UNIVERSIDADE FEDERAL DO CEARÁ
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Uso do ultrassom seguido de secagem para o aproveitamento do bagaço do pedúnculo do caju (*Anacardium occidentale* L.): Efeitos sobre o potencial antioxidante e bioacessibilidade de nutrientes

Orientadora: Dra. Sueli Rodrigues

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Tese apresentada a Coordenação do Programa de Pós-Graduação em Engenharia Química do Departamento de Engenharia Química da Universidade Federal do Ceará, como parte dos requisitos para a obtenção do título de Doutor em Engenharia Química.

Área de Concentração: Processos Químicos e Bioquímicos

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Sumário

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ABTS	2,2´-azinobis (3-etilbenzotiazolina-6-ácido sulfônico)
DPPH	2,2-diphenyl-1-picrylhydrazyl
PPO	Polifenoloxidase
G-POD	Guaicol peroxidase
APX	Ascorbato peroxidase
SOD	Superóxido dismutase
CAT	Catalase
TPC	Total phenolic compounds
CCD	Central Composite Design
ΔC	Delta Chroma
ΔE	Total Color Difference
L*	Luminosidade
a*	Coordenada de cromaticidade de verde ao vermelho
b*	Coordenada de cromaticidade de azul ao amarelo
DLP	Double Log Polynomial
BET	Brunauer, Emmet e Teller
GAB	Guggenheim, Anderson, de Boer
ANOVA	Análise de Variância
R^2	Coeficiente de determinação
TROLOX	6-hidroxi-2,5,7,8-tetrametilcromo-2-ácido carboxílico
NBT	Nitro-blue tetrazolium
ROS	Reactive oxygen species

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Resumo

Esta tese descreve alguns efeitos da utilização do ultrassom de alta intensidade para o aproveitamento do bagaço do pedúnculo de caju. Desta forma, os objetivos deste trabalho foram: 1) Desenvolver um processo otimizado para o processamento ultrassônico do bagaço de caju, avaliando o efeito do ultrassom em compostos antioxidantes enzimáticos e não enzimáticos e 2) estudar os efeitos do ultrassom no processo de secagem avaliando os efeitos em compostos bioativos e na bioacessibilidade de nutrientes após os processamentos. Para definir as melhores condições de sonicaçãofoi utilizado o planejamento fatorial composto central 2^3 tendo como variáveis independentes, razão bagaço:água, intensidade de energia ultrassônica (W/cm^2) e tempo de processamento (min). As amostras foram submetidas aos ensaios de quantificação de compostos antioxidantes não enzimáticos como vitamina C, compostos fenólicos totais e β-caroteno. A capacidade antioxidante total (ABTS e DPPH) e a atividade de enzimas antioxidantes superóxido dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) e ascorbato peroxidase (APX, EC 1.11.1.1) e das enzimas peroxidase (EC 1.11.1.7) e polifenoloxidase também foram avaliadas. O processamento do bagaço formou um purê homogêneo de cor amarela intensa, apresentando um alto rendimento de extração de compostos bioativos. A máxima retenção de vitamina C, compostos fenólicos e β-caroteno foi obtida para as seguintes condições operacionais: proporção bagaço:água de 1:4, intensidade da energia ultrassônica de 226 W/cm^2 e 6 minutos. As elevadas concentrações de compostos fenólicos (2186,05 mg de ácido gálico/100 g base seca), vitamina C (148,32 mg/100 g BS) e β-caroteno (12,38 mg/100g BS), além da elevada atividade antioxidante, confirmaram a possibilidade de uso da tecnologia ultrassônica para o preparo de um purê de bagaço de caju rico em antioxidantes. O ultrassom teve um efeito direto sobre a concentração de peróxido de hidrogênio e, consequentemente, sobre as atividades da superóxido dismutase, catalase e ascorbatoperoxidase. O processamento resultou em mudanças perceptíveis na cor do bagaço do caju, porém, não houve evidências de escurecimento uma vez que o ângulo de tonalidade (h°) das amostras sonicadas estava próximo da cor característica de polpa de caju. O efeito do ultrassom em retardar o escurecimento pode ser correlacionado com o aumento da atividade das enzimas antioxidantes e diminuição da atividade da enzima guaiacol peroxidase. Em uma segunda etapa, o efeito do ultrassom no processo de secagem do bagaço de caju foi avaliado. Os parâmetros de secagem difusividade efetiva da água, atividade de água e as isotermas de sorção foram avaliados. Ao longo da secagem, as concentrações de vitamina C, compostos fenólicos, capacidade antioxidante total e a atividade das enzimas peroxidase e polifenoloxidase foram determinadas. A bioacessibilidade *in vitro* de vitamina C e de compostos fenólicos foi avaliada. O ultrassom induziu a ruptura de células, que favoreceu uma menor resistência à difusão da água durante a secagem, menor efeito de histerese e aumento da taxa de reidratação. Observou-se para as amostras sonicadas, uma retenção mais elevada de vitamina C e de compostos fenólicos em comparação à secagem convencional. O processamento ultrassônico reduziu a bioacessibilidadede vitamina C, por outro lado, observou-se um aumento na bioacessibilidade de compostos fenólicos, em relação ao controle. O uso do ultrassom reduziu a severidade do tratamento convencional de secagem e, por conseguinte, melhorou a qualidade do produto seco. Os resultados indicaram que a sonicação aumentou a concentração de compostos funcionais no bagaço de caju, tornando este recurso subutilizado, uma matéria-prima potencial para diversos usos na indústria alimentícia.

Palavras-chave: Ultrassom, bagaço de caju, compostos bioativos, atividade antioxidante.

