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Effects of supercritical fluid extraction on *Curcuma longa L.* and *Zingiber officinale R.* starches

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Abstract

Ginger and turmeric tubers have approximately 45 and 40% of starch, respectively. These starches were analyzed before and after ginger and turmeric were subjected to supercritical fluid extraction to obtain oleoresin and essential oil. The starches were isolated and analyzed with respect to purity, amylose/amylopectin content, X-ray pattern, viscosity, swelling factor, granule morphology by scanning electron microscopy, gelatinization temperature by differential scanning calorimetry and turbidity. Supercritical fluid extraction process did not alter the starchy matrix showing small physical rearrangement of the starch molecules; this effect was more intense in the ginger starch, as observed by X-ray diffraction. The ginger starch became less resistant, in other words, there was a starchy structure relaxing after supercritical fluid extraction, evidenced by the lower setback value in the gelatinization process and nonetheless, it did not alter the granule morphology as observed by microscopy. This study reveals similar characteristics of these starches with commercial starches, indicating their potential for industrial applications.

Keywords: Curcuma longa L.; Ginger; Starch; Supercritical fluid extraction; Turmeric; Zingiber officinale R.

1. Introduction

Starch is a predominant food reserve substance in plants and provides 70–80% of the calories consumed by humans worldwide. It has unique chemical and physical characteristics and its nutritional quality set it apart from all other carbohydrates. Some modified starches with specific physical characteristics can even play a role in lowering the fat content of prepared foods by providing a similar sensory perception of fattiness or creaminess (Whistler & BeMiller, 1999).

Many of the granules in tuber and root starches, such as potato and cassava starches, tend to be larger than those of seed starches and are generally less dense and easier to gelatinize (Colonna, Gallant, & Mercier, 1980).

X-ray diffraction has been used to reveal the presence and characteristics of the starch crystalline structure (Singh, Singh, Kaur, Sodhi, & Gill, 2003). For tubers and roots, the X-ray pattern is B-type with water molecules columns substituting one of the double helices (Hoover, 2001). The viscosity properties are supposed to depend on changes in structure, size and composition of the starch granules (Madsen & Christensen, 1996). The enthalpy (ΔH_{gel}) and temperature of gelatinization of starch depend on the microstructure, degree of crystallinity within the granule and also on granule size and shape, and the amylose/amylopectin ratio. Starches of tubers contain fewer short chains of amylopectin and more long chains than the amylopectin from cereals (Hizukuri, 2004).

Originated from India and found in South America countries, turmeric (*Curcuma longa* Linneu) is largely used in medicinal and food preparations. Due to its easy digestibility, turmeric has been used in industry to prepare special foods and children's foods (Jyothi, Moorthy, & Vimala, 2003). Ginger (*Zingiber officinale* Roscoe), originated from South Asia (Govindarajan, 1980) is used in food, beverage, pharmaceutical and cosmetics industries (Mustafá & Srivastava, 1990). Phenotype and agronomic properties of tropical tubers and roots have been well documented (Whistler & BeMiller, 1999). On the other hand, physicochemical properties of many starchy tubers and roots have not yet been studied extensively; there is a need to intensify the researches on products development to explore these starches to use in food industry (Whistler & BeMiller, 1999).

The behavior of the starch structure under high pressure depends on its crystalline structure. Modifications have been reported to occur at very high pressures on the order and over 1000 MPa [Mertens (1993) cited by Francisco and Sivik (2002)].

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Pressures of 80–300 bar used in cassava, wheat and potato starches gelatinization with supercritical carbon dioxide did not change X-ray patterns of these starches, but differences in the gelatinization degree occurred: increasing of the pressure caused decreasing of the gelatinization degree (Francisco & Sivik, 2002).

The SFE starchy residues of turmeric and ginger have around 30–50% of starch (Braga, Leal, Carvalho, & Meireles, 2003). Turmeric starch can contain traces of oleoresin and curcuminoids (yellow color); therefore, it can be classified as a special starch with high commercial potential. Jyothi et al. (2003) studied the physicochemical properties of turmeric starch, and their results indicated a stable viscosity, a resistant gel and easily digestible; nonetheless, properties as swelling volume and viscosity were modified when the color was removed from the starch. This emphasizes that colored turmeric starch can potentially find application in the food industry.

