Brazilian Journal of Chemical Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 23, No. 02, pp. 235 - 242 April - June, 2006

GINGER AND TURMERIC STARCHES HYDROLYSIS USING SUBCRITICAL WATER + CO_2: THE EFFECT OF THE SFE PRE-TREATMENT

S. R. M. Moreschi, J. C. Leal, M. E. M. Braga and M. A. A. Meireles*

LASEFI - DEA / FEA (College of Food Engineering), UNICAMP (State University of Campinas),
Phone: +(55) (19) 3788-4033, Fax: +(55) (19) 3788-4027,
C. P. 6121, 13083-862, Campinas, SP, Brazil
E-mail: meireles@fea.unicamp.br

(Received: October 20, 2004 ; Accepted: February 14, 2006)

Abstract - In this work, the hydrolysis of fresh and dried turmeric *(Curcuma longa* L.) and ginger *(Zingiber officinale R.*) in the presence of subcritical water + CO_2 was studied. The hydrolysis of ginger and turmeric bagasses from supercritical fluid extraction was also studied. The reactions were done using subcritical water and CO_2 at 150 bar, 200 °C and reaction time of 11 minutes; the degree of reaction was monitored through the amount of starch hydrolyzed. Process yields were calculated using the amount of reducing and total sugars formed. The effects of supercritical fluid extraction in the starchy structures were observed by scanning electron microscopy. Higher degree of hydrolysis (97- 98 %) were obtained for fresh materials and the highest total sugar yield (74%) was established for ginger bagasse. The supercritical fluid extraction did not significantly modify the degree of hydrolysis in the tested conditions.
Keywords: Ginger; Subcritical water; Hydrolysis; Supercritical fluid extraction; Turmeric.

INTRODUCTION

Lately, it has been observed an increasing interest in using super and subcritical water as reaction medium because of its reactivity. Using water satisfies today's pursuit for "green" transformation processes, i.e., beneficial to the environment. Subcritical water (150 < T < 370 °C, 4 < P < 220 bar) can act as a basic or acid catalyst. Subcritical water has being used by itself or with other reactants such as CO2 (Siskin, 2000).

Ginger (Zingiber officinale Roscoe) and turmeric (Curcuma longa L.) are widely used in cooking and phytotherapy because of their volatile oils and oleoresins (Zancan et al., 2002; Braga et al., 2003). These rhizomes have a considerable amount of starch (30 - 40%, dry basis) that can be used as substrate for hydrolysis reactions to obtain new molecules from oligosaccharides to glucose or even smaller molecules (Moreschi et al., 2004). Starch is not soluble in supercritical CO2, therefore, the starch content of the raw material should not be modified by the supercritical fluid extraction (SFE).

Hydrolysis of ginger and turmeric bagasses using subcritical water with or without CO2 can benefit from the effects of the SFE process. SFE can act as a pre-treatment to the hydrolysis because the high pressure process loosens the starch (Zheng et al, 1995). Additionally, CO2 contributes to the decrease of the pH, thus, increasing hydrolysis reaction rate. Hydrolysis with pressurized water, also known as hydrothermolysis, autothermolysis

*To whom correspondence should be addressed

and autohydrolysis, uses hydron ion as catalyst, which is supplied, initially, by the water autoionization, and then by the acids generated by the reaction. This reaction has a complex mechanism that involves glucose degradation products (Krammer and Vogel, 2000).

The starch macromolecule is formed by two polysaccharides: amylose and amylopectin. The majority of the starches consist of approximately 75% of semi-crystalline amylopectin and 25% of amorphous amylose. Amylose is a linear chain polymer formed by several hundreds of glucose units connected by alpha-D-(1→4) links (BeMiller, 1984). Amylopectin is a ramified polymer containing from hundreds of thousands to millions of units of D-glucose joined by alpha-D (1→4) and alpha-D-(1→6) links. The acid hydrolysis of starch produces D-glucose and it's degradation products, like 5-hydroxylmethyl furfural (5-HMF), levulinic acid, and formic acid; 5-HMF is the precursor of the last two substances (Theander and Nelson, 1988). The degree of the acid hydrolysis of starch depends on: (i) the effect of the acid to rupture the starch granule, (ii) the degree of hydrolysis of each starch component (amylose and amylopectin) that vary with the starch nature, (iii) the degree of hydrolysis of the polysaccharide itself, which is a function of the physical distribution of the amylose and amylopectin structures (van Beynum, 1985). Partial starch hydrolysis produces maltdextrins (DE < 20), nutritive saccharide polymers, non-sweet substances used as thickening agents, texturizers, auxiliary substances for dry powders, fat substitutes, imitation cheese, sauces, freezing and film production control agents, substances used to prevent crystallization, and nutritional additives.

