

# HYDROLYSIS OF GINGER (*ZINGIBER OFFICINALE* ROSCOE) STARCH: SEARCH FOR PROCESS CONDITIONS USING THE SURFACE RESPONSE METHODOLOGY

Silvânia R. M. Moreschi<sup>1</sup>, Ademir J. Petenate<sup>2</sup>, M. Angela A. Meireles<sup>1,\*</sup>

<sup>1</sup>LASEFI - DEA / FEA (College of Food Eng) – UNICAMP, Cx. P. 6121, 13083-970 Campinas, São Paulo, Brazil; <sup>2</sup>Department of Statistics / IMECC – UNICAMP, Cx. P. 6065, 13083-970, Campinas, São Paulo, Brazil

[meireles@fea.unicamp.br](mailto:meireles@fea.unicamp.br); phone: 55 19 37884033; fax: 55 19 37884027

The use of subcritical water hydrolysis can considerably decrease the reaction time for several systems. Ginger (*Zingiber officinale*) besides being a source of volatile oil and oleoresin has as much as 40% of starch. In the present work subcritical water was used to hydrolyze the ginger starch after the solid matrix was subjected to SFE using CO<sub>2</sub> and mixtures of CO<sub>2</sub> + ethanol or CO<sub>2</sub> + isopropyl alcohol. Because no information related to this system ginger starch + H<sub>2</sub>O + CO<sub>2</sub> was found in literature, the surface response methodology was used to identify the best operation temperature and pressure. The reactions were carried out in a Spe-ed unit; the reactor mixture consisted of ginger bagasse with 30 % of water (mass); the reaction time was held constant at 15 minutes. The search for the best temperature and pressure started using a factorial experimental with 2 levels of pressure (100 and 200 bar), two levels of temperature (140 and 180°C) and a central point (150 bar and 160°C); the assays were made in triplicates and quintuplicate at the central point; the axial points were done at 132 and 188°C and 80 and 220 bar. The reaction products were characterized with respect to the content of starch and reducing sugar. The maximum amount of reducing sugar was detected at 188°C and 150 bar while the largest amount of hydrolyzed starch was obtained at 180°C and 200 bar. These results indicate that part of the reducing sugar is being transformed to other reaction products such as furfural compounds, acids and gases.

Keywords: *Zingiber officinale*, ginger bagasse, hydrolysis, subcritical water, SFE

## INTRODUCTION

Lately, it has being observed an increasing interest in using super and sub-critical water as reaction medium because of its reactivity. Sub-critical water (150 < T < 370 °C, 4 < P < 220 bar) can act as a basic or acid catalyst. Sub-critical water has being used by itself or with other reactants such as CO<sub>2</sub> [1].

Ginger (*Zingiber officinale* Roscoe) is widely used in cooking and phytotherapy because of its volatile oil and oleoresin. Nonetheless, ginger has a considerable amount of starch (up to 40%, dry basis) with potential applications, thus, the residue from SFE of ginger or the ginger bagasse can be used as a source of starch and also as substrate for hydrolysis reactions to obtain new molecules from oligosaccharides to glucose or even smaller molecules. Hydrolyzes of ginger bagasse using sub-critical water with or without CO<sub>2</sub> can be favored by the treatment of the material suffer during the extraction process [2] [3].

The starch macromolecule is formed by two polysaccharides: amylose and

amylopectin. The majority of the starches consist of 75% of amylopectin semi-crystalline and 25% of the amorphous amylose. Amylose is a linear chain polymer formed by several hundreds of glucose units connected by alpha-D-(1→4) links. The amylopectin is a ramified polymer containing some hundreds of thousands to some millions of units of D-glucose joined by alpha-D (1→4) e alpha-D-(1→6) links [4]. The acid hydrolysis of starch produce D-glucose besides the D-glucose degradation products: 5-hydroxymethyl furfural (5-HMF), levulinic acid, and formic acid; the 5-HMF is the precursor of the last two substances [5]. The rate of the starch acid hydrolysis depends on: (i) the effect of the acid to rupture the starch granule, (ii) the rate of hydrolysis of each starch component (amylose and amylopectin) that vary with the starch nature, and (iii) the rate of hydrolysis of the polysaccharide itself, which is a function of the physical distribution of the amylose and amylopectin structures [6].