Rosa and Meireles (2005) have estimated that the cost of manufacturing (COM) ginger oleoresin by SFE is approximately US$ 99.80/kg, which is virtually equal to the selling price of ginger oleoresin; a similar behavior is expected for turmeric. Therefore, in both cases the SFE residue must find use in order to decrease the COM. Two options can be envisaged: (i) to use the SFE starchy residue to produce others chemical (Moreschi, Petenate, & Meireles, 2004) or, (ii) The direct use of the starch present in the SFE residue. Thus, the objective of this work was to study the influence of SFE process on the starches of turmeric and ginger, in order to evaluate the use of these residual starches by the food industry.

2. Material and methods

The ginger (*Z. officinale* R.) and the turmeric tubers (*C. longa* L.) used on this work came from previous work done in our laboratory by Braga et al. (2003); Zancan, Marques, Petenate, and Meireles (2002), respectively. These raw materials were used to obtain their extracts by SFE. For ginger, SFE was done using CO_2 and the cosolvent isopropyl alcohol (1.5% v/v) at 250 bar and 35 °C (Zancan et al., 2002). For turmeric, the SFE was done with CO_2 plus a mixture (1:1) of ethanol/isopropyl alcohol as cosolvent (10% v/v) at 300 bar and 30 °C (Braga et al., 2003). The residues from the triplicate SFE assays of turmeric and ginger were used in this work; the residues were denoted as C_b (turmeric starch before SFE), C_a (turmeric starch after SFE), Z_b (ginger starch before SFE) and Z_a (ginger starch after SFE).

2.1. Starch isolation

The isolation of starches was obtained following the procedure of Perez, Bahnassey, and Breene (1993) using a centrifuge (model BR4i, Jouen, St Herblain, France). The isolation method uses NaOH 0.25% [wt] to break the vegetal structure.

2.2. Composition

The isolated starches were characterized using the AOAC methods (AOAC, 1995): starch (Method No. 32.2.05), protein (Method No. 32.1.22), ash (Method No. 4.1.10), fibers (Method No. 4.6.01), lipids (Method No. 32.125), and humidity (Method 4.1.03); reducing sugar was determined by Somogy–Nelson method (Nelson, 1994).

2.3. Amylose content

The amylose content was analyzed as apparent amylose, because it is known that tuber has small content of lipids (Sowbhagya & Bhaattachrya, 1997). The amylose contents of the starches were determined by the spectrophotometric method using a spectrophotometer (Beckman, model DU70, Fullerton, USA), with amylose standard 96.8% (Sigma–Amylose type II-from potato, Lot 63H3910, USA) at 610 nm. The amylopectin content was obtained by difference.

2.4. Swelling factor

The capacity of swelling of the starch granules was determined using the method of Anderson, Conway, Pfeifer, and Fand Griffin (1969).

2.5. Scanning electron microscopy (SEM)

The vegetal structure and the granule morphology were examined using SEM. Starch samples were applied on circular aluminum stubs with double sticky tape and the starch was coated with 24 nm of gold. The micrograph was obtained by scanning electron microscopy (Jeol, model SM 5800 LV, Tokyo, Japan) and accelerating potential of 15 kV, this analysis was made in the Laboratory of Scanning Electron Microscopy of the Biology Institute, Unicamp. The images were analyzed by Scion Image software—version Beta 4.0.2 (Scion Corporation, 2005).

2.6. X-ray diffraction

X-ray diffractograms were obtained by X-ray diffractometer (Shimadzu, model XRD 600, Tokyo, Japan), at 40 kV, signals of the reflection angle of 1θ, from 5 to 50° and copper irradiation. The analysis was made at the Analytical Center of the Institute of Chemistry, Unicamp.

2.7. Differential scanning calorimetry

Gelatinization temperature was measured with modulate differential scanning calorimeter (MDSC) (TA Instruments Thermal Analysis, model 2920, New Castle, UK). The starch to water ratio used was 1:3 (wt:wt) (Jyothi et al., 2003) and the sample pans were heated at a rate of 10 °C/min from 20 to 120 °C. Onset temperature (T_o), peak temperature (T_{peak}), conclusion temperature (T_c) and entalphy of gelatinization (ΔH_{gel}) were obtained.

