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Structural characteristics and in vitro digestibility of Mango kernel starches (*Mangifera indica* L.)

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Abstract

Structural characteristics and digestibility of starches isolated from the kernels of two mango cultivars (Chausa and Kuppi) were studied and compared with those of a commercial normal corn starch. Mango kernel starches showed an A-type X-ray diffraction pattern, with relative crystallinities of 35.4% and 38.3%, respectively for Kuppi and Chausa cultivars. The structural characterisation obtained, using high performance size exclusion column chromatography connected to multi-angle laser light scattering and refractive index detectors (HPSEC-MALLS-RI), revealed that the mango kernel starches had lower molecular weight (M_w) and radius of gyration (R_g) of amylopectin and amylose compared to those of corn starch. The M_w of amylopectin for Chausa and Kuppi starches were 179×10^6 and 140×10^6 g/mol, respectively. The amounts of readily digestible starch (RDS) and slowly digestible starch (SDS) were lower for mango kernel starch than those of corn starch. Resistant starch (RS) contents in the mango kernel starches (75.6% and 80.0%, respectively) were substantially higher than those of corn starch (27.3%). The glycemic index (GI) values for mango kernel starches were 48.8 and 50.9 (for Chausa and Kuppi, respectively), whereas that of corn starch was 74.8, indicating that the mango kernel starch granules were highly resistant to digestion with significant contents of RS.

Keywords: Mango kernel starch; Crystallinity; Molecular weight; Digestibility

1. Introduction

Mango (*Mangifera indica* L.) is one of the most favoured and commercially valuable fruit growing throughout the tropics and is used in a variety of food products. Considerable amounts of mango kernels (seeds) are discarded as waste after industrial processing of mangoes (Puravankara, Bohgra, & Sharma, 2000). Approximately 40–60% waste is generated during processing of mangoes, 12–15% and 15–20% of which consists peels and kernels, respectively (Kaur, Singh, Sandhu, & Guraya, 2004). Depending on the variety, mango kernels contain 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash, based on the dry weight average (Zein, El-Bagoury, & Kassab, 2005).

Kaur et al. (2004) studied the physicochemical, morphological, thermal and rheological properties of mango kernel starches and found their properties to be comparable with starches from other commercial sources. Velan, Krishnan, and Lakshmanan (1995) studied the conversion of mango kernel starch to glucose syrups by enzymatic hydrolysis. Chowdary, Hari Krishna, and Hanumantha Rao (2000) studied optimisation of enzymatic hydrolysis of mango kernel starch by response surface methodology. Tavares, Bathista, Silva, Filho, and Nogueira (2003) reported molecular dynamic study of the starches obtained from the mango and the *Espada* seeds by ^{13}C solid state NMR.

In recent years, glycemic index (GI) has become a potentially useful tool in planning diets for patients suffering from diabetes, dyslipidemia, cardiovascular disease and even certain cancers (Jenkins et al., 1981). The digestibility of starch in foods varies widely according to the nature of the foods (Björck, Granfeldt, Liljeberg, Tovar, & Asp,

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1994). Therefore, a nutritional classification of dietary starch that takes into account both the kinetic component and the completeness of its digestibility has been proposed. This classification consists of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992).

Starches from fruits such as mango (Iagher, Reicher, & Ganter, 2002; Millan-Testa, Mendez-Montealvo, Ottenhof, Farhat, & Bello-Perez, 2005), apple (Singh, Inouchi, & Nishinari, 2005), and banana (Núñez-Santiago, Bello-Pérez, & Tecante, 2004; Zhang, Whistler, BeMiller, & Hamaker, 2005) have been characterised however, no studies concerning the structural characterisation and digestibility of mango kernel starches have been reported to date. Therefore, in this study structural properties and in vitro digestibility of mango kernel starches were investigated and compared with those of normal corn starch.

2. Materials and methods

2.1. Materials

Starches from the kernels of two mango cultivars (cv.), i.e. Chausa and Kuppi were isolated as described previously (Kaur et al., 2004). Normal corn starch was provided by Samyang Genex Co. (Seoul, Korea).

2.2. X-ray diffraction analysis

X-ray diffraction analysis was performed using an X-ray diffractometer (Philips, X'pert MPD high resolution XRD, Almelo, Netherlands) operated at 40 kV and 40 mA. Diffractograms were obtained from 4° (2θ) to 30° (2θ) using a scanning speed of 4°/min. The degree of relative crystallinity was quantitatively estimated following the method described by Nara and Komiya (1983) using peak-fitting software (Origin-Version 6.0, Microcal Inc., Northampton, MA).