Abstract

This thesis describes some effects of high power ultrasound to the reuse of cashew apple bagasse. Thus, the objectives of this study were: 1) Develop a optimized process for sonication of cashew apple bagasse, evaluating the effect of ultrasound on enzymatic and non-enzymatic antioxidant compounds and 2) study the effects of ultrasound in drying process evaluating the effects on bioactive compounds and bioaccessibility after processing. To define the best conditions for sonication, a 2^3 factorial central composite design was used with independent variables: bagasse:water ratio, ultrasonic power intensity (W/cm^2) and processing time (min). Nonenzymatic antioxidants such as vitamin C, β-carotene and total phenolic compounds were determined. The total antioxidant capacity (ABTS and DPPH) and the activity of antioxidant enzymes such superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.1) and peroxidase enzymes (EC 1.11.1.7) and polyphenol oxidase were also evaluated. Sonication changed the bagasse aspect from a fibrous residue to a pleasant yellow puree. The maximal concentration of vitamin C, phenolics and β-carotene was obtained when the bagasse:water ratio was 1:4, the ultrasound power intensity 226 W/cm^2 and 6 minutes of processing. The high total phenolic content (2186 mg of gallic acid/100 g DW), vitamin C (148 mg/100 g DW) and β-carotene (12 mg/100g) obtained proved the sonication efficiency. Furthermore, the antioxidant activity determined by the DPPH and ABTS assays confirmed the suitability of ultrasound for the preparation of antioxidant-rich cashew apple bagasse puree. 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. Sonication resulted in perceptible color changes in cashew apple bagasse. Despite this result, there were no evidences of browning since h° of sonicated samples was close to the characteristic color of cashew apple pulp. The effect of ultrasound in delaying browning could be correlated to the enhanced antioxidant enzyme activity and decrease in guaiacol peroxidase activity. In a second stage, the effect of ultrasound on drying of cashew bagasse was evaluated. The parameters effective diffusivity of water, water activity and the sorption isotherms were evaluated. During drying, the concentration of vitamin C, phenolics, total antioxidant activity and the enzyme activities of peroxidase and polyphenol oxidase were determined. The in vitro bioaccessibility of vitamin C and phenolic compounds was evaluated. Sonication induced disruption of cashew bagasse parenchyma, which resulted in lower resistance to water diffusion, less hysteresis effect and increased rehydration rate. Sonication did not affect the lignocellulosic fibers, or eschlerenquima cells. For sonicated samples, in the first 2 hours of drying, water activity reached values below 0.4, considered appropriated in order to prevent bacterial and fungi growth. The increase of the mass transfer due to ultrasound processing can be attributed to the reduction on the boundary layer thickness produced by pressure variations, oscillating velocities and microstreaming. The sorption isotherms of cashew apple bagasse had sigmoid-shape for all samples and followed the type II of BET classification. Sonicated cashew apple bagasse presents high antioxidant activity, and high total phenolic compounds (TPC) and vitamin C values. The increase in phenolic compounds and the high final amount of vitamin C may lead to enhance the antioxidant activity. The ultrasound processing caused some reduction of bioaccessibility of vitamin C but increase on TPC bioaccessibility, compared to control. Moreover, sonication reduces the severity of conventional drying treatments, improving the quality of the dried product.

Keywords: Ultrasound, cashew apple bagasse, bioactive compounds, antioxidant activity.

Introdução Geral

As tecnologias não térmicas de processamento de alimentos surgiram com o intuito de ser uma ferramenta auxiliar ao processamento térmico, uma vez que as altas temperaturas utilizadas afetam a qualidade sensorial e nutricional dos alimentos. De acordo com o conhecimento científico recente, o emprego de ondas sonoras no processamento de alimentos representa uma alternativa rápida, eficiente e confiável para atender à demanda por alimentos nutritivos com características similares às do alimento *in natura.*

Diversas pesquisas têm revelado o potencial do ultrassom para a otimização de processos na indústria de alimentos como, por exemplo, na melhoria de processos convencionais de secagem (FERNANDES; GALLÃO; RODRIGUES, 2009; GAMBOA-SANTOS *et al.*, 2014; IGUAL *et al.*, 2012; NOSHAD *et al.*, 2012; RODRIGUES *et al.*, 2009a). A aplicação do ultrassom antes da secagem com ar quente reduz o tempo de processamento total, entre 10% e16%, efeito que se deve principalmente ao aumento da difusividade da água (ROMERO; YÉPEZ, 2015). O ultrassom induz alterações físicas como perda de adesão celular, formação de espaços intercelulares e a ruptura das paredes celulares (RODRIGUES *et al.*, 2009b), responsáveis pelos efeitos observados na taxa de secagem. O pré-tratamento ultrassônico tem um impacto direto na economiado processo, uma vez que a secagem convectiva é uma operação de alto gasto energético.

As condições extremas de temperatura e pressão que ocorrem durante a sonicação também podem desencadear reações sonoquímicas, que dependendo do processamento podem ser desejáveis ou indesejáveis (ROMERO; YÉPEZ, 2015). ABID *et al.*(2014) atribuíram um aumento na concentração de compostos fenólicos em suco de maçã sonicado a uma possível ligação de radicais hidroxila formados durante o processamento ao anel aromático dos compostos fenólicos. Por outro lado, o processamento ultrassônico também pode levar à perda de vitamina C (ADEKUNTE *et al.*, 2010) e de compostos fenólicos (FONTELES *et al.*, 2012) devido à interação desses compostos com os radicais livres formados durante a sonicação (HART; HENGLEIN, 1985).

Uma melhor compreensão dos mecanismos físico-químicos, a otimização de parâmetros operacionais e a avaliação dos possíveis efeitos no alimento são fundamentais para que se possa explorar o potencial de uso desta tecnologia para melhorar características tecnológicas e funcionais de alimentos como os frutos tropicais e seus derivados.