Hydrothermolysis of materials with aromatizing properties, such as ginger and turmeric, can result in products with special colors and aroma characteristics, presenting potential for applications in a variety of food formulations, besides, their hydrolysis products can be used as substrates for several enzymatic processes. Moreschi et al. (2004) studied the hydrolysis of ginger bagasse produced by SFE, a process that can be employed for other starchy or cellulosic raw materials such as the biomass from sugar cane industry, increasing the aggregated value of these products.

The cost of manufacturing (COM) of some SFE extracts, such as ginger, may not be attractive unless, an application, which uses the bagasse produced, can be found. The use of ginger SFE bagasses in subcritical hydrolysis would promote the decrease in the COM of the SFE ginger oleoresin. Rosa and Meireles (2004) analyzed this cost and considered the SFE waste treatment cost negligible once the ginger bagasse was used as raw material to produce oligosaccharides and other lower molecular mass substances with subcritical water (Moreschi et al., 2004). Ginger oleoresin produced by SFE contains high purity nutraceuticals and the utilization of starchy bagasse for hydrolysis can reduce the COM (US$99.80/kg), which is similar to its selling price (Rosa and Meireles, 2004).

Therefore, the objectives of this work were to study the hydrolysis of starchy materials such as ginger and turmeric (fresh, dried and SFE bagasse) and to compare the influence of the drying process as well as the influence of the SFE step in the degree of hydrolysis.

MATERIALS AND METHODS

Raw Material Identification and Characterization

Ginger (Zingiber officinale Roscoe) was obtained in the municipal market of Campinas (state of São Paulo) and turmeric (Curcuma longa L.) was supplied by a producer located in Maringá (state of Paraná). The fresh ginger and turmeric were triturated in a domestic food processor (Wallita, model Master, São Paulo, SP) for 5 s, and their average particles sizes were calculated considering the particle as an ellipse. In the present work, these materials are called fresh ginger and turmeric.

A part of the triturated ginger and turmeric was dried in an oven (Fanem, model 320-SE, São Paulo, SP) at 32 °C; comminuted in a knife mill (Tecnal, model TE 87631, Piracicaba, SP) and classified using an agitator (Bertel, Model 1868, Caieiras, SP), for 10 min, containing sieves of the Tyler series. Equal amounts of particles mesh sizes 22, 32, and 48 were used in the assays. These materials, denoted as dried ginger and turmeric, were packed in plastic bags and kept in a freezer at –5 °C.

The SFE assays were done in a SFE unit containing an extraction cell of 221 × 10-6 m3 (length of 37.5 × 10-2 m, internal diameter of 2.74 × 10-2 m) described by Pasquel et al. (2000). The extraction procedure was as described by Braga et al. (2003). The ginger bagasse was obtained at 250 bar and 35 °C using CO2 (99.0% purity, Gama, S.S.

ONU 1013, Campinas, SP) as solvent and isopropyl alcohol (Merck, lot K 27434734, Germany) as cosolvent (1.17% v/v). Turmeric bagasse was obtained at 300 bar, 30 °C and a mixture (1:1, v/v) of ethanol (Merck, lot K 32173883325, Germany) and isopropyl alcohol (Merck, lot K 27434734, Germany) was used as cosolvent (10% v/v). These conditions were chosen based on the results of Zancan et al. (2002) and Braga et al. (2003), respectively. After the SFE, the ginger and turmeric bagasses were packed in plastic bags and kept in a freezer at –5 °C. The residues of SFE (denoted as ginger or turmeric bagasses) were used as hydrolysis substrates.