2.3. Molecular weight analysis

For measuring the structural properties, the starch sample was purified following the method described by Han and Lim (2004). For starch dissolution, the dry and pure starch (5 mg, dry solids), in a glass vial, was wetted with ethanol (50 μl) and then 1 M NaOH (1 ml), 50 mM $NaNO_3$ (3 ml) and 1 M HCl (1 ml) was added and mixed thoroughly. The starch solution was then autoclaved at 121 °C/20 min and filtered through the mixed cellulose ester filter (5 μm) while hot (≈70 °C), before injection into the high performance size exclusion column chromatography (HPSEC) system. The molecular structure of the starch was analysed using a HPSEC, connected to a multi-angle laser light scattering detector (MALLS) and a refractive index (RI). The mobile phase used for HPSEC was an aqueous $NaNO_3$ solution (0.15 M) that had been filtered through 0.1 μm cellulose acetate filters (Whatman, UK) and degassed with a vacuum pump for 4 h before use. The SEC column (2.6 × 70 cm) contained Toyopearl HW 65 F resins (Tosoch Corp. Tokyo, Japan) of particle and pore sizes, 30–60 μm and 1000 A, respectively. The system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 1 ml loop (model 7072, Rheodyne, Cotati, CA), a SEC column, a multi-angle laser light scattering detector (MALLS, 632.8 nm, DAWN DSP-F, Wyatt Technology, Santa Barbara, CA) and a refractive index detector (RI, Optilab DSP, Wyatt Technology, Santa Barbara, CA). The flow rate and pump pressure were 0.8 ml/min and 40 psi.

2.4. In vitro digestibility

In vitro starch digestibility was analysed following the method described by Englyst et al. (1992) as modified by Chung, Lim, and Lim (2006). Porcine pancreatic alpha-amylase (No. 7545, Sigma–Aldrich, St. Louis, MO) and amyloglucosidase (No. 9913, Sigma–Aldrich) (3.89 g) was dispersed in water (25.7 ml) and centrifuged for 10 min at 2500*g* and 18.7 ml of supernatant was collected. Amyloglucosidase (No. 9913, Sigma–Aldrich) (1 ml) and deionised water (2 ml) were added to enzyme solution. The solution was freshly prepared for the digestion analysis.

Aliquots of guar gum (10 ml, 5 g/l) and sodium acetate (5 ml, 0.5 M) were added to the starch samples (0.5 g, db) in a test tube. Seven glass balls (10 mm diameter) and 5 ml of enzyme solution were then added to each tube, followed by incubation in a water bath (37 °C) with agitation (170 rpm). Aliquots (0.5 ml) were taken at intervals and mixed with 4 ml of 80% ethanol and the glucose contents in the mixture were measured using glucose oxidase and peroxidase assay kits (No. GAGO-20, Sigma–Aldrich). The total starch content in the starch samples was measured according to Englyst et al. (1992). The starch classification based on its digestibility was: RDS as the starch that was hydrolysed within 20 min of incubation, RS as the starch not hydrolysed with 120 min, and SDS as the starch digested during the period between 20 and 120 min.

2.5. Estimated glycemic index (GI)

Using the hydrolysis curve (0–180 min), the hydrolysis index (HI) was calculated as the percentage of total glucose released from the sample, to that released from white bread (Goñi, Garcia-Alonso, & Saura-Calixto, 1997; Granfeldt, Björck, Drews, & Towar, 1992). The glycemic indices of the samples were estimated according to the equation proposed by Goñi et al. (1997): $GI = 39.71 + 0.549\ HI$.

2.6. Statistical analysis

The data reported in all the tables were subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Software version 15 (Minitab, Inc., State College, USA).

3. Results and discussion

3.1. Size distribution and amylose content

The mean granule diameters of the mango kernel starches were slightly higher than those of corn starch (Table 1): 15.8 and 16.3 μm, respectively, were observed for Chausa and Kuppi cultivars. The amylose content of the mango kernel starches (28.8% and 33.6%, respectively for Chausa and Kuppi cultivars) measured using the HPSEC-MALLS-RI system was slightly higher than that of corn starch (26.7%).