Table 1
Composition of *Curcuma longa* Linneu (C) and *Zingiber officinale* Roscoe (Z) tubers starches

Dry basis (%)	Turmeric		Ginger	
	C_b	C_a	Z_b	Z_a
Starch	76±4	77±2	85±2	84±1
Total Protein	0.6±0.1	0.5±0.1	0.53±0.01	0.56±0.01
Ash	1.5±0.2	1.43±0.04	0.91±0.04	0.16±0.03
Reducing Sugar	tr	tr	tr	tr
Moisture	11.8±0.4	12.1±0.5	8.2±0.8	9.7±0.1
not quantified	10.1	8.97	5.36	5.58

tr = trace(0.01%; the subscripts b and a means before and after SFE, respectively.

2.8. Rapid visco analyzer

Gelatinization parameters were determined using a rapid visco analyser (RVA) (Newport Scientific Pty Ltd, Warriewood, Australia), following AACC method (1995) (method no. 22). The pasting temperature (°C), Peak time (min), viscosity peak (cP), breakdown (cP), final viscosity (cP) and setback (cP) were obtained.

2.9. Turbidity measurements

The starches turbidity was measured using Perera and Hoover (1999) method.

2.10. Statistic analysis

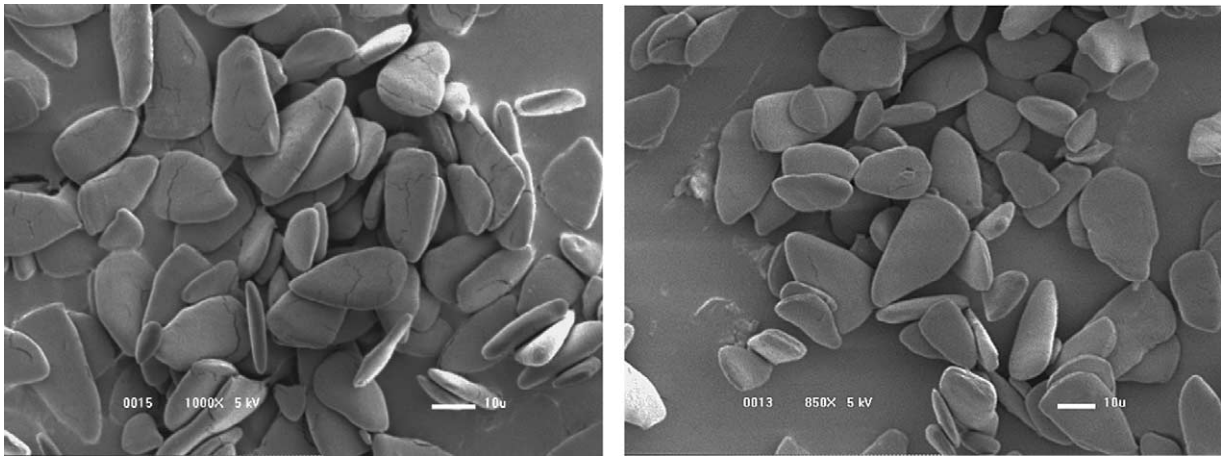
The analysis of variance (Tukey test) was done with MiniTab 2.0 software.

3. Results and discussions

Table 1 shows the chemical composition of ginger and turmeric isolated starches. Data show similarity between starch contents before and after supercritical process, indicating that the starch was not solubilized by the solvent (CO_2 + cosolvent). Isolated turmeric and ginger starches had purity of approximately 77 and 85%, respectively. The C_a sample showed an intense yellow color, after exhaustive washes and centrifugation during the starch isolation process, which indicates the presence of curcuminoids. It can be explained by lipid–amylopectin complexation (Hoover & Vasanthan, 1994), which made difficult the starch clarification by washing. As observed by Jyothi et al. (2003), some properties are modified with the presence and absence of curcuminoids, the pigments responsible for the color of turmeric. The percentage of unidentified compounds was approximately 8–10% for the turmeric and about 5% for the ginger. In spite of the relatively low level of purity (approximately 76 and 85% for turmeric and ginger, respectively), these starches can find use as special starches in industry due to their soft smell, flavor and color (turmeric). These special starches can be used in soup preparation, pasta, jellies (Calvo & Salvador, 2000) and even on biofilms with anti-oxidants characteristics, because of the presence of curcuminoids.