The substrates of hydrolysis were characterized with respect to humidity (Jacobs, 1981), reducing sugars (Nelson, 1944), total sugars (Miller, 1958) and starch content (Method Nº 32.2.05, AOAC, 1997). The amounts of oleoresin present in the rhizomes were estimated as the global yield obtained by SFE.

Experimental Procedure of the Hydrolysis Tests

The hydrolysis tests were made using a Speed SFE unit (Applied Separations, Inc., Model 7071, Allentown, USA) and a 5 mL reactor (Thar Designs, Inc., Pittsburgh, USA) as described by Moreschi et al. (2004): The reactor was filled with substrate for the fresh ginger and turmeric, and with a mixture of substrate and distilled water (3:7, mass) for dried ginger and turmeric as well as for ginger and turmeric bagasses. The reactor was assembled in the SFE unit oven. Keeping the inlet and outlet valves closed, the heating system was turned on, and when the desired temperature was reached, the system was pressurized to 150 bar by simultaneously turning on the CO2-pump and opening the inlet CO2-valve. CO2 of purity 99.0% (Gama, S.S. ONU 1013, Campinas, SP) was used. It took 39 ± 2 min to heat up the system. The reactor was kept still at the desired temperature and pressure for 11 minutes. Afterwards, the outlet CO2-valve was opened and the reaction products were collected. The oven was opened and an external fan helped cooling the system. The process losses, that is, the mass losses of the hydrolysis process due to leakages, dragging of liquid and gaseous products by the CO2-stream, etc, were kept below 10 %. After each assay, the process losses were calculated. If the losses were above 10%, the assay was discharged and a new one was performed. Losses below 10% do not interfere in the conclusions related to the effects of the operating variables in the response variables (degree of hydrolysis, reducing sugars, and total sugars) (Moreschi et al., 2004). All hydrolysis experiments were made in triplicate.

Characterization of The Reaction Products and Residues

The reaction products were characterized with respect to the quantities of reducing (Nelson, 1944) and total (Miller, 1958) sugars; pH (AOAC, 1997) (Method 32.1.20). The reaction residues were characterized with respect to the amounts of starch (AOAC, 1997), reducing (Nelson, 1944) and total (Miller, 1958) sugars, and with respect to the humidity (AOAC, 1997) (Method 4.1.03). Analyses were made in triplicate.

Scanning Electron Microscopy

The vegetal structures and the granule morphologies were examined using scanning electron microscopy (SEM). Samples were applied on circular aluminium stub with double sticky tape and the sample was coated with 24nm of gold. The micrograph was obtained by SEM (Jeol, model SM 5800 LV, Tokyo, Japan) and accelerating potential of 15 kV. This analysis was made in Laboratory of Electronic Microscopy of the Biology Institute, Unicamp.

Calculation Procedure

The overall extraction curves (OEC) were fitted to a spline using two straight lines accordingly to Rodrigues et al. (2003). The procedures PROC REG and PROC NLIN of SAS 6.12 (Freund and Littell, 1995) were used. From the spline, the extraction rate for the constant extraction rate period (MCER) was calculated, as well as the length of the CER period, which is time corresponding to the interception of the two lines (tCER). The mass ratio of solute in the supercritical phase at the extraction cell outlet (YCER) was obtained dividing MCER by the mean solvent flow rate (QCO2) for the CER period. The yield corresponding to the CER period was denoted as RCER. The global yields were estimated as the yields obtained at the end of the SFE assays. The extraction degrees were calculated as:

$$\text{Extraction Degree} = \frac{m_{extrat}(t)}{m_{extract}(t \to \infty)} \times 100 \quad (1)$$

where:

mextract(t) is the mass of extract from time t=0 to time t;
mextract(t→∞) is the mass of extract from time t=0 to time t=∞.