Por esta razão, este trabalho visa avaliar o uso do ultrassom para o processamento do bagaço do pedúnculo do caju (*Anacardium occidentale* L.), uma fruta tropical muito cultivada no Brasil, cuja produção na região do Nordeste brasileiro é uma importante fonte de renda, emprego rural e fonte de alimento (SANTOS *et al.*, 2008).

O processo foi avaliado considerando duas características importantes: a preservação de compostos antioxidantes presentes do resíduo após a aplicaçãode ultrassom e o efeito do processamento ultrassônico nos parâmetros de secagem com ar quente.

O trabalho apresenta-se subdividido em quatro capítulos. No Capítulo 1 é apresentada uma revisão bibliográfica que aborda os fundamentos básicos e aplicações da tecnologia ultrassônica. Nos Capítulos de 2 a 4 almejou-se atingir os seguintes objetivos específicos:

I. Desenvolver um processo otimizado para o processamento ultrassônico do bagaço do pedúnculo do caju visando a preservação de vitamina C, compostos fenólicos e β-caroteno;
II. Avaliar os efeitos do ultrassom no sistema antioxidante enzimático;
III. Avaliar os efeitos do ultrassom nos parâmetros de secagem avaliando os efeitos em compostos bioativos e na bioacessibilidade dos nutrientes após os processamentos.

Capítulo 1

Revisão Bibliográfica

1.1 Fundamentos básicos sobre tecnologia ultrassônica

Ondas sonoras com frequência acima de 20 kHz são denominadas ultrassônicas (KWIATOWSKA *et al.*, 2011) (Fig. 1). O uso mais difundido do ultrassom é como ferramenta na medicina diagnóstica, porém, pesquisas têm revelado diversas aplicações industriais desta tecnologia como, por exemplo, no processamento de alimentos.

Figura 1. Escala de frequência do som.

As aplicações da tecnologia ultrassônica podem variar de acordo com a frequência utilizada. No processamento de alimentos, é feita uma divisão entre o ultrassom de baixa intensidade (potência < 1 W/cm^2 e frequência 100 kHz – 1 MHz), utilizado como ferramenta analítica não destrutiva, e o ultrassom de alta intensidade (potência entre 10-1000 W/cm^2), usado em frequências mais baixas (20–100 kHz) para causar o rompimento físico de tecidos, criar emulsões ou promover reações físicas e químicas (SORIA; VILAMIEL, 2010).

Os efeitos provocados pelo ultrassom ocorrem devido ao fenômeno da cavitação acústica, que é o processo de nucleação, crescimento e colapso de bolhas transientes em líquidos expostos a ondas ultrassônicas de baixa frequência.

As ondas ultrassônicas geram pressão acústica no meio de propagação (P_a). A pressão acústica é uma onda senoidal dependente do tempo (t), frequência (f) e da amplitude máxima da onda (P_{aMAX}) (MUTHUKUMARAN *et al.*, 2006):

$$P_a = P_{aMAX} sen\,(2\pi f t) \qquad \textbf{Eq. 1}$$

Caso a onda acústica atinja pressão suficientemente alta, os gases e vapores formados durante os ciclos alternados de expansão e compressão não retornam completamente para o líquido, gerando um aumento do tamanho da cavidade até que seja atingido seu diâmetro crítico, entrando em violento colapso. O colapso das bolhas transientes provoca a liberação de grande quantidade de energia, gerando temperaturas locais instantâneas muito elevadas (5000 K) e pressões da ordem de centenas de atmosferas (1000 atm) (O'DONNELL *et al.*, 2010).

Quando o ultrassom é aplicado a sistemas sólido-líquido (como no caso da desidratação osmótica) o colapso das bolhas de cavitação difere em relação a sistemas puramente líquidos. Devido à proximidade da superfície sólida, as bolhas colapsam assimetricamente, resultando em irrupção de fluido da bolha para a superfície, fenômeno conhecido como *microstreaming* (microjatos). Este efeito causa o aumento das taxas de transferência de massa e calor na superfície sólida quando a camada limite é rompida. O fenômeno de *microstreaming* também pode destruir estruturas como as paredes celulares de micro-organismos (BHASKARACHARYA; KENTISH; ASHOKKUMAR, 2009).

1.2 Aplicações do ultrassom de alta potência em engenharia de alimentos

Muitos processos na indústria de alimentos podem ser conduzidos com o auxílio da tecnologia ultrassônica de alta intensidade como o congelamento, secagem, emulsificação, esterificação, extração ou pasteurização (CHEMAT; HUMA, KHAN, 2011). Porém, os possíveis efeitos do processamento ultrassônico na qualidade sensorial e nutricional são muito importantes na avaliação do seu potencial para a preservação de alimentos.

Nos tópicos a seguir serão abordados os principais e os mais inovadores efeitos decorrentes da sonicação de alimentos reportados recentemente na literatura científica.

1.2.1 *Uso do ultrassom na preservação de alimentos*

1.2.1.1 *Inativação de micro-organismos*

A atividade microbiana é a principal responsável pela deterioração de alimentos além de causar inúmeras doenças. Tradicionalmente, a estabilidade microbiológica dos alimentos é alcançada através do uso de altas temperaturas como nos processos de pasteurização ou esterilização térmica. Porém, a demanda por produtos de qualidade nutricional e sensorial, com características mais próximas às do produto *in natura*, tem levado à busca e ao aperfeiçoamento de processos capazes de preservar os alimentos contra agentes deteriorantes, sem acarretar os efeitos adversos comuns aos processamentos convencionais, principalmente os que envolvem tratamento térmico.