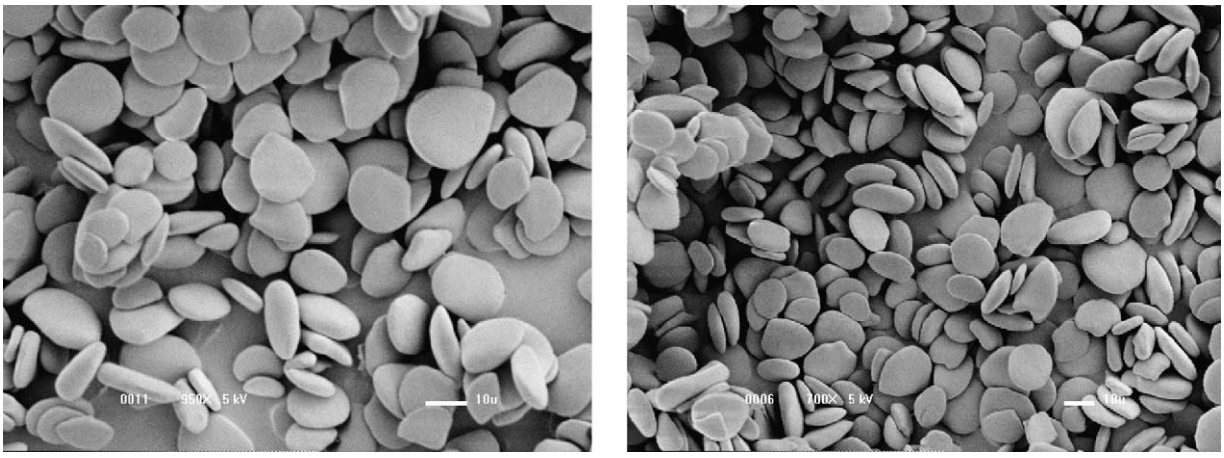
The Tukey test for data in Table 1 demonstrated that for the ash content of C_b and C_a, the differences were not statistically significant (5%) while for the ginger samples (Z_b and Z_a) the differences were statistically significant; the differences in ash content of ginger and turmeric samples were statistically significant. The differences of the starch content in turmeric and ginger samples before and after SFE were not statistically significant (5%), while between the species (turmeric and ginger) the differences were statistically significant. The differences in total protein, reducing sugars and the other analyzed components were not statistically significant.

Microscopy studies revealed that the SFE process using CO_2 and cosolvents (isopropyl alcohol or the mixture 1:1 of ethanol/isopropyl alcohol) just mixed the cellular structures of turmeric and ginger particles after the SFE process, and did not alter the surface and morphology of the granules (Figs. 1 and 2). Jyothi et al. (2003) got average granules size of 33 μm for *Curcuma zedoaria* and *Curcuma malabarica* starches. Potato starch granules may be as large as 100 μm along the major axis (Whistler & BeMiller, 1999). The ginger starch



C_b (900x) C_a (850x)

Fig. 1. *Curcuma longa* starches before (C_b) and after (C_a) SFE process analyzed by SEM.



Z_b (950x) Z_a (700x)

Fig. 2. *Zingiber officinale* starches before (Z_b) and after (Z_a) SFE process analyzed by SEM.

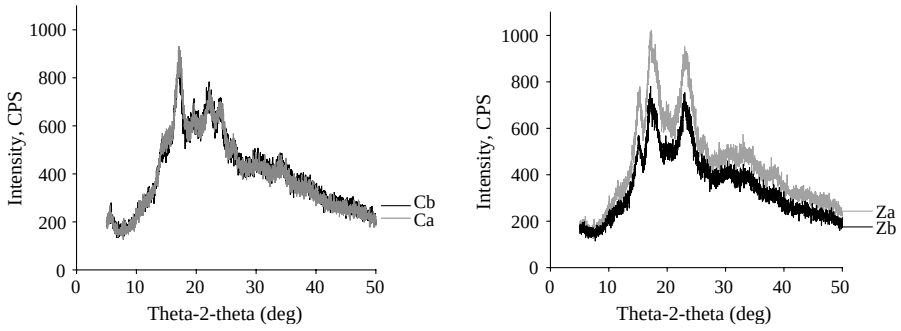


Fig. 3. Diffractograms of *C. longa* L. and *Z. officinale* R. starches (before SFE process: C_b and Z_b; after SFE process: C_a and Z_a).