The dried material was the basis of the mass balance for the hydrolysis process. The process losses (L, %) were evaluated using the following equation:

$$L = \left[1 - \frac{(m_P + m_W)}{(m_B + m_A)}\right] \times 100 \quad (2)$$

where mA is the mass of water in the feed, mB is the mass of substrate in the feed, mP is the mass of products, and mw is the mass of reaction residue or unreacted material.

The degree of hydrolysis (X, wt %.) or the starch conversion was defined as:

$$X = \frac{St_B - St_W}{St_B} \times 100 \quad (3)$$

where StB is the initial mass of starch in the feed and StW is the mass of starch in the unreacted material.

The reaction yield (yRS, wt %) was calculated with respect to reducing sugars formed as:

$$y_{RS} = \frac{RS_W + RS_P}{St_B} \times 100 \quad (4a)$$

where RSP is the mass of reducing sugar in the product stream, RSW is the mass of reducing sugar in the reaction residue.

In addition, with respect to total sugars formed (yTS, wt %) was calculated as:

$$y_{TS} = \frac{TS_W + TS_P}{St_B} \times 100 \quad (4b)$$

where TSP is the mass of total sugar in the product stream, TSW is the mass of total sugar in the reaction residue.

An analysis of variance was done to determine the effects of the type of substrate (ginger or turmeric) and the pretreatment (fresh, dried, SFE bagasse) on the degree of hydrolysis, total sugars yield, and reducing sugars yield. Minitab v. 12 was used.

RESULTS AND DISCUSSIONS

The average particle sizes of the triturated fresh ginger and turmeric particles were 1.2 ± 0.5 and 3.0 ± 0.6 mm, respectively, and their humidity content were 73 ± 2% and 62 ± 2%, respectively. Table 1 shows the compositions of the hydrolysis substrates. The starch content was slightly higher for turmeric than for ginger, and the observed increase for the SFE bagasses was due to oleoresin removal in the SFE step. Total sugars were approximately equal for ginger and turmeric, while the content in reducing sugars was higher for turmeric.

Figure 1 shows the OECs, in terms of the extraction degrees for ginger and turmeric. The OECs have the typical shape presenting the 3 periods: (1) constant extraction rate period (CER); falling rate period (FER), and diffusion controlled (DC) rate period. The behaviors of these curves were as reported in literature for these raw materials (Zancan et al., 2002; Braga et al., 2003), except that, in the present work, larger values for the global yields (Xo) were determined for both substrates (Table 1). Since the extraction conditions were the same of that of Braga et al (2003) and Zancan et al. (2002), the observed differences can be attributed to the origin of these material. Table 2 shows the kinetic parameters for ginger and turmeric OECs. The length of the CER period for the system ginger + CO2 + isopropyl alcohol was 64 min with 5.6 % of extract yield (representing about 70% of the global yield). For the system turmeric + CO2 + ethanol + isopropyl alcohol it was necessary 137 min to obtain 4.2 % of extract (representing 70% of the global yield). The YCER was higher for turmeric than for ginger. Considering that about 70% of the global yields for ginger and turmeric were obtained in approximately 1 hour and 2 h and 17 min, respectively, and that to extract the remaining 30% would require additional time of 1h and 30 and 4h for ginger and turmeric, respectively, it would not be advisable, from an economical point of view, continuing with extraction during the falling and diffusion-controlled rate periods. Rosa and Meireles (2004) observed that the extraction process should be stopped at a time equal or near the tCER and the SFE residues should be used as substrate of other processes. For instance, since the turmeric residue retained its yellowish color even after washing with NaOH (0.25%, 1:5wt/wt) and successive washing with water, turmeric bagasse could be added to starchy foods to impart color in addition to the use as substrate for hydrolysis reactions.