Novas tecnologias de processamento não térmico têm recebido avanços significativos para uso em escala comercial, dentre as quais, o processamento ultrassônico tem se destacado com resultados promissores na inativação de micro-organismos (LEE *et al.*, 2009; SALLEH-MACK; ROBERTS, 2007; UGARTE-ROMERO *et al.*, 2006).

A eficácia do ultrassom na inativação de micro-organismos é uma função da linhagem microbiana, tempo de exposição/contato, amplitude e potência ultrassônica, volume processado, composição do alimento assim como da temperatura (PIYASENA *et al.*, 2003).

Diferentes mecanismos são propostos para elucidar o efeito do ultrassom sobre micro-organismos, porém a formação de bolhas de cavitação tem sido o mecanismo mais aceito. As forças de cisalhamento e as mudanças rápidas de pressão criadas pelas ondas de ultrassom são eficazes na destruição de células microbianas.

CAMERON, MCMASTER e BRITZ (2008) mostraram através de microscopia de transmissão eletrônica que células sonicadas apresentaram-se desprovidas de conteúdo interno. Apesar de não garantirem que a fragmentação seja a única causa de morte celular, os autores também atribuem a morte celular à formação de radicais H_2O_2.

LUO *et al.* (2012) avaliaram a susceptibilidade de contaminantes comuns do vinho ao processamento ultrassônico. Nesse estudo, suspensões celulares (leveduras e bactérias) em solução salina, suco de uva e vinho, foram submetidas ao processamento ultrassônico durante 20 minutos com potência de 126 W, 131 W e 118 W, respectivamente. Os autores comprovaram que a eficácia da sonicação varia de acordo com o meio para micro-organismos do mesmo gênero. A variabilidade dentre os diferentes gêneros pode ser atribuída à

configuração da parede celular de bactérias e leveduras que confere maior ou menor proteção ao tratamento. Os resultados apontaram que bactérias gram-positivas, que apresentam camada mais espessa de peptideoglicanos em relação a bactérias gram-negativas, são mais resistentes ao processamento ultrassônico. É bem estabelecido que o fenômeno cavitacional seja afetado pelos parâmetros: reologia do fluido, em particular pela viscosidade, pressão de vapor e tensão superficial, que em conjunto com as particularidades de cada micro-organismo pode influenciar na susceptibilidade à sonicação.

Sabe-se ainda que as leveduras apresentam maior sensibilidade às ondas ultrassônicas. ADEKUNTE *et al.* (2010) estudaram o efeito do processamento ultrassônico na inativação da levedura *Pichia fermentans* em suco de tomate avaliando diferentes níveis de amplitude e tempos de tratamento com frequência constante de 20 kHz. O uso do ultrassom, em temperaturas moderadas, permitiu uma redução de 5 ciclos logarítmicos, redução mínima exigida pelo FDA para sucos de frutas, em 7,5 minutos na amplitude de 61 μm. Os autores atribuem a inativação da levedura a fenômenos físicos e químicos que ocorrem durante a cavitação. Porém, os mecanismos físicos não são tão efetivos para a destruição de células de levedura devido à relativa rigidez da sua parede celular que dificulta o rompimento da célula pelo *microstreaming*.

1.2.1.2 *Inativação/Ativação de enzimas*

Muitas características de qualidade dos alimentos como sabor, cor, viscosidade ou textura são afetadas pela atividade de enzimas específicas. A atividade da enzima peroxidase, por exemplo, é responsável pelo surgimento de *off-flavour* e mudanças de cor indesejáveis em vegetais (OMS-OLIU *et al.,* 2008).

Tecnologias não térmicas são empregadas com o intuito de reduzir ou mesmo inativar uma enzima indesejável sem a perda do sabor e/ou textura do alimento *in natura.*

Vários estudos comprovaram a eficácia do ultrassom para a inativação de enzimas em sistemas alimentícios (TEREFE *et al.*, 2009; JANG; MOON, 2011; FONTELES *et al.*, 2012). No estudo publicado por ERCAN e SOYSAL (2011) foi observada uma redução de 100% da atividade peroxidásica de tomate utilizando amplitude de 10 μm por 90 segundos a 70 °C. Os autores afirmaram, ainda, que a taxa de inativação enzimática aumenta proporcionalmente com a potência ultrassônica.

TIWARI *et al.*, (2009) investigaram a inativação termo-ultrassônica de pectinametilesterase (PME) em suco de laranja com frequência de 20 kHz, amplitude de 65μm e temperaturas entre 50 e 75 °C. A temperatura e o ultrassom combinados agiram sinergicamente na redução da atividade de PME. Além da inativação enzimática, os autores observaram uma maior estabilidade das partículas em suspensão do suco. A maior estabilidade das partículas em suspensão do suco à redução das mesmas após o tratamento ultrassônico. Além disso, os autores sugerem que a atividade da enzima PME e suas interações com o seu substrato (pectina) tenham um grande impacto na estabilidade do suco de laranja.

Por outro lado, diversas pesquisas têm comprovado o aumento da atividade de enzimas livres sob irradiação ultrassônica (SAKAKIBARA *et al.*, 1996). O aumento de atividade enzimática pode ser atribuído ao rompimento das células ocasionado pelas ondas ultrassônicas que proporciona maior contato da enzima com o substrato. O'DONNELL *et al.*, (2010) ressaltaram que a estabilidade das enzimas ao tratamento ultrassônico varia de acordo com o tipo de enzima, das condições de tratamento, da composição do meio, do pH e do tipo de ligação que elas estabelecem com outras moléculas.

