Effect of enzymatic treatment on the rheological behavior and vitamin C content of *Spondias tuberosa* (umbu) pulp

Rodrigo F. Gouvêa¹ · Leilson O. Ribeiro² · Érika F. Souza² · Edmar M. Penha² ·
Virgínia M. Matta² · Suely P. Freitas¹

Revised: 6 April 2017 / Accepted: 7 April 2017 / Published online: 24 April 2017
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Abstract Umbu is a native fruit of the semi-arid Northeastern region of Brazil, which presents an exotic and differentiated flavor. Containing vitamin C and presenting a high potential of consumption, no appropriated technology has been developed to process this fruit and expand its commercialization to other markets. The enzymatic treatment of fruit pulps leads to viscosity reduction, which makes possible an efficient processing for obtaining high quality umbu juices. In order to contribute to the valorization of this underexploited culture, two commercial pectinolytic enzymes, Pectinex Ultra SP-L[®] and Rapidase TF[®], were used to promote viscosity reduction of umbu pulp. The effect of reaction temperature (35, 45 and 55 °C) and enzyme concentration (100, 200 and 300 ppm) on the rheological properties of the fruit pulp was evaluated. In relation to the viscosity of the original pulp (84.8 mPa s at 100 s⁻¹ shear rate), a significant, four times lower, viscosity reduction of 18.9 mPa s was observed. Under optimum process condition (35 °C and Rapidase at 100 ppm concentration), the lowest viscosity was achieved after 40 min of reaction. Under these reaction conditions, no significant change was found in the vitamin C content,

indicating the preservation of functional and nutritional properties of umbu pulp.

Keywords Umbu pulp · Enzymatic treatment · Viscosity · Vitamin C

Introduction

The unique health-promoting benefits of fruits have favored their commercialization as frozen pulp, jams and jellies, dairy products and juices. In the case of juices, clarification consists of an important step for its production (Sandri et al. 2011; Tastan and Baysal 2015). Due to the presence of polysaccharides (mainly pectin, cellulose and hemicelluloses), proteins, polyphenols and inorganic materials, fruit juices present undesirably turbidity and high viscosities. These characteristics cause, in general, negative impact during food products industrialization. Contrarily to conventional and more expensive methods, enzymatic treatment of pulp had shown to lead to lower viscosities (Abbès et al. 2015; Kaur et al. 2011) and higher yields (Landbo et al. 2007), as well as improvements in flavor, and in the contents of bioactive substances (Kaur et al. 2011; Landbo et al. 2007).

The umbu tree (*Spondias tuberosa* Arruda Camara) is a native Anacardiaceae of the semi-arid northeastern region of Brazil. Its green-yellow fruits are highly appreciated, mainly in the production regions, because of their pleasant and mild sweet and sour taste (Galvão et al. 2011). By these characteristics, umbu became known as a fruit of exotic and differentiated flavor. Moreover, umbu fruits are the main source of income for many farmers; home-made juice, jam, ice-cream and other desserts are prepared from them, and commercialized regionally (Lins Neto et al.

Electronic supplementary material The online version of this article (doi:10.1007/s13197-017-2630-8) contains supplementary material, which is available to authorized users.

✉ Rodrigo F. Gouvêa
rfgouvea@hotmail.com

¹ Departamento de Engenharia Química, Escola de Química, Universidade Federal do Rio de Janeiro, Avenida Athos da Silveira Ramos 149, Rio de Janeiro 21941-909, Brazil

² Embrapa Agroindústria de Alimentos, Avenida das Américas 29501, Rio de Janeiro 23020-470, Brazil

2010). Nevertheless, a high quantity of umbu is wasted every year, since no appropriated technology has been developed to processing all the fruit production and to expand its commercialization to other markets.