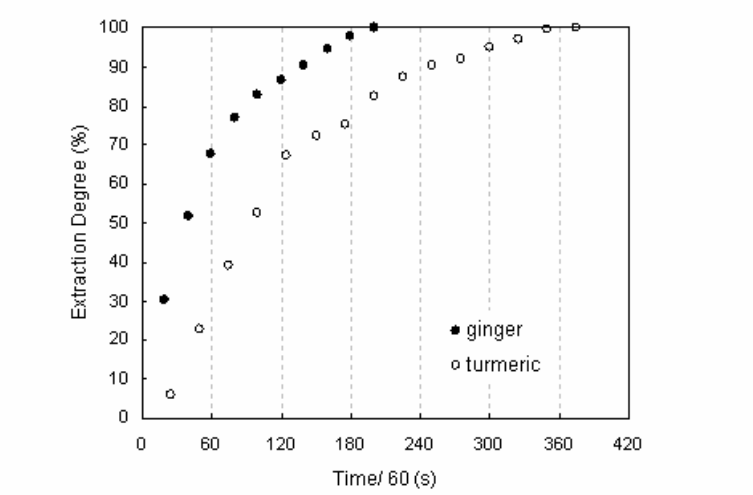

Figure 1: Extraction degree obtained by SFE process of ginger (•) at 250 bar and 35 ºC, 1.7% (v/v) of isopropyl alcohol as cosolvent; and turmeric (o) at 300 bar and 30 ºC, 10% (v/v) of ethanol/isopropyl alcohol (1:1) as cosolvent.

Table 1: Composition of the dried milled ginger and turmeric rhizomes and ginger and turmeric SFE bagasses

% (dry basis)	Dried ginger	Ginger SFE bagasse	Dried turmeric	Turmeric SFE bagasse
Starch	25 ± 3	29.4 ± 0.4	29.2 ±. 0.6	33 ± 2
Total sugar	1.15 ± 0.03	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.1
Reducing sugar	0.20 ± 0.02	0.21 ± 0.01	0.5 ± 0.1	0.4 ± 0.1
SFE Global yield	8.2	-	6.0	-
Humidity	4.4 ± 0.2	9.2 ± 0.2	8.2 ± 0.3	11.7 ± 0.1

Table 2: Kinetic Parameters of ginger and turmeric OECs obtained by SFE process

SFE substrate and conditions	M_{CER} (kg/s)×10^7	Q_{CO2} (kg/s)×10^5	Y_{CER} ×10^3	t_{CER} (s/60)	R_{CER} (%)	X_o (%)	Extraction time (min)
Ginger 250 bar, 35 ºC	3.5	7.4	4.7	64	5.6	8.2	200
Turmeric 300 bar, 30 ºC,	5.2	6.0	8.6	137	4.2	6.0	375

Table 3 shows the values of the degree of hydrolysis, total and reducing sugars yields. The pH measured in the reacting medium for the ginger substrates varied from 3.5 to 4.0. For ginger substrates, the fresh ginger showed the highest degree of hydrolysis. This behavior is probably a result of the interaction between the water and the starchy-cellulosic structure in the fresh material, which contributed for the improvement of hydrolysis. On the other hand, the differences in the degree of hydrolysis between the dried ginger and SFE ginger bagasse were not statistically significant (p_{value} = 0.486). As the degree of hydrolysis increased the reducing sugar yield decreased; this can be explained by the fact that the increase in the hydrolysis rate increased the reducing sugar yield and consequently its degradation rate. The difference in total sugar yields between fresh and dried ginger substrates was not statistically significant (p_{value} = 0.349). In Table 3, fewer results were shown for turmeric substrates, due to the impossibility of performing the assays in triplicates keeping the losses bellow 10%; therefore, these results were not reported. The explanations found for the turmeric substrates behavior were connected to the larger degree of hydrolysis as compared to the ginger substrates. Larger degree of hydrolysis would result in larger amounts of gaseous products, which were not quantified in the present work. The pH of the reacting medium was near to 3.0 for the turmeric substrates, and, at 200 ^oC it was impossible to collect products at the SFE system outlet: the reaction products obstructed the tubing lines with products resembling caramelized sucrose. Considering that the temperature of 200 ^oC could be very high for hydrolyzing turmeric bagasse, hydrolysis reactions were done at lower temperatures of 180, 150, and 130 ^oC. At 180 ^oC, the behavior of the system was

the same as that observed at 200 °C. The degrees of hydrolysis (duplicates) quantified at 150 °C and 130 °C were 80% and 76%, respectively (Table 3); nonetheless, the reducing and total sugars could not be quantified. The difference in reducing sugars yields between dried turmeric and its SFE bagasse was not statistically significant (p_{value} = 0.141). The larger total sugars yield can be a consequence of the availability of polysaccharides, other than starch resulting of the physical-chemical modifications of the starchy structure that might occur during the SFE process.