3.2. Crystallinity by X-ray diffraction

The X-ray diffractograms of the corn and mango kernel starches are shown in Fig. 1. Both corn and mango kernel starches showed A-type X-ray diffraction patterns, which are typically found in many cereal starches. Millan-Testa et al. (2005) reported A-type X-ray diffraction patterns for mango starch. Both corn and mango kernel starches showed strong reflections at 15° and 23° (2θ) and an unresolved doublet at 17° and 18° (2θ). Mango kernel starches showed an additional peak at 10° (2θ) which was slightly different from that of normal corn starch. The relative crystallinity, which was measured, based on diffraction intensity was higher for mango kernel starches than corn starch (Table 1). Chausa starch had a higher crystallinity (38.3%) than Kuppi starch (35.4%). The side chains of amylopectin form the crystalline structure, it is therefore expected that the crystallinity will be inversely related to amylose content. It has been reported that the relative crystallinity of normal corn starch was 30.3% (Cheetham & Tao, 1998) and that of mango starch was 35% (Millan-Testa et al., 2005).

3.3. Molecular characteristics

The structural data measured using an HPSEC-MALLS-RI system for corn and mango kernel starches are shown in Table 2 and their representative curves are shown in Fig. 2. Significant differences in the M_w of amylopectin and amylose were observed between corn and mango kernel starches. Corn starch showed the higher value of M_w of amylopectin (200×10^6 g/mol) and amylose (9.5×10^6 g/mol) than the mango kernel starches. Comparing the mango kernel starches, the M_w of amylopectin was higher for Chausa (179×10^6 g/mol) than for Kuppi (140×10^6 g/mol). The radius of gyration (R_g) of amylopectin was the highest for corn starch (348 nm) and the lowest for Kuppi starch (293 nm) in the same order for M_w. A similar relation between M_w and R_g has been

Table 1
Mean granule diameter, amylose content and crystallinity of starches from normal corn and mango kernels of different cultivars

Starch	Mean granule diameter (μm)	Amylose content (%)	Relative crystallinity (%)
Normal corn	13.6 ± 0.2^a	26.7 ± 0.6^a	30.1 ± 0.4^a
Chausa	15.8 ± 0.3^b	28.8 ± 0.7^b	38.3 ± 0.7^c
Kuppi	16.3 ± 0.3^c	33.6 ± 0.8^c	35.4 ± 0.9^b

Values with the same superscript within a column do not differ significantly ($p < 0.05$).
Mean (±standard deviation) of triplicate analysis.

Table 2
Molecular characteristics of starches from normal corn and mango kernels of different cultivars

Starch	M_w ($\times 10^6$ g/mol)		R_g (nm)	
	Amylopectin	Amylose	Amylopectin	Amylose
Normal corn	200 ± 2.1^c	9.5 ± 0.9^c	348 ± 2.8^c	145 ± 0.9^c
Chausa	179 ± 2.5^b	4.8 ± 0.5^a	318 ± 2.1^b	124 ± 2.3^a
Kuppi	140 ± 1.7^a	6.2 ± 0.7^b	293 ± 1.9^a	134 ± 1.2^b

Values with the same superscript within a column do not differ significantly ($p < 0.05$).
Mean (±standard deviation) of triplicate analysis.

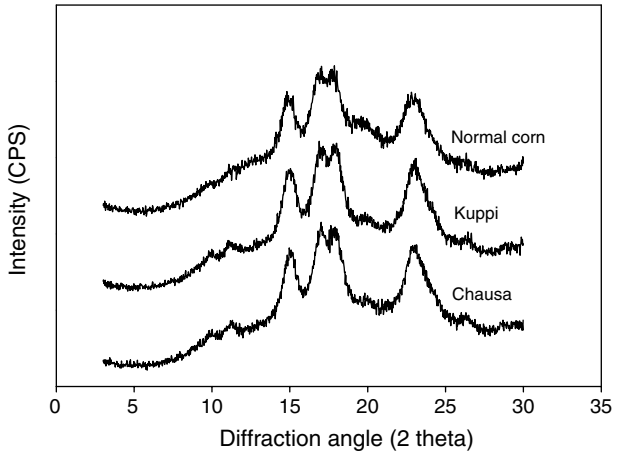

Fig. 1. X-ray diffractograms of starches from normal corn, Chausa and Kuppi.