The volatile (Galvão et al. 2011) and bioactive (Almeida et al. 2011) substances present in umbu fruits were identified and quantified. In addition to flavor, the presence of considerable amounts of micronutrients strongly recommends umbu fruits for a healthy diet and suggests promising perspectives for the technological development of new products. Among the main antioxidant substances already determined in umbu fruit, ascorbic acid (vitamin C) is one of the most important. According to Spínola et al. (2014), vitamin C takes part in several biochemical processes and in prevention of chronic diseases. In umbu, vitamin C reaches 12.5 mg/100 g of pulp (Almeida et al. 2011). As a micronutrient component, ascorbic acid is an important parameter to evaluate the juice quality. It is known that vitamin C retention is extremely influenced by processing temperature, pH, light and presence of oxygen and metals, as well as ascorbic acid oxidase (Bai et al. 2013; Zhao et al. 2014) and can be used as an indicator of food nutrients preservation (Xiao et al. 2010, 2014; Valente et al. 2014). In this context, pretreatment methods for juice production, which associate viscosity reduction and antioxidant retention, should contribute to facilitate umbu processing and the valorization of this underexploited culture.

In this work, two commercial pectinolytic enzymes, Pectinex Ultra SP-L® and Rapidase TF®, were used to hydrolyze polysaccharides from the cell wall, as a pre-treatment clarification method. To the authors' knowledge, although these microbial-derived enzymes were commonly cited for other fruits processing, no such procedure was applied to umbu pulp. Following an experimental design, the effect of the enzymes on the rheological properties was evaluated. Vitamin C was selected as an indicator of functional and nutritional umbu pulp quality. Its content was quantified and correlated to the best hydrolysis results.

Materials and methods

The non-pasteurized umbu pulp was provided by the frozen pulp industry, situated in Bahia state, stored in polyethylene bags, and maintained at -18°C until use. Before processing, the pulp was slowly thawed. The commercial enzymatic preparations Pectinex Ultra SP-L® and Rapidase TF® were acquired from Novozyme (Bagsvaerd, Denmark) and DSM Food Specialties B.V. (Delft, The Netherlands), respectively.

A center composite design was performed, using hydrolysis temperature (35 and 55°C), and enzyme

concentration (100 and 300 ppm) as independent variables and viscosity and vitamin C content as response variables.

For each experiment, 250 g of pulp were used at the natural pH of 2.8. The samples were maintained in 500 mL erlenmeyers under stirring at 110 rpm, and controlled temperature. The essays were performed in a shaker for 2 h, to ensure that the hydrolysis reaction had reached equilibrium conditions. Then, the hydrolysates were stored at 8°C until further analysis.

Steady-shear experiments were performed at 20°C , in an ARG2 rheometer (TA Instrument, New Castle, DE, USA), using a coaxial cylinder geometry. Steady state flow was performed from 1.0 to $6 \cdot 10^2 \text{ s}^{-1}$ shear rates. The Ostwald de Waele model (Eq. 1) was fitted to the shear stress versus shear rate data by nonlinear regression.

$$\tau = K \left(\frac{dv}{dy} \right)^n \quad (1)$$

where τ is the shear stress, dv/dy is the shear rate, K is the consistency index and n is the viscosity index.

The kinetics of the hydrolysis process was followed for the best experimental conditions (in 100 ppm Rapidase TF® at 35°C), by viscosity measurements.

Vitamin C was determined by the 2,6-dichlorophenol indophenol (DCPI) titrimetric method with modification (AOAC 1990; Contreras-Calderón et al. 2011). By this method, a 0.01% DCPI water solution was calibrated with a 0.03% L-ascorbic acid (AA) solution. This DCPI solution was used to titrate 2.0 g of umbu pulp diluted with 50 mL of oxalic acid at 1% concentration, up to a slight pink color. Results were expressed as mg of ascorbic acid equivalents per 100 g of pulp sample X , according to Eq. 2.

$$X = \frac{v \cdot m \cdot 100}{V \cdot M} \quad (2)$$

where v is the consumed volume of DCPI (mL) relative to 1 mL of titrated sample, m is amount of AA (mg) in 1 mL of AA standard solution, V is the consumed volume of DCPI (mL) relative to 1 mL of titrated AA standard solution, and M is the mass of sample (g).

All statistical analyses were performed with Statistica software v. 7.0. Differences at $p \leq 0.05$ were considered to be significant. The Tukey test was applied for the means comparison of two independent samples.