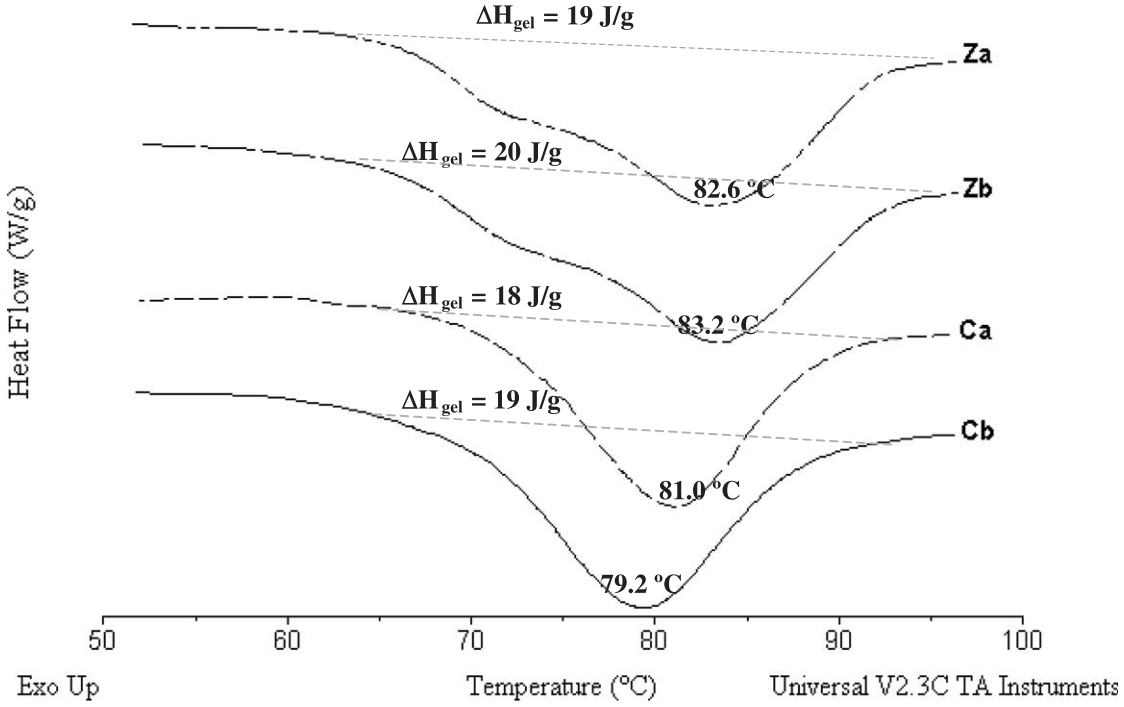


Fig. 4. DSC gelatinization curves of *C. longa* L. and *Z. officinale* R. starches (before SFE process: C_b and Z_b; after SFE process: C_a and Z_a).

Table 2
Starch gelatinization enthalpy of *Curcuma longa* Linneu (C) and *Zingiber officinale* Roscoe (Z) obtained by DSC

Sample	T_o (°C)	T_{peak} (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH_{gel} (J/g)[a]
C_b	69.3±0.1	79.2±0.2	96±1	27±1	19±1
C_a	70.8±0.4	81.0±0.2	97±1	26±1	18±1
Z_b	69±2	83.24±0.05	99±1	30±3	20±1
Z_a	66.3±0.4	82.6±0.5	96±1	30±1	19±1

The subscripts b and a means before and after SFE, respectively, T_o, onset temperature; T_{peak}, peak temperature; T_c, conclusion temperature; ΔH_{gel}, entalphy of gelatinization.
[a] dry basis.

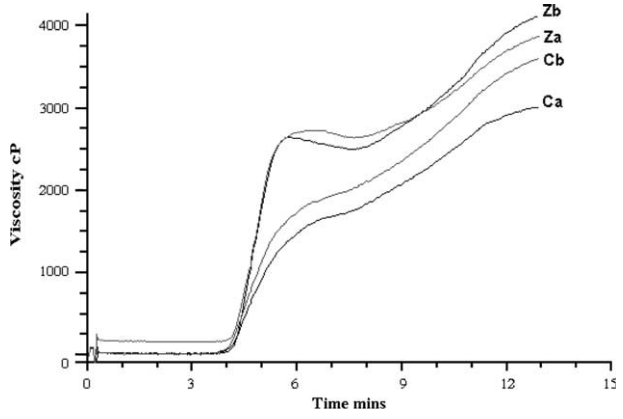


Fig. 5. Viscosity of *C. longa* L. and *Z. officinale* R. starches (before SFE process: C_b and Z_b; after SFE process: C_a and Z_a).