Aintensidade cavitacional tem sido um dos principais mecanismos propostos de inativação enzimática de alimentos submetidos ao tratamento ultrassônico. A formação de bolhas de cavitação resulta em força de atrito que pode desnaturar as proteínas. As enzimas também podem ser desnaturadas pelos radicais livres gerados durante a sonólise das moléculas de água (SUSLICK, 1989).

1.3 Efeito sobre compostos bioativos

A relação entre alimentação e saúde tornou-se uma das principais preocupações da população e da comunidade científica, que buscam nos componentes bioativos dos alimentos a capacidade de reduzir o risco de doenças. Compostos bioativos são definidos como substâncias presentes nos alimentos que exercem influência em processos biológicos do organismo que por sua vez, resultam em benefícios à saúde humana (MÖLLER *et al.*, 2008).

O processamento dos alimentos através de tratamentos térmicos geralmente reduz o seu valor nutritivo pela destruição ou remoção parcial de seus nutrientes. O desenvolvimento de novos processos capazes de tornar o alimento seguro e que possa adicionalmente, proteger os compostos bioativos é um desafio para a indústria de alimentos.

O ultrassom é considerado uma tecnologia não-térmica aplicável em diversos processos na indústria de alimentos como, por exemplo, na extração de compostos de interesse.

DUBROVIĆ *et al.,* (2011) investigaram a influência do processamento ultrassônico na concentração e estabilidade de antocianinas em suco de morango. Diferentes parâmetros de processamento ultrassônico (amplitude, tempo e temperatura) foram otimizados e o efeito do processamento comparado à pasteurização (85°C/2 min). Foi observado um decréscimo de 0.7–4.4% na concentração de antocianinas relacionado ao aumento de temperatura durante a sonicação. A amplitude também apresentou efeito significativo na resposta estudada. A concentração total de antocianinas diminuiu quando foram utilizados amplitude e tempo de tratamento maiores. As cavidades formadas durante a sonicação podem ser preenchidas com vapor d'água e outros gases dissolvidos no suco como O_2 e N_2. Quando a amplitude ultrassônica é maior, uma maior quantidade de energia e intensidade é aplicada no sistema sonicado (suco), causando o colapso das bolhas de cavitação. Como consequência, o vapor e os gases contidos nas cavidades são dissipados no sistema, acelerando muitas reações químicas como a degradação de antocianinas. Somente quando o tempo e a temperatura de processamento foram maiores a degradação de compostos bioativos foi maior do que nos sucos pasteurizados.

No estudo de SUN *et al.,* (2010) foi demonstrado que a taxa de degradação do β-caroteno é menor quando há um aumento na intensidade ultrassônica e temperatura. Os autores afirmaram que em maiores potências de ultra-som, as bolhas formadas durante a cavitação podem ser muito grandes para colapsarem ou essas bolhas colapsam menos violentamente, o que pode causar uma redução dos efeitos da cavitação. Nessas condições, as bolhas podem ainda, atrapalhar a propagação das ondas ultrassônicas.

A extração de compostos bioativos assistida por ultrassom tem sido reportada como um processo eficiente que pode ser conduzido em um tempo reduzido com baixo consumo de solvente. O aumento da extrabilidade através do ultrassom é atribuído ao efeito cavitacional e a um efeito mecânico, permitindo maior penetração do solvente na matriz sólida, aumentando a superfície de contato entre a fase sólida e líquida (ABID *et al.*, 2014; ARAÚJO *et al.*, 2013; WANG *et al.*, 2008).

O processo de transferência de massa em sistemas de extração sólido-líquido envolve duas etapas principais. A primeira etapa envolve o processo de lixiviação de compostos solúveis aderidos à superfície sólida. A segunda etapa consiste na difusão de substâncias

intracelulares do sólido para o solvente. Esta etapa é considerada como um fator limitante em muitos processos de extração sólido-líquido devido à reduzida taxa de extração. O ultrassom pode aumentar a eficiência de extração uma vez que, devido à implosão das bolhas de cavitação, ocorre a ruptura de estruturas celulares (CHEUNG *et al.*, 2013). A Figura 2 ilustra a formação e o colapso das bolhas de cavitação.

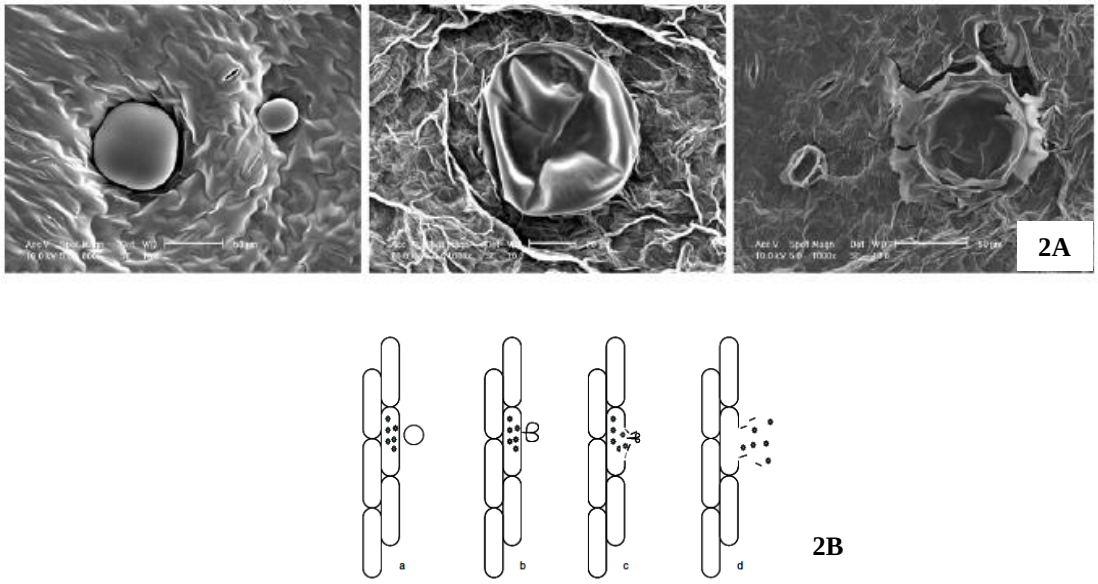

Fig.2 Colapso da bolha de cavitação (2A) e exemplo de como a bolha de cavitação age no tecido da planta (2B).