In the present work subcritical water in the presence of CO<sub>2</sub> was used to hydrolyze the starch of ginger bagasse after the solid matrix was subjected to SFE using CO<sub>2</sub> and mixtures of CO<sub>2</sub> + ethanol or CO<sub>2</sub> + isopropyl alcohol.

## MATERIALS AND METHODS

### Raw material identification and characterization

The ginger bagasses used on this work were from Zancan et al [7]. The experimental runs were performed using 03 lots ginger bagasse from distinctive SFE assays as indicated in Table 1. The bagasse was characterized with respect to its centesimal composition including: humidity [8], starch content [9], [10], [11], lipids [12], ashes [13], proteins [14], fibers [15] and reducing sugar [16], [17].

**Table 1: Identification and characterization of the ginger bagasse used in the hydrolysis studies; SFE performed at 10<sup>-5</sup> kg/s using equal amounts of particles sizes 16, 22, 32, and 48 mesh.**

Ginger bagasse code	SFE operating conditions	Solvent
KB	250 bar and 25 °C	CO <sub>2</sub> + Ethanol
KC	250 bar and 25 °C	CO <sub>2</sub> + Ethanol
KD	250 bar and 35 °C	CO <sub>2</sub> + Isopropyl alcohol

### Experimental procedure

The hydrolysis tests were done using a Speed SFE unit (Applied Separations, Inc., model 7071, Allentown, PA, USA); an 8 mL reactor (Thar Designs, Inc., Pittsburgh, PA, USA) was used. The reactor was filled with ginger bagasse and distilled water (3:7, mass) and assembled in the SFE unit oven. Keeping the inlet and outlet valves closed the heating system was turned on, when the desired temperature was reached the system was pressurized by opening the inlet CO<sub>2</sub> valve and CO<sub>2</sub> was admitted into the reactor up to reach the operating pressure. The reactor was kept still during 15 minutes, without CO<sub>2</sub> draining, and then the reaction products were withdrawn from the reactor using a CO<sub>2</sub> flow rate of 3.83×10<sup>-3</sup> kg/s. The process losses were kept above 10%. And it indicates the mass loss of the hydrolysis process and it can be due leakages, dragging of liquid and gaseous products by CO<sub>2</sub>, etc.

The surface response methodology was used to search for the conditions of temperature and pressure that maximizes the starch hydrolysis without producing to many degradation products. The first set of assays was done using a factorial design with 2 levels of pressure (100 and 200 bar), two levels of temperature (140 and 180°C) and a central point (150 bar and 160°C); the assays were made in triplicates and quintuplicate at the central point. The assays at the axial points were done at 132 and 188°C and 80 and 220 bar, again the assays were made in triplicates.

## Characterization of the reaction products

The reaction residues were characterized with respect to the amount of reducing sugar and starch. The reaction products were characterized with respect to the amount of reducing sugar. The amount of reducing sugar was determined by the Somogyi & Nelson method [16], [17] and the starch content by a modified AOAC method [9], [10], [11].

## Calculation procedure

The dry ginger bagasse was the basis for the mass balance for the process. The process losses were evaluated using the following equation:

$$\% \text{ Losses} = \left[ 1 - \frac{(m_p + m_w)}{(m_B + m_A)} \right] \times 100 \quad (1)$$

where:  $m_A$  is the mass of water in the feed,  $m_B$  is the mass of bagasse in the feed,  $m_p$  is the mass of products, and  $m_w$  is the mass of reaction residue or un-reacted material.

The reaction yield ( $y$ ) or amount of reducing sugar was calculated as:

$$y = \frac{RS_w + RS_p}{(St + RS)_B} \quad (2)$$

where:  $RS_p$  is the mass of reducing sugar in the product stream,  $RS_w$  is the mass of reducing sugar in the reaction residue,  $(St + RS)_B$  is the mass starch and reducing sugar in the ginger bagasse.

The amount of hydrolyzed starch defined as:

$$HS = \frac{St_B - St_w}{St_B} \times 100 \quad (3)$$

## Statistical analysis

The analysis of variance (ANOVA) and the Fitted Response Surface were done to analyze the influence of temperature and pressure in hydrolyzed starch contents, reducing sugar yield and process losses.