Results and discussion

Enzymatic hydrolysis of pectic substances is known to depend on the type of enzyme, enzyme concentration, incubation temperature, contact time and pH. Commercial pectinolytic enzymes were used successfully to pretreat

pulps from numerous fruits (Abbès et al. 2011; Abbès et al. 2015; Dongowski and Sembries 2001; Kaur et al. 2011). In particular, Pectinex Ultra SP-L[®] was used for the clarification of Indian gooseberry (Manjuantha et al. 2012b), white pitaya (Nur'Aliaa et al. 2010) and lime (Manjuantha et al. 2012a) juices. On the other hand, Rapidase TF[®] in combination with another enzyme (Viscozyme) led to the lowest viscosity and the highest levels of total sugars and polyphenols a mucilage from cactus cladodes (Kim et al. 2013).

Figure 1a, b shows the variation of viscosity as a function of shear rate for umbu pulp treated with Pectinex and Rapidase, respectively. Independently of enzyme concentration and reaction temperature, the viscosity curves shows the pseudoplastic behavior of the hydrolysed pulps. Both enzymes promoted significant reductions ($p \leq 0.05$) of umbu original pulp viscosity. At 100 s^{-1} shear rate, the viscosity observed for the pulp was 84.8 mPa s . At the same shear rate, the average values

(central point) observed for the samples treated with Pectinex and Rapidase were 51.0 and 18.9 mPa s , respectively.

As it can be observed, some of the hydrolysed pulps showed higher viscosities than the original one. This result might be related to interactions between charged pectin oligomers and proteins (Pinelo et al. 2010) or proteins and polyphenols (Sieber 2006).

It is worth noting that the choice of hydrolysis conditions range was based on previous observations. For example, the temperature conditions were selected according to the optimum activity temperatures of the enzymes. Also, it is well known that high methoxyl pectin is present in all pulps, and that this pectic substance becomes gels by a complex mechanism, which depends on temperature. The intermolecular gel network is stabilized by hydrophobic interactions (stable at high temperatures) and by hydrogen bonds (stable at low temperatures). Moreover, the use of temperatures below or above the $35\text{--}55^\circ\text{C}$ range would mask the viscosity results. As for the chosen concentration range, the economic factor has a fundamental role. Higher enzymes concentrations would turn out to be financially impracticable, at least in the case of umbu pulp. Furthermore, reported data showed that increasing enzyme concentrations have a limited effect. In this case, a plateau was reached, from which the effect of concentration was no more effective (Maktouf et al. 2014).

Although viscosity reduction was significant, the enzymatic treatments with Pectinex and Rapidase, at the different concentrations and temperatures, had no significant effect on the consistence and flow index values (data not shown). On the other hand, temperature had a significant effect ($p \leq 0.05$) on vitamin C contents (Fig. S1 of supplementary material). This latter result was expected due to the degradation effect of temperature. Surprisingly, no significant effect of enzyme concentration and temperature was observed on the pulp viscosity. In this point, some characteristics of the systems and their analysis should be addressed. Probably, as a result of pulp heterogeneity, due to suspended particles in the medium, the viscosity values at the central point led to a high standard deviation. In this way, taking $p \leq 0.05$, all viscosity values were found within the confidence interval considered. The other fact to be considered was related to the narrow range of concentrations ($100\text{--}300 \text{ ppm}$), which might had not been sufficient to affect significantly the viscosity of umbu pulp. Even if the increase in enzyme concentration affected positively the pulp viscosity (by decreasing their values), the effect of temperature could mask the results. The increase in temperature would contribute to the viscosity increase, as a result of pectin gelatinization.