Fonte: CHEMAT, HUMA, KHAN, 2011.

LE e LE (2012) utilizaram o processamento ultrassônico para extrair vitamina C e compostos fenólicos de acerola utilizando como solvente a água. O ultrassom (15 $W.g^{-1}$/6min a 50°C) proporcionou um aumento de 40 e 12,5% nos teores de vitamina C e compostos fenólicos, respectivamente em relação à fruta *in natura*. Os autores compararam os resultados à extração enzimática observando um maior rendimento em um menor tempo de extração para a extração assistida por ultrassom. A redução do tempo de reação é um fator importante para a extração de compostos bioativos termolábeis, pois implica em menor tempo de reação.

O processamento ultrassônico também pode levar à perda de vitamina C (ADEKUNTE *et al.*, 2010) e de compostos fenólicos (FONTELES *et al.*, 2012). A degradação de ácido ascórbico se deve principalmente a reações sonoquímicas e a condições de temperatura e pressão extremas que ocorrem durante a sonicação.

Radicais livres, íons H^+ e peróxido de hidrogênio formados durante a cavitação podem afetar a bioatividade de componentes alimentícios, como os compostos fenólicos (WAN *et al.*, 2005). ABID *et al.,* (2014) atribuíram um aumento na concentração de compostos fenólicos em suco de maçã sonicado à ruptura de paredes celulares, liberação de compostos fenólicos associados e possível ligação de radicais hidroxila ao anel aromático dos compostos fenólicos.

1.4 Efeito sobre características sensoriais

Durante a escolha de uma potencial tecnologia para o processamento de alimentos devem ser avaliados os possíveis efeitos adversos à qualidade sensorial, parâmetro crucial para a aceitação do produto pelo consumidor.

ADEKUNTE *et al.,* (2010) reportaram mudanças na cor de suco de tomate após tratamento ultrassônico. A sonicação resultou em decréscimo nos valores dos parâmetros L*, a* e b* e um aumento no valor de ΔE, indicando haver um escurecimento do suco resultante da degradação de compostos carotenóides (licopeno). COSTA *et al.,* (2011) atribuíram as mudanças de cor de suco de abacaxi submetido ao processamento ultrassônico ao rompimento celular que libera o conteúdo intracelular podendo assim,desencadear alterações na cor do produto. Por outro lado, o ultrassom permitiu a estabilização da cor do suco de abacaxi durante 42 dias de estocagem refrigerada devido à inativação da enzima polifenoloxidase, principal responsável pelo escurecimento enzimático de vários produtos de origem vegetal.

Características de textura dos alimentos também podem ser modificadas através do uso do ultrassom. DAY *et al.*, (2012) aplicaram o ultrassom como pré-tratamento com o intuito de melhorar as características mecânicas de cenouras enlatadas e autoclavadas. Cenouras sonicadas por 10 minutos a 60 °C em banho ultrassônico (40 kHz/ 0,06 W/mL) apresentaram maior força mecânica da parede celular comparado ao branqueamento pelo mesmo período de tempo. Após o pré-tratamento, as células apresentaram redução da pressão de turgescência, devido à mudança de pressão durante a sonicação. Logo, as cenouras tornavam-se mais deformáveis e menos resistentes à compressão e alongamento quando comparadas às cenouras não tratadas. Um material mais deformável irá requerer uma maior força para fraturar do que um material rígido.

Dureza e crocância são parâmetros de textura muito apreciados pelos consumidores de produtos desidratados. SHAMAEI *et al.,* (2012) verificaram um aumento na maciez (redução da dureza) de *cranberry* desidratada osmoticamente submetida ao pré-tratamento ultrassônico.

O efeito na textura da fruta variou de acordo com a frequência ultrassônica utilizada. Em frequências mais baixas (35 kHz) observou-se a formação de canais microscópicos, porém a utilização da frequência de 130 kHz causou danos e rupturas mais intensas ao tecido levando ao colapso da estrutura celular.

1.5 Ultrassom como ferramenta auxiliar nos processos de secagem

A retirada da água por um método de secagem adequado é de grande vantagem no que diz respeito à diminuição de custos com transporte, armazenamento e embalagem, facilitando assim, sua manipulação e distribuição. Com a diminuição da atividade de água também ocorre um aumento do tempo de vida útil do produto, uma vez que são inibidas reações enzimáticas e as atividades microbianas (GOULA; ADAMOPOULOS, 2010).

Por outro lado, a redução do conteúdo de água do produto leva a alterações de textura e sabor que muitas vezes impactam na aceitação do alimento pelo consumidor. Logo, o desafio dos pesquisadores é implementar melhorias no processo de secagem a fim de que o produto seja apreciado no que se refere à qualidade nutricional e sensorial além de atender aos requisitos necessários para que se mantenha estável durante o armazenamento.

O uso de pré-tratamentos tem sido investigado como uma forma de auxiliar o processo de secagem e consequentemente melhorar a qualidade final do produto. A tecnologia ultrassônica, por exemplo, foi utilizada por diversos pesquisadores de forma a diminuir o tempo e os custos de secagem (TELES *et al.*, 2006; OLIVEIRA *et al.*, 2006; NOGUEIRA *et al.*, 2010).