## RESULTS AND DISCUSSIONS

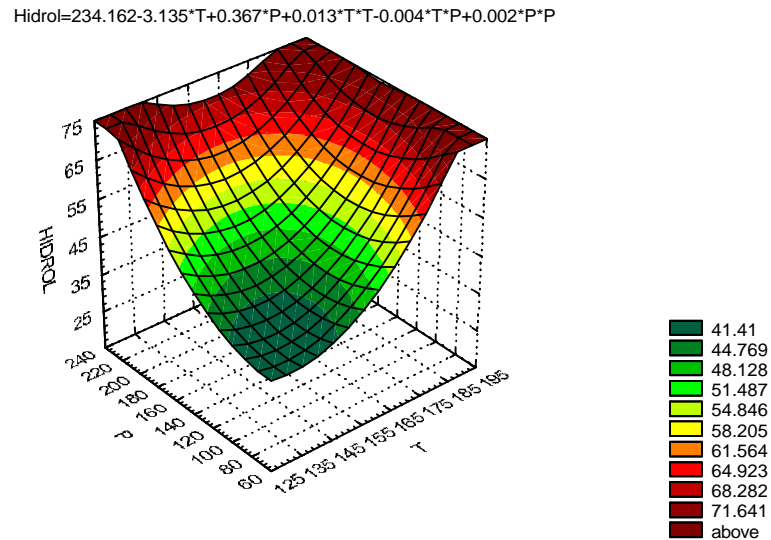
The compositions of ginger bagasse used in the hydrolysis reactions are given in Table 2. The starch and the reducing sugar contents were analyzed for each ginger bagasse lot, their variation were used as a measure of the extent of reaction.

**Table 2: Composition of Ginger Bagasse used in the Hydrolysis Reactions**

Dry basis (%)	Ginger bagasse			Ginger before SFE
	KB	KC	KD	
Starch	56.5	48.5	31.3	44
Reducing Sugar	6.1	5.2	5.3	5
Humidity	19	16	14.6	12
Fiber	3			5
Ash	5			6
Lipid	1			2
Protein	12			10

## Statistical Analysis

The influence of the temperature and pressure is visualized in the response surfaces in Figure 1.

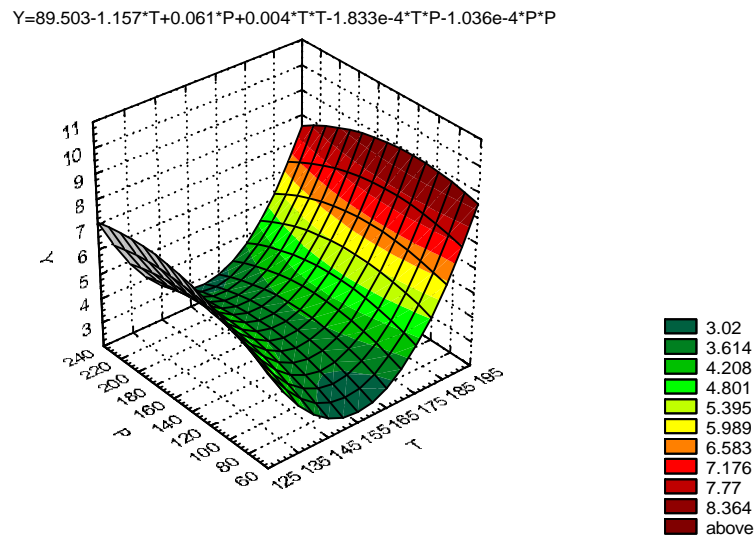


**Figure 1: Hydrolyzed starch response surface in ginger bagasse hydrolysis reaction**

The R-square for the fitted model was 50.1%. Both factors (temperature and pressure) were statistically significant.

### Yield (Y) (in reducing sugar)

The influence of the temperature and pressure is visualized in the response surfaces in Figure 2.