After processing for 2 h with both enzymes, the vitamin C contents were reduced, as shown in Table 1. Generally, fresh fruits contain more vitamin C than even those cool-

Fig. 1 Apparent viscosity as a function of shear rate for the hydrolysed umbu pulp treated with the enzymes Pectinex (a) and Rapidase (b)

Table 1 Ascorbic acid contents for umbu pulp after enzymatic treatments during 2 h

Treatment	Ascorbic acid content (mg 100 g ⁻¹)	
Original pulp	10.06 ± 0.39 ^a	10.06 ± 0.39 ^a
	Pectinex	Rapidase
100 ppm/35 °C	9.10 ± 0.57 ^{b,c}	9.33 ± 0.16 ^{a,b}
300 ppm/35 °C	9.54 ± 0.20 ^{a,b}	8.52 ± 0.23 ^c
200 ppm/45 °C	7.43 ± 0.42 ^d	7.35 ± 0.46 ^{d,e}
200 ppm/45 °C	7.25 ± 0.19 ^{d,e}	7.20 ± 0.30 ^{d,e,f}
200 ppm/45 °C	7.26 ± 0.26 ^{d,e}	7.18 ± 0.27 ^{d,e,f}
100 ppm/55 °C	6.91 ± 0.59 ^{d,e,f}	6.67 ± 0.30 ^{e,f}
300 ppm/55 °C	6.47 ± 0.51 ^f	6.66 ± 0.58 ^{e,f}

Tests were conducted in triplicate. Average values on the same column and with the same letter do not differ to the level of significance of 5%

stored (Lee and Kader 2000) and, consequently, the results obtained for the enzyme-treated pulp were expected. Recently, the vitamin C content of litchi pulp, 17.7 mg/100 g, was reported to decrease to 11.8 mg/100 g after treatment with pectinase at 40 °C for 2 h. This decrease was attributed to thermal oxidation (Vijayanand et al. 2010).

The kinetics of the hydrolysis reaction was followed by viscosity measurements. As shown before, a better effect on viscosity reduction was observed using Rapidase, but no significant effect on pulp viscosity was detected with increasing concentration. More important, at the hydrolysis temperature of 35 °C, the retention of vitamin C was higher. Thus, considering the most friendly and least costly conditions, the pulp sample was treated with 100 ppm of Rapidase at 35 °C. The viscosity results at 10, 100 and 600 s⁻¹ were plotted against the reaction time (Fig. 2). It is worth observing in the graph of Fig. 2

Fig. 2 Effect of enzymatic hydrolysis at 35 °C by Rapidase at 100 ppm concentration on the apparent viscosity of umbu pulp

that the viscosity values presented a tendency to be stabilized after 40 min of processing. However, the curve corresponding to viscosity values at 600 s⁻¹ shear rate showed a 66% decrease in viscosity in relation to the original pulp, leading to the lowest viscosity among the three conditions of shear rate evaluated. This result may be attributed to the turbulent regime, which promoted a better mixture between components, but does not favor the enzymatic hydrolysis. On the other hand, at an intermediate shear rate (100 s⁻¹), a highest decrease in viscosity, about 71%, was observed, which strongly recommends this condition to be used for umbu pulp enzymatic pretreatment.

The kinetics of the enzymatic hydrolysis was also monitored by the vitamin C content. Although a significant reduction ($p \leq 0.05$) in vitamin C content was observed for the 2-h processing with Rapidase at 100 ppm and 35 °C (Fig. S2 of supplementary material), no significant decrease was detected up to 90 min of processing. This is a good result because, as discussed above, relatively short periods of time would be sufficient for the pretreatment of the umbu pulp.

Conclusion

The enzymatic treatment before juice extraction has become a very attractive processing step in the last decades. It's mainly due to the productivity and quality gain reached by the juice industry. The pretreatments carried out with the commercial pectinolytic enzymes Pectinex Ultra SP-L[®] and Rapidase TF[®] promoted a significant reduction of umbu original pulp viscosity. The enzymatic hydrolysis using 100 ppm of Rapidase at 35 °C, carried out during 40 min, has been shown to be the best process conditions. These conditions enabled the effective viscosity reduction, preserving the vitamin C of umbu pulp. This work showed a tailor-made alternative for umbu processing, capable to add value to this exotic fruit and contribute to the economic development in the production rural areas.

Acknowledgements The authors thank the financial support of Embrapa Agroindústria de Alimentos.

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