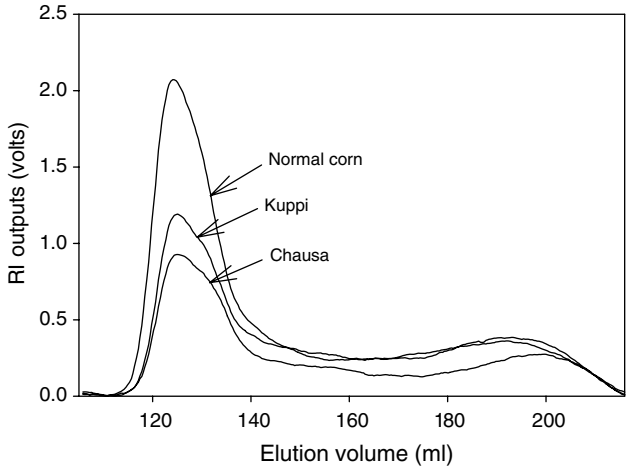

Fig. 2. Molecular characterisation of starches from normal corn, Chausa and Kuppi.

reported earlier for okenia, mango and banana starches by Millan-Testa et al. (2005). It was noteworthy that the mango kernel starch from the Kuppi cultivar contained the greater amylose content and the molecular size of the amylose was also greater in comparison to that of the Chausa cultivar. The M_w and R_g of amylopectin were negatively correlated to the amylose content ($r = -0.999$ and -0.963, $p < 0.01$). A similar inverse relationship between the M_w of amylopectin and amylose content has been previously reported for mango and banana (Millan-Testa et al., 2005) and wheat starches (Yoo & Jane, 2002).

3.4. Digestibility studies

The digestibilities of the corn and mango kernel starches and starch fractions differ in digestion behavior (readily digestible starch, RDS; slowly digestible starch, SDS; and resistant starch, RS) as shown in Table 3 and Fig. 3. RDS is rapidly and completely digested in the small intestine and is associated with more rapid elevation of postprandial plasma glucose. The mango kernel starches showed lower values for RDS and SDS but very high RS compared to normal corn starch. The Chausa starch showed the lowest amounts of RDS and SDS (4.7% and 15.3%, respectively). These values were substantially lower than those for normal corn starch (27.4% and 45.3%, respectively). SDS is completely, but more slowly, digested in the small intestine and attenuates postprandial plasma glucose and insulin levels. It is generally the most desirable form of dietary starch (Jenkins et al., 1981). RS has been defined as the sum of starch and the product of starch degradation not absorbed in the small intestine but is fermented in the large intestine of healthy individuals (Asp, 1992). The lowest RS (27.3%) was observed for corn starch, whereas Chausa starch contained a RS more than three times that of corn starch (80.0%). Zhang, Venkatachalam, and Hamaker (2006) followed the method of Englyst et al. (1992) to determine the RDS, SDS and RS (%) for native normal corn starch and reported values of 24.4%, 53.0% and 22.6%. Corn starch granules had channels connecting the internal cavity with the external environment (Huber & BeMiller, 1997) therefore the hydrolytic enzymes had access to the interior of the granules via channels (Hood & Liboff, 1983), which results in its high digestibility. Conversely, mango kernel starches contain a high proportion of starch granules with smooth surfaces, with very few granules having surface pores (Kaur et al., 2004). Hydrolytic enzymes act primarily through surface erosion of the starch granules, which may be the cause of their lower digestibility. High RS contents due to hydrolytic enzymes acting on the surfaces of starch granules have been reported for banana (Zhang et al., 2005), yam and lily starches (Jane, Wong, & McPherson, 1997). The differences in the in vitro digestibility of native starches among species have been attributed to the interplay of many factors, including starch sources (Ring, Gee, Whittam, Orford, & Johnson, 1988), granule size (Snow & O'Dea, 1981), amylose/amylopectin ratio (Hoover & Sosulski, 1985), degree of crystallinity (Chung et al., 2006; Hoover & Sosulski, 1985) and type of crystalline polymorphic forms (Jane et al., 1997). The total amount of digestible starch (RDS + SDS) was negatively correlated to starch granule diameter ($r = -0.969$, $p < 0.05$). The size of starch granules may affect digestibility because as the size of the starch granule increases, the contact between the enzyme and substrate decreases (Svihus, Uhlen, & Harstad, 2005).