Table 3: Degree of hydrolysis (X %), total sugar yield (y_{TS} %) and reducing sugar yield (y_{RS} %) at 200 °C, 150 bar and 11 min reaction time

Substrate of hydrolysis	Degree of hydrolysis X (%)	TS Yield y_{TS} (%)	RS Yield y_{RS} (%)
Fresh ginger	97 ± 1	46 ± 2	4.1 ± 0.1
Dried ginger	90.5 ± 0.1	43 ± 2	7.1 ± 0.4
Ginger bagasse	89 ± 2	74.2 ± 0.4	6.4 ± 0.2
Fresh turmeric	98	34	9.6
Dried turmeric	98	49	10.6
Turmeric bagasse (150 ºC)	80	nq	nq
Turmeric bagasse (130 ºC)	76	nq	nq

nq - not quantified

Scanning electronic microscopy permitted observing the ginger and turmeric substrate (dried) as well as the SFE bagasses. Figures 2-5 show the cellulosic walls and starch granules. Figures 3 and 5 show the disarrangement suffered by the cellulosic structure as the result of the pressure applied during the SFE process, and, at the same time, that the starch granules remained intact. Because, the starch granules are enclosed by the cellulosic structure, they did not suffer or suffered to a lesser extend the action of pressure. For the turmeric substrates, it is clearly seen, Figures 4-5, the smaller proportion of cellulosic walls; or, starch in the surface of the analyzed fragments of ginger was present in a larger quantity when compared to the amount of starch present in an equal amount of turmeric fragment analyzed. The dimensions and the morphology of both starch granules are well defined: ginger and turmeric starch granules have a spherical to ellipsoidal shapes (ginger: 10-28 mm for the larger axis; turmeric: 10-33 mm for the larger axis). Thus, the larger quantities of starch in turmeric (Table 1) and the stronger effects of pressure over its cellulosic-starchy structure could have contributed to the obstructions of the SFE unit tubing lines as well as the formation of caramelized sugars. And, also, the turmeric starch granules were apparently deformed to a larger extended than the ginger starch granules during the SFE process. Among the ginger substrates, the degrees of hydrolysis were smaller for the dried and SFE bagasse, indicating the difficulties encountered by the water to be re-incorporated to the starchy-cellulosic matrix. In spite of this, the total sugars yield was larger for SFE bagasse as compared to the fresh and dried substrates.

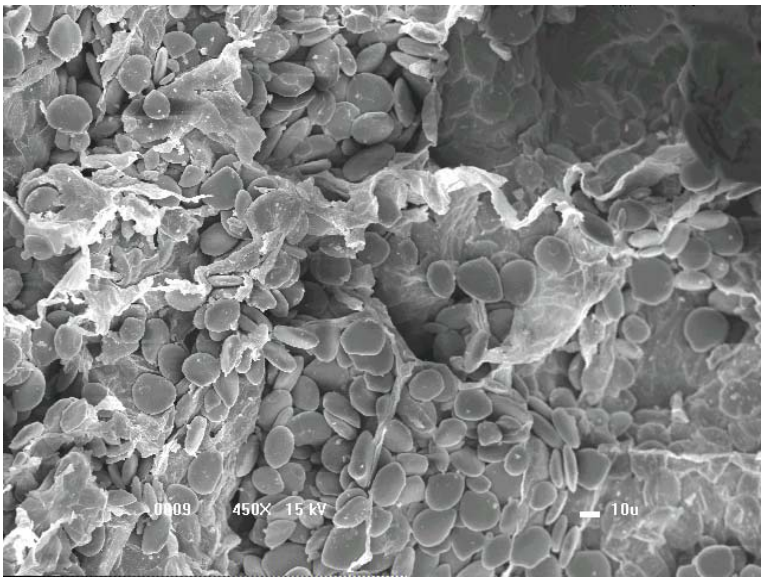

Figure 2: Starchy cellulosic structure in milled dried ginger by SEM 450×.

