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Behavior of antioxidant enzymatic system of sonicated cashew apple bagasse

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ABSTRACT

The effects of ultrasound processing on the activity of antioxidative enzymes of cashew apple bagasse (Anacardium occidentale L.) were investigated. The changes in the activities of superoxide dismutase, catalase, and ascorbate peroxidase were evaluated for either sonicated or control samples. The stimulus from sonication has a direct effect on the production of reactive oxygen species such hydrogen peroxide, and consequently, on the activities of superoxide dismutase, catalase, and ascorbate peroxidase.

1. INTRODUCTION

High power ultrasound has been applied as a green, an inexpensive and an easy-to-use method for food processing with good preservation of bioactive compounds). The effects caused by sonication came from two hydrodynamic events: acoustic cavitation and induced microstreaming cavitation (Bermúdez-Aguirre et al., 2011).

Plants can protect themselves against oxidative damage using their antioxidant system, including non-enzymatic compounds and antioxidative enzymes (Yang et al., 2012). Superoxide dismutase is the first enzyme to convert superoxide anion into peroxides, which are scavenged by catalase and ascorbate peroxidase (Gawlik-Dziki, 2014). According to Oliveira et al., (2011) the impact of food processing on antioxidant capacity may be evaluated through the activity of enzymes used as damage markers. Thus, in this study, the effect of sonication of cashew apple bagasse on enzymes involved in the antioxidative system was investigated.

2. MATERIAL AND METHODS

2.1 Sample preparation

Red cashew apples were harvested at commercial maturity stage in Ceará State, Brazil. The fruits were sanitized, the nuts were removed, and the peduncles were reserved. The cashew apple juice was extracted pressing the peduncles in an expeller press, and the bagasse was reserved.

2.2 Sonication

The experiments with the ultrasound were carried out in 600 mL Becker flasks with a final sample volume of 200 mL (bagasse+water), under ambient temperature (20°C) in a 500W ultrasound processor with a 1.3 cm diameter probe tip without mechanical agitation or temperature control. The ultrasound frequency was 20 kHz. The calculated intensities were 75, 226 and 373 W/cm^2, respectively. A central composite experimental design was used to evaluate the effect of sonication and bagasse to water ratio.

2.5 Enzymatic assay

For superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.1) determinations, enzyme extraction was done according to the methodology described by Wissemann and Lee (1980). APX activity was assayed according to the method of Nakano and Asada (1981). CAT activity was measured according to the method of Beers and Sizer (1952). SOD activity was determined by the inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) (Giannopolitis and Ries, 1977).One unit of enzyme activity (1 UEA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance (395 nm) per minute.Results were expressed as mean ± SD. F-test and ANOVA analysis were used as significant criteria for the fitted models. Tukey's test was used to determine the significant differences among means ($p<0.05$). Statistical analysis of the experimental data was carried out using the software Statistica 7.0 (Statsoft).

3. RESULTS AND DISCUSSION

The activity of antioxidant enzymes of control samples is summarized in Table 1. Control samples consisted of cashew apple bagasse submitted to only water immersion without sonication, kept at ambient temperature (20°C) for 10 min. The exposure of cashew apple bagasse to ultrasound substantially affected the activity of antioxidant enzymes. SOD activity increased by 276% compared to control (Table 1). The response surface graph for residual SOD, CAT and APX activity are presented

in Figure 1. The surface graphs were built using fitted models statistically significant at 95% of confidence level (results not shown).

Table 1 Enzymatic activity of control (non-sonicated) samples

	SOD	CAT	APX
Bagasse:water ratio	**UEA. mg^{-1} P**	**µmol H_2O_2 $mg^{-1}P.min^{-1}$**	**µmol H_2O_2 $mg^{-1}P.min^{-1}$**
1:2	4.61 ± 0.09	69.56 ± 0.53	0.39 ± 0.01
1:3	4.63 ± 0.87	65.23 ± 0.00	0.37 ± 0.01
1:4	2.30 ± 0.04	65.78 ± 0.05	0.59 ± 0.00

UEA=Unity of enzymatic activity

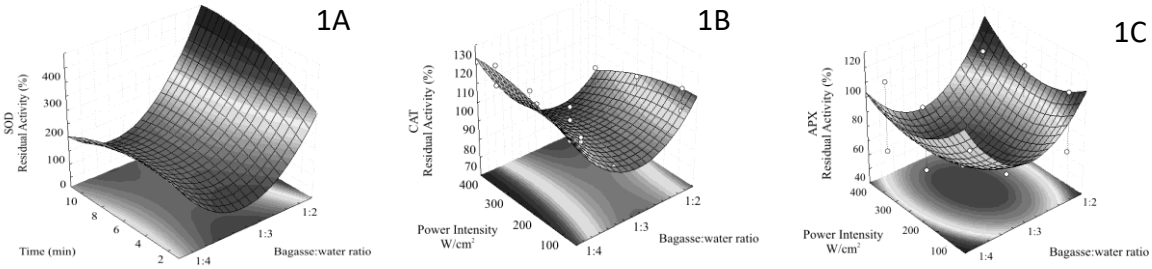

Fig.1 Response surface for the effect of sonication on SOD (1A), CAT (1B) and APX (1C) activities of cashew apple bagasse.

The surface for SOD activity showed a saddle point near to the central point of the experimental domain. This saddle point is a local minimum of processing time and a local maximum for bagasse: water ratio, showing that when keeping the processing time near to 6 min, a residual activity increased for bagasse to water ratio higher and lower than 1:3. The SOD activity is increased by a variety of chemical and physical stimuli (Chen *et al.*, 2008). SOD scavenges the radical superoxide catalyzing its conversion to H_2O_2, which subsequently is neutralized by CAT or APX.

The same tendency was observed for CAT activity of sonicated bagasse which was 19% greater than the control sample. The response surface graph for CAT presented a well-defined for maximum. The process parameters for maximal CAT residual activity were: power intensity of 236.12 W/cm^2, 1:3 of bagasse: water ratio and 6.21 minutes of processing time, determined by the critical point (zero derivate) of the fitted equation.

The minimum APX residual activity (≈50%) was at the central point, the same conditions that maximized the CAT activity. Chen *et al.* (2008) suggested that at least two different factors are involved to prevent the oxidative damage under ultrasound stress: rise in the activities of antioxidant enzymes such as superoxide dismutase, catalase and increase in the content of carotenoids and

glutathione. So, higher activities of scavenging antioxidant enzymes found for sonicated cashew apple bagasse may be a defense against oxidative stress.

4. Conclusions

The results indicated that the physical stimulus from sonication can increase the activities of SOD, CAT and APX in cashew apple bagasse. The enzymes were resistant to the processing conditions.

5. Acknowledgments

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