Table 3
Digestibilities of starches and starch fractions from normal corn and mango kernels of different cultivars

Starch	Digested starch		RS (%)
	RDS (%)	SDS (%)	
Normal corn	27.4 ± 0.7^{b}	45.3 ± 2.6^{c}	27.3 ± 2.1^{a}
Chausa	4.7 ± 0.4^{a}	15.3 ± 1.1^{a}	80.0 ± 2.9^{c}
Kuppi	5.2 ± 0.3^{a}	19.2 ± 1.3^{b}	75.6 ± 1.7^{b}

Values with the same superscript within a column do not differ significantly ($p < 0.05$).
Mean (±standard deviation) of triplicate analysis.

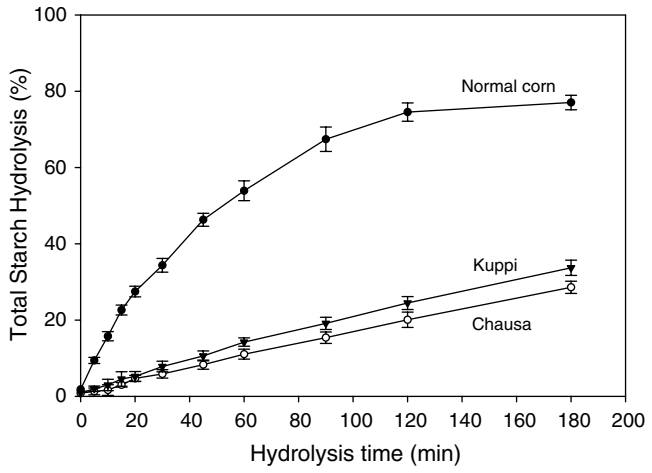

Fig. 3. Enzymatic digestion of starches from normal corn, Chausa and Kuppi.

3.5. Hydrolysis index (HI) and estimated glycemic index (GI)

The hydrolysis index (HI) is a useful tool, from a nutritional point of view, for comparison of starch digestibility. This index expresses the digestibility of the starch in foods in relation to the digestibility of starch in a reference material, namely white bread. Glycemic index (GI) is calculated from HI and is defined as the incremental postprandial blood glucose area after injection of the test product, as a percentage of the corresponding area after injection of an equicarbohydrate portion of the reference product (Jenkins et al., 1983). The HI and GI of corn and mango kernel starches are shown in Table 4. Corn starch showed a higher

Table 4
Hydrolysis index (HI) and estimated glycemic index (GI) of starches from normal corn and mango kernels of different cultivars

Starch	HI	GI[d]
Normal corn	64.0 ± 1.5^{c}	74.9 ± 1.1^{c}
Chausa	16.7 ± 0.9^{a}	48.8 ± 0.6^{a}
Kuppi	20.5 ± 1.3^{b}	50.9 ± 1.0^{b}

Values with the same superscript within a column do not differ significantly ($p < 0.05$).
Mean (±standard deviation) of triplicate analysis.
[d] GI was calculated by the equation proposed by Goñi et al. (1997): GI = 39.71+0.549 HI.

HI and GI (64.0% and 74.9%, respectively) compared to mango kernel starches. The HI of mango kernel starches was 16.7 and 20.5, respectively and the estimated GI based on HI were 48.8% and 50.9%, respectively for Chausa and Kuppi. The HI and GI of Kuppi were higher than those of Chausa. The hydrolysis rate was significantly different for corn and mango kernel starches: the highest rate was for corn (77.0%) and the lowest was for Chausa (28.6%). After 20 min of hydrolysis, mango kernel starches showed a hydrolysis rate of less than 5.3%, whereas that of corn starch was 27.5%. After 120 min, the hydrolysis rate of corn starch (72.7%) remained substantially greater than those of the mango kernel starches (24.5% and 20.1%, respectively). The greater amylose content and crystallinity observed for the mango kernel starches, in comparison to the normal corn starch, may be a major contributor to the greater resistance of the digestive enzymes for mango kernel starches.

4. Conclusion

Mango kernel starches showed an A-type X-ray diffraction pattern, typically found in many cereal starches. The M_w of the mango kernel starches were lower whereas the amylose content and crystallinity were higher than that of normal corn starch. The mango kernel starches contained higher RS leading to lower GI values compared to normal corn starch. Therefore, mango kernels wasted after industrial processing of mango could become a useful source of starch, especially in terms of its beneficial digestibility behavior and high RS content.

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