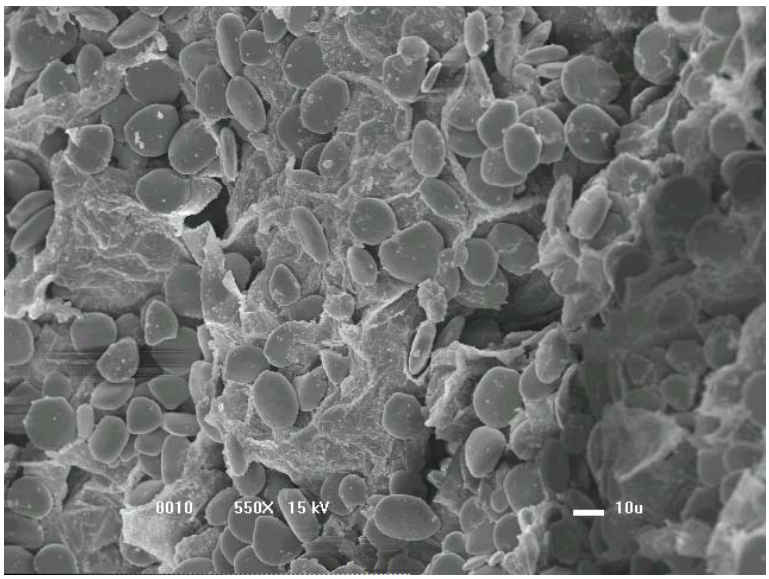

Figure 3: Starchy cellulosic structure in milled dried SFE ginger by SEM 550×.