O efeito cavitacional produzido pelas ondas ultrassônicas pode ser benéfico para a remoção de umidade que é fortemente ligada à matriz sólida. A deformação de materiais sólidos porosos, tais como frutas, causada por ondas ultra-sônicas, é responsável pela criação de canais microscópicos que reduzem a camada limite de difusão e aumentam a transferência de massa por convecção no fruto (FUENTE-BLANCO *et al.*, 2006).

Ainda no que tange aos efeitos promovidos pelas ondas ultrassônicas, OLIVEIRA *et al.*, (2011) mostraram que a aplicação dessa tecnologia previamente à secagem de jambo promoveu aumento de aproximadamente 30% na difusividade efetiva da água, reduzindo assim, o tempo necessário para a secagem. Este resultado corrobora com a observação de RODRIGUES *et al.*, (2009b) de que o uso do ultrassom induz a perda de adesão celular, formação de espaços intercelulares e a ruptura das paredes celulares (Fig. 3).

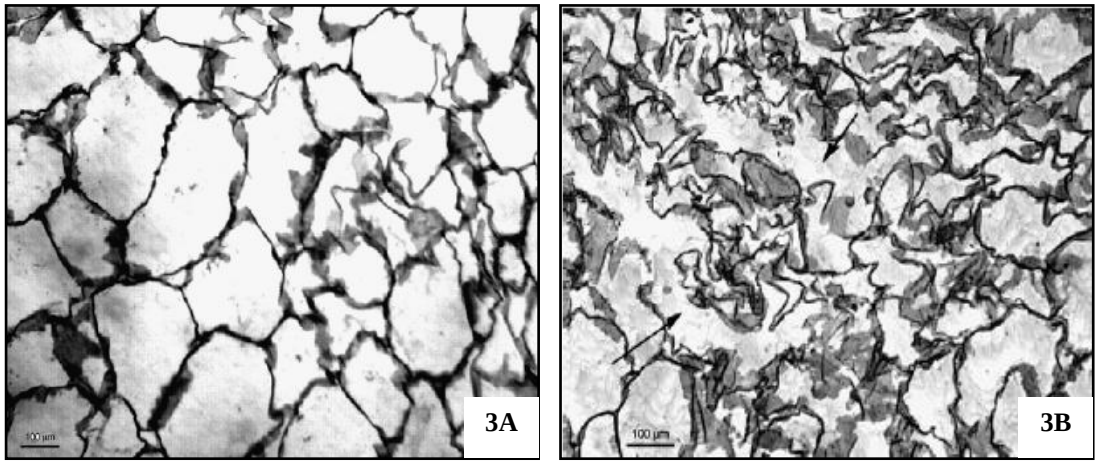

Fig.3 Micrografia de células de abacaxi evidenciando a formação de canais microscópicos após processamento ultrassônico (4870 W/m^2 por 30 minutos). Controle (3A). Células submetidas ao ultrassom (3B). As setas indicam os canais microscópicos.

Fonte: FERNANDES; GALLÃO; RODRIGUES (2009).

As mudanças estruturais acima mencionadas desencadeiam no aumento na difusividade da água uma vez que o tecido da fruta oferece baixa resistência à difusão, facilitando assim a perda de água. Os autores observaram ainda uma maior perda de água durante a desidratação osmótica de mamão pré-tratado por ultrassom.

RODRIGUES *et al.*, (2009a) obtiveram um aumento de 23,1% na taxa de difusividade efetiva durante a secagem de sapota por ar forçado, após o pré-tratamento ultrassônico. Este aumento foi menor do que os valores reportados para abacaxi (64,3%) e melão (39,3%). Segundo os autores, a diferença observada pode estar relacionada ao comprimento das células alongadas da sapota, que é menor em relação às células de abacaxi ou melão.

NOSHAD *et al.*, (2013) estudaram o impacto do pré-tratamento ultrassônico nas isotermas de dessorção de marmelo desidratado osmoticamente. O estudo das isotermas de equilíbrio é de fundamental importância na prática de armazenamento ou secagem de alimentos. Através do calor isostérico de sorção, por exemplo, é possível estimar a demanda de energia necessária para o processo de desidratação. Os resultados mostraram que o processamento ultrassônico diminuiu a umidade de equilíbrio, o calor isostérico, a entropia diferencial e a *spreading pressure*[1] do produto o que significa que a energia necessária para

[1]*Spreadingpressure*(φ) é uma nova constante termodinâmica definida como a energia responsável pela difusão da água pelos poros do material durante o processo de sorção e depende da temperatura e da atividade de água (Aw), sendo usado na interpretação da cinética de sorção (ASCHERI *et al.*, 2009).

desidratar o marmelo até um valor de atividade de água que torne o alimento estável será menor em relação ao marmelo sem pré-tratamento.

1.6 Tendências futuras

As tecnologias não térmicas de processamento de alimentos surgiram com o intuito de ser uma ferramenta auxiliar ao processamento térmico, uma vez que as altas temperaturas utilizadas afetam a qualidade sensorial e nutricional dos alimentos. De acordo com o conhecimento científico recente, o emprego de ondas sonoras no processamento de alimentos representa uma alternativa rápida, eficiente e confiável para atender à demanda por alimentos nutritivos com características similares às do alimento *in natura.* Uma melhor compreensão dos mecanismos físico-químicos, a otimização de parâmetros operacionais e a avaliação dos possíveis efeitos no alimento são fundamentais para que se possa explorar o potencial de uso desta tecnologia para melhorar características tecnológicas e funcionais de alimentos como os frutos tropicais e seus derivados.