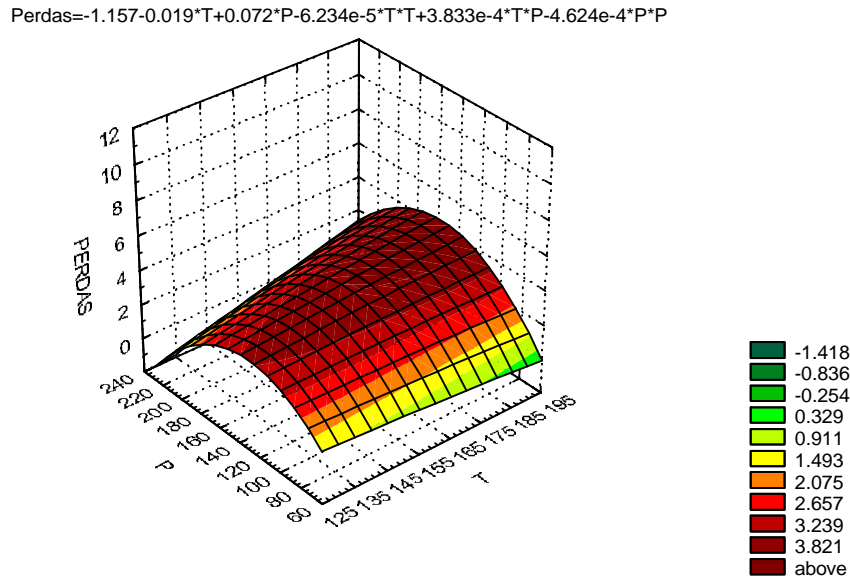


**Figure 2: Yield response surface for the ginger bagasse hydrolysis reaction**

The R-square for the fitted model was 68.8%. Only the temperature was statistically significant (for both coefficients, Linear and Quadratic, the p-value < 0.05).

## Process losses

The influence of the temperature and pressure is visualized in the response surfaces in Figure 3.



**Figure 3: Process losses response surface in ginger bagasse hydrolysis reaction**

The R-square for the fitted model is 13%, a very low value, and none of the coefficients are statistically significant ( $p$ -value  $> 0.14$ ).

## CONCLUSION

The hydrolysis of ginger bagasse in subcritical medium is a promising technique for the production of the reducing sugar because it can occur in relatively short times. The hydrolysis rate can be increased due the pretreatment of ginger in the extraction step.

The higher values of hydrolyzed starch content can be gotten at any pressure for temperatures up to 188°C or at any temperature for pressure up to 220bar. The better values of the yield were gotten in temperatures above 188 °C. The pressure was not relevant in the tested interval. It is possible that degradation of reducing sugar occurred at higher pressures. Although the process losses were relatively high this did not affected the conclusions, because, process losses were not significantly affected by either the temperature or the pressure.

## REFERENCES

- [1]. M. Siskin, Naturwissenschaftliche Rundschau cited by P. Krammer, H.Vogel, Vol.16, **2000**, p.189
- [2]. Y. Zheng, H.M. Ln, J. Wen, N. Cao, X. Yu and G.T. Tsao, Biotechnology Letters, Vol.17 **1995**, p.845
- [3]. H.S. Lee, Y.W. Ryu and C. Kim, Journal of Microbiology and Biotech, Vol. 4 **1994**, p. 230.
- [4]. J. N. BeMiller in R. L.Whistler and E. F. Paschall (Eds.), Vol. 1, Academic Press, London, New York, NY, **1965**, p.495.

- [5]. O. Theander and D.A. Nelson, Vol. 46, Academic Press, London, **1988**, p. 273.
- [6]. G.M.A. van Beynum, J.A. Roels, Marcel Dekker, New York and Brazil, **1985**, p. 143.
- [7]. K. C. Zancan, M. O. M. Marques, A. J. Petenate, M. A. A. Meireles, Journal of Supercritical Fluids, Vol. 24, **2002**, p. 57.
- [8]. M.B. Jacobs, Robert Krieger Publishing Co, New York, NY, **1981**, p.21.
- [9]. Association of Official Analytical Chemists, Washington, 1975, p.1094 in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [10]. Brasil. Resolução nº 12/78 da Comissão Nacional de Normas e Padrões para Alimentos. in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [11]. International Organization for Standardization. Norme Internacionale, 1987, p.4 (ISO, 6647) in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [12]. Association of Official Analytical Chemists, 12<sup>a</sup> ed, Washington, 1975, p.1094. in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [13]. ISO Method 3593. (1981). in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [14]. Association of Official Analytical Chemists, 1995, Method 32.1.22, in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [15]. American Association of Cereal Chemists, St. Paul, 1975, in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [16]. Nelson, N.A., J. Biol. Chem., Baltimore, nº 153, p. 375-380, 1944, in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [17]. Somogy, M., J. Biol. Chem., Baltimore, nº 160, p. 69, 1945, in: Methodology of CERAT (Centro de Raízes Tropicais), Unesp, Botucatu, SP, **1995**.
- [18]. G.E. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters, John Wiley and Sons, New York, USA, **1978**.