stored at -20 ºC/30d RS).

by crystalline regions that correspond to amylose and amylopectin short chains. Compared to native, the processed starch displayed higher melting temperature but lower enthalpy. The enthalpy of native kodo and kutki starch was 15.6 J/g and 14.3 J/g respectively, while roasted and cooked starch recorded 5.6 J/g and 5.5 J/g for kodo and 2.4 J/g and 1.5 J/g for kutki respectively. Such decrease in enthalpy energy may indicate the weakening of bonds and realignment of amylose and amylopectin chains during processing. Change in enthalpy is attributed to the double helical structure, crystalline order after different processing and parameters used to measure the heat capacity. Enthalpy for kodo and kutki starch stored under -20 ºC/30 days are found to be increased to 7.3 J/g and 14.6 J/g respectively than gelatinized and roasted starch samples. This may be due to recrystallization occurred in stored starch samples and probably due to an increased number of hydrogen bonds compared to cooked and roasted samples. This suggests that increased molecular ordering and glucan interaction during storage at lower temperature and for longer period of time. This result is in agreement with the results of Marina Garcia-Rosas *et al.* (2009). Christopher Mutungi *et al.* (2009) observed increase in change in enthalpy from 8.5 J/g to 12.7 J/g for debranched starch to debranched retrograded cassava starch. Enzyme resistant starch of cooked and stored samples exhibited multiple endotherms over a broader temperature range. The higher temperature range for the retrograded resistant starch is observed for both the samples. Two multiple endotherm were observed for kodo at the peak temperatures 130 °C and 156.9 ºC whereas three transitions were observed for kutki at Tp 61.11 ºC, 109.55 ºC and 140 ºC. Seivert and Pomeranz (1990) reported melting endotherm of amylomaize resistant starch over a range of 120 ºC-165 ºC. R C Erlingen observed endotherm transition of resistant starch from wheat at 150 ºC. These multiple endotherm and broad nature of transition indicate the crystalline inhomogeniety and formation of crystallites of varying molecular stabilities after processing. The higher melting temperature of the resistant starch from retrograded kodo and kutki also indicates thermal stability.

CONCLUSION

The granular structure of millet starches have been evaluated by scanning electron microscopy with and without α-amylase attack. Processed starch granules showed significant degree of enzymatic erosion. RS derived from gelatinized and retrograded starch showed non-granular structures and not a well-defined entity. DSC was used to study thermal behaviour of starches. Notable changes in To, Tp and Tc values were observed in native as well as processed starches. Retrograded RS recorded significant increase in melting temperature which indicates the thermal stability of resistant starches. Starch provides energy in its different forms; therefore it is of importance to increase our understanding of granular structure of starch after processing.

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