granules have different dimensions from those of turmeric and their morphological differences are very well defined: ginger granules are spherical (10–28 μm along the major axis) and turmeric granules are elliptical (10–33 μm along the major axis). The surface of a turmeric starch granule showed fissures in C_b sample (Fig. 1); this behavior has been reported to be indicative of a strong bond between the starch and the protein matrix (Jyothi et al., 2003).

Fig. 3 shows the X-ray patterns of the samples. Ginger starch was identified as C-type, similar to Ginko biloba and Manihot esculenta starches (Gunaratne & Hoover, 2002), and the turmeric starches as B-type, such as ordinary potato (Solanum tuberosum) (Hoover & Vasanthan, 1994).

The samples C_b and C_a presented similar peaks at 17, 22 and 24° at 2θ with slight difference on intensity, what reflects that the SFE process altered minimally the physical structure of the starch macromolecule. The maximum intensity was 926 cps for C_a. The peaks of the ginger samples were at 15, 17 and 23° with the maximum intensity at 1015 cps for Z_a, but the intensities between the samples showed differences that varied from 200 to 240 cps; these differences cannot be attributed to X-ray patterns or to amylopectin content. Probably, these differences are due to the manner in which double helices are arranged within the crystalline granule regions, indicating that the SFE process caused the disarrangement of the granule double helices.

Fig. 4 and Table 2 shows the gelatinization behavior for both turmeric and ginger starches samples. The differences in peak temperature ($T_{peak,}$ °C) (Table 2) between $C_b - C_a$ and $Z_b - Z_a$ were not statistically significant (5%), this is also true for the differences among turmeric and ginger samples. The values of T_{peak} for ginger were higher than that for turmeric. The onset temperature (T_o, °C) of turmeric samples were lower than the values of literature for *C. zedoaria* (79.7 °C), but T_{peak} and T_c were similar (Jyothi et al., 2003). The differences among the gelatinization enthalpy data (Table 2) of all samples were not statistically significant (5%) by Tukey test. All samples showed higher values than that for potato starch published by Hoover and Vasanthan (1994) (T_{peak} = 58.8 °C and ΔH = 16.8 J/g) as well as for the cassava starch (T_{peak} = 71.5 °C and ΔH = 12.3 J/g) (Gunaratne & Hoover, 2002) (The enthalpy was similar to the values for *C. zedoaria* (Jyothi et al., 2003). Aggarwal, Singh, Kamboj, and Brar (2004) concluded that the differences of gelatinization enthalpy values for pea starches maybe attributed to lengthy chains of amylopectin; then, longer chains require much higher temperature to break them. Although the amylopectin content values have showed statistical difference for both species, the gelatinization enthalpy values are similar and then, probably both species have similar chain lengths of amylopectin. According to literature, tuber starches need higher temperatures to break the structures and high enthalpy to gelatinize (Aggarwal et al., 2004; Hizukuri, 2004).

The viscography characteristics of starches can be observed in Fig. 5 and Table 3. The pasting temperature, the peak time and the viscosity peak of turmeric starch were similar to *C. zedoaria* studied by Jyothi et al. (2003), although C_a had a decreasing of the viscosity peak value. The difference of the viscosity peak between C_b and C_a were statistically significant. The breakdown in viscosity of pasting curves could be occurring, and the reduction rate depends on the temperature

Table 3
Viscosity parameters of *Curcuma longa* L. (C) and *Zingiber officinale* R. (Z) starches

Sample	Pasting temperature (°C)	Peak time (min)	Viscosity peak (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
C_b	85	7	1951	199	3631	1879
C_a	86	7	1632	116	3045	1529
Z_b	86.6	5.9	2650	148	4175	1673
Z_a	86.5	6.0	2769	172	4060	1463

The subscripts b and a means before and after SFE, respectively.