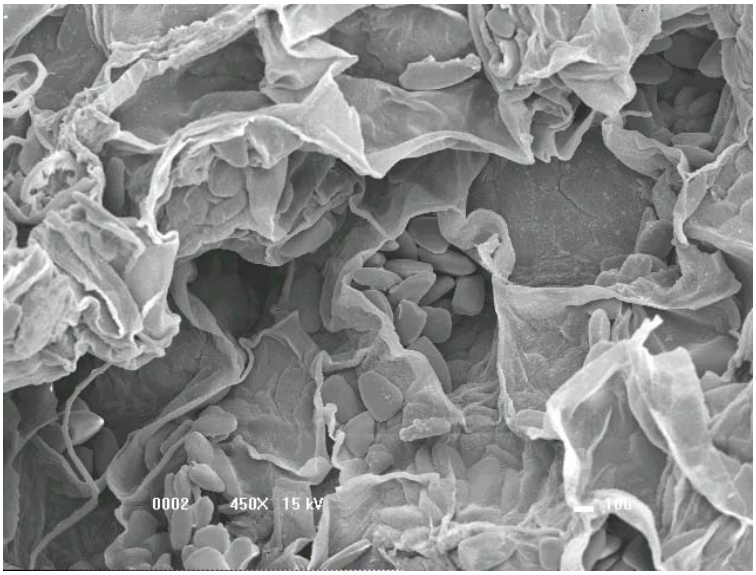

Figure 4: Starchy cellulosic structure in milled dried turmeric by SEM 450×

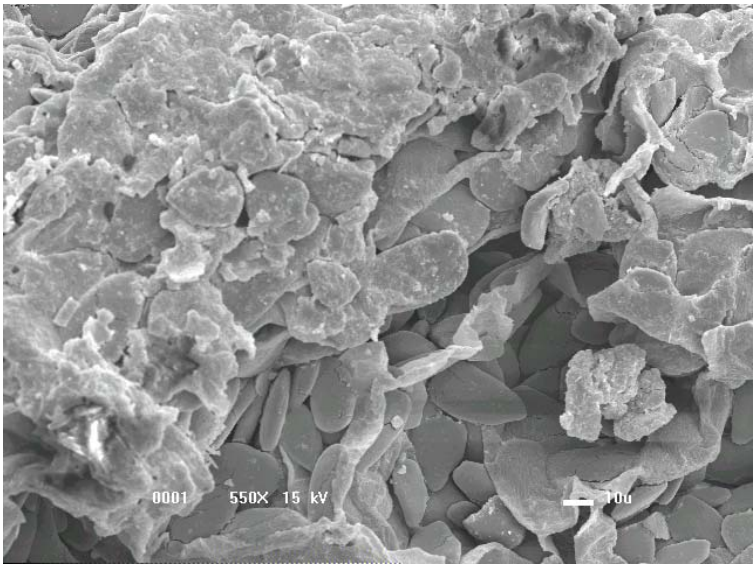

Figure 5: Starchy cellulosic structure in milled dried SFE turmeric bagasse by SEM 550×

CONCLUSIONS

Fresh substrates resulted in larger degree of hydrolysis, nonetheless, the use of these materials implicates in loosing the ginger and turmeric oleoresins, because the high temperatures required by the hydrolysis process would impart permanent damage to these thermo-labile mixtures. Although, SEM has shown that small alterations were observed in the surface and morphology of the starch granules, the cellulosic structure was disarranged. So, it seems a more appropriate process sequence to remove the oleoresin and use the ginger and turmeric SFE bagasses as hydrolysis substrates.

REFERENCES

AOAC, Association of Official Analytical Chemists, Cuniff, P., AOAC International, Gaithersburg, Maryland (1997).

BeMiller, J.N, Acid hydrolysis and other lytic reactions of starch in Whistler, R.L., BeMiller, J.N. and Paschall, E.F. Starch: chemistry and technology, Vol. 1, Academic Press, London, New York (1984).

Braga. M.E.M., Leal, P. F., Carvalho, J. E. and Meireles, M.A.A., Comparison of the yield, composition, and antioxidant activity of turmeric (*Curcuma longa* L.) extracts obtained using various techniques, J. Agric. Food Chem., 51, 6604, (2003).

Freud, R. J. and Littell, R. C. SAS system for regression. SAS series in statistical applications, 2nd ed.; SAS Institute: Cary, NC, 211, (1995).

Jacobs, M.B., The chemical analysis of foods and food products, Robert Krieger Publishing Co, New York, NY (1981).

Krammer, P and Vogel, H., Hydrolysis of esters in subcritical and supercritical water, J. Supercrit. Fluids, 16, 189, (2000).

Miller, G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analyt. Chem., 31, 426 (1958).

Moreschi, S.R.M., Petenate, A.J. and Meireles, M.A.A., Hydrolysis of ginger bagasse starch in subcritical water and carbon dioxide, J. Agric. Food Chem., 52, 1753 (2004).

Nelson, N.A., A photometric adaptation for Somogyi method for determination of glucose, J. Biol. Chem, 153, 375 (1944).

Pasquel, A., Meireles, M.A.A., Marques, M.O.M., Petenate, A.J., Extraction of stevia glycosides with CO_2 + ethanol and water and ethanol, Braz. J. Chem. Eng., 271 (2000)

Rosa,P.T.V. and Meireles, M.A.A., Rapid estimation of the manufacturing cost of extracts obtained by supercritical fluid extraction, J. Food Eng, available online (www.sciencedirect.com.), (2004).

Siskin, M., cited by Krammer,P and Vogel, H., Hydrolysis of esters in subcritical and supercritical water, J. Supercrit. Fluids, 16, 189 (2000).

Theander, O. and Nelson, D.A., Aqueous, high-temperature transformation of carbohydrate relative to utilization of biomass, Adv Carbohyd Chem. Bi., 46, 273 (1988).

Zancan, K. C., Marques, M.O.M., Petenate, A. J. and Meireles, M. A. A., Extraction of ginger (*Zingiber officinale* Roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant action of the extracts. J. Supercrit. Fluids, 24, 57 (2002).

Zheng.Y., Lin, H.M., Wen, J., Cao,N., Yu, X. and Tsao, G.T., Supercritical carbon dioxide explosion as a pretreatment for cellulose hydrolysis, Biotech. Letters, 17, 845 (1995).