Table 4
Swelling factor, amylose and amylopectin contents of *Curcuma longa* L. (C) and *Zingiber officinale* R. (Z) starches

Sample	Amylose (%)	Amylopectin (%)	Swelling factor	Turbidity (ABS)
C_b	48±3	52±3	2.3±0.2	2.93±0.03
C_a	48±3	52±3	2.11±0.04	2.43±0.01
Z_b	34±2	66±2	4.45±0.04	2.20±0.02
Z_a	34±±1	66±1	4.41±0.02	2.25±0.04

The subscripts b and a means before and after SFE, respectively.

and degree of mixing or shear applied to the mixture, and the nature of the material itself. The lower value of the breakdown indicates the higher resistance and cross-linked linkages of the starch. The turmeric breakdown value decreased after SFE from 199 to 116 cP and ginger breakdown value increased from 148 to 172, ginger starch became slightly less resistant. The setback, that involves retrogradation or reordering of the starch molecules, was reduced after SFE for both ginger and turmeric. The viscosity peak of the turmeric starches was closer to that of maize starch (∼2100 cP) and lower than that of ginkgo biloba starch (∼4200 cP) (Spence & Jane, 1999), and much lower than that of potato starch (∼4680 cP) (Erlingen, Jacobs, Block, & Delcour, 1997).

The swelling factor values are showed in Table 4. The data showed no difference after the SFE process for both species, however, the ginger swelling factor was higher than that of turmeric, this behavior is probably due to the fact that ginger starch pattern is C-type (as shown by X-ray, Fig. 3), which have higher water contents.

The estimated contents of amylose and amylopectin present in the ginger and turmeric starches are presented in Table 4. The difference between turmeric and ginger starches was statistically significant (5%), but the SFE process did not alter the amylopectin content for both species. The ginger starch had higher amylopectin content, thus higher crystallinity. *C. malabarica* and *C. zedoaria* have 25 and 27% of apparent amylose content, respectively (Jyothi et al., 2003), thus, the amylose content of *C. longa* was higher than other curcuma species in literature. Amylose content of potato starch varies from 23 to 31% for normal potato genotypes (Kim, Wiesenborn, Orr, & Grant, 1995).

Turbidity values of ginger and turmeric starches (Table 4) differed significantly, but the SFE process did not alter the starches turbidity. Turmeric starch turbidity was higher than *C. zedoaria* and *C. malabarica*. These values were approximately equal to that of pea (Aggarwal et al., 2004).

Robyt, Choe, Fox, Hahn, and Fuchs (1996) studied the acid modification of starch in alcohols (methanol, ethanol, 2-propanol, butanol) and observed a decreasing of the degree of polymerization of the limit dextrins as the alcohols content increased. They propose that these alcohols or combination of alcohols exposed and made available different types of linkages in starch granule to acid hydrolysis; this involves the conversion of the crystalline regions in the granule into amorphous regions. These acid susceptible linkages are dependent on the type of alcohols and their relative ratios, the temperature, starch type and the acid concentration. The acid modification of starch in presence of alcohols (ethanol and 2-propanol) at 25 °C, showed the formation of series of limit dextrins formed in 12 h, with alcohol mixtures at 0–100% (v/v), 0.36% (wt/v) HCl and starch/alcohol ratio 1:4 resulting 245–235° of polymerization from potato starch. The amount of cosolvents used in the SFE process was low (alcohol 5:1 wt/wt for ginger and alcohol 1:1 w/w for turmeric) and the processing time was very short (200 min for ginger and 375 min for turmeric). Therefore, it is possible that the cosolvent imparted a small modification in the starch granules.

4. Conclusions

The pressure applied on SFE step and co-solvent ratio did not alter morphologically the starch granules, observed by SEM, although the starch structure was physically altered as demonstrated by X-ray (variation of peak intensities). A rearrangement of the starch molecules with slight variation of the starch behavior was observed; it was also revealed by setback value in viscosity analyze, but the starches did not suffer strong modifications under the pressure of 250 and 300 bar. The turmeric starch (B-type) demonstrated more resistance under the pressure than ginger starch (C-type), in spite of its lower amylopectin content (52%) as compared to ginger starch (66%). The curcuminoids in the turmeric starch, which give a yellow color, can be a differential factor for its utilization as starch with special characteristics. These results revealed that *C. longa* Linneu and *Z. officinale* Roscoe starches have similar characteristics with other in literature, and consequently high potential for using of the SFE starchy residues for several industrial processes.

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