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Capítulo 2

Sonication effect on bioactive compounds of cashew apple bagasse

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Abstract

In this study, high intensity ultrasound was applied on cashew apple bagasse (*Anacardium occidentale* L.) changing the bagasse to water ratio, the ultrasound power intensity (W/cm^2) and the processing time (min) according to a factorial experimental planning. Bioactive compounds such as vitamin C, phenolic compounds and β-carotene were the evaluated responses. Total antioxidant capacity was determined by 2,2-diphenyl-1- picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammoninum salt (ABTS) methods. A thermal treatment was done at the highest temperature reached after sonication (51^oC) to evaluate the heat effect due to temperature increase during the sonication. Sonication changed the bagasse aspect from a fibrous residue to a pleasant yellow puree. The maximal concentration of vitamin C, phenolics and β-carotene was obtained when the bagasse:water ratio was 1:4, the ultrasound power intensity 226 W/cm^2 and 6 minutes of processing. The high total phenolic content (2186 mg of gallic acid/100 g DW), vitamin C (148 mg/100 g DW) and β-carotene (12 mg/100g) obtained proved the sonication efficiency. Furthermore, the antioxidant activity determined by the DPPH and ABTS assays confirmed the suitability of ultrasound for the preparation of antioxidant-rich cashew apple bagasse puree.

Keywords: high intensity ultrasound, extraction, antioxidants, cashew apple bagasse, bioactive compounds

2.1 Introduction

Cashew (*Anacardium occidentale* L.) is an important tropical fruit crop in Brazil. According to the official data, Brazil processes around 250 thousand tons of cashews every year (CASHEWINFO, 2014). The most valuable product of cashew industry is the cashew nut. The cashew apple, also known as cashew peduncle or pseudofruit, corresponds to about 90% of the whole fruit weight and is composed of carbohydrates, dietary fiber, vitamins (mainly vitamin C), minerals and antioxidant phenols (ASSUNÇÃO; MERCADANTE, 2003; RABELO; FONTES; RODRIGUES, 2009). The cashew apple is juicy, has a pleasant flavor and, besides being direct consumed, it can be industrialized as juice, jams and ice creams.

The main product obtained from the cashew apple is the cashew juice commercialized as frozen pulp, read to drink juice and nectar (QUEIROZ *et al.*, 2011).The juice is obtained by pressing the apple into an expeller press (GUILHERME *et al.*, 2009). The residue of the juice production is the cashew apple bagasse, which correspond to about 20% of the cashew apple weight (SANTOS *et al.*, 2007). After the industrial processing, only small amounts of this by-product is used, usually as nutritional complement for animal feed. The cashew apple bagasse is rich in fibers and retains high levels of nutrients and bioactive compounds. Such residue could be utilized by the food industry, minimizing possible environmental problems and generating a new food source.

Ultrasound processing has been studied in several food processing applications because it is able to intensify the color and the flavors of food matrices. This technology may also contribute to the food stabilization and preservation with good retention of bioactive compounds (ABID *et al.*, 2014; ASHOKKUMAR *et al.*, 2008; FONTELES *et al.*, 2012; KEENAN *et al.*, 2012). The effects caused by sonication in aqueous media are mainly attributed to the cavitation phenomenon that can be useful for food quality enhancement.

Ultrasound produces repeated cycles of compression and decompression called acoustic cavitation. The cavitation can generate high local temperatures and high pressures, which can accelerate the heat and mass transfer rate to disrupt cell walls and facilitate the release of the target extractable compounds (CHEMAT; HUMA; KHAN, 2011). The sonication can be affected by several factors, such as particle size, solvent, temperature, processing time, power intensity level and others (FONTELES *et al.*, 2012). The high potency ultrasound processing has been applied to extract bioactive compounds from vegetal tissues due to its ability of cell disruption.

As cashew apple bagasse represents a potential, abundant and low cost source of bioactive compounds, in this study, ultrasound processing was applied to release antioxidant compounds enhancing the nutritional value of this residue. Despite several works have been published on ultrasound processing of fruits and fruit products, very few are addressed to sonication of fruit wastes to be used as food source, and the sonication of cashew apple bagasse was not reported elsewhere. In addition, there is a growing scientific interest on the influence of ultrasound on nutritional quality of food matrix. Therefore, the purpose of the present work was to develop an ultrasound processing for the cashew apple bagasse, to be used a nutritious raw material in the food industry. The parameters that could potentially influence the process were optimized using a statistical experimental design approach.

2.2 Materials and Methods

2.2.1 Sample preparation

Red cashew apples were harvested at commercial maturity stage in Ceará State, Brazil, from September to December 2012. 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. The peduncles were not protected from light to simulate the conditions that the fruit may be exposed in the postharvest period. The solid residues (bagasse) were packaged in polyethylene bags, vacuum sealed and stored at −18°C. Prior to the experiment, the cashew apple bagasse was thawed at 4°C.

2.2.2 Ultrasound processing

The processing was carried out with a 500W ultrasound processor (Unique® DES500, São Paulo, Brazil) with a 1.3 cm diameter probe tip, changing the bagasse to water ratio, the ultrasound power and the processing time. The experiments were carried out in separate 600 mL Becker flasks (80mm diameter x 165mm height). The ultrasound probe was submerged to a depth of 25 mm in the sample (200 mL). The intensity of ultrasound power dissipated from the probe tip was calculated by Eq. 1(LI; PORDESIMO; WEISS, 2004).

$$I = \frac{P}{\pi r^2} \qquad \textbf{Eq. 1}$$

Where r is the radius of the titanium tip (cm) and P is the input power level (W). The input power was controlled through the amplitude setting and the power levels were adjusted to 20%, 60%, and 100% of total input power (500 W), which was equal to 100, 300 and 500 W. The calculated intensities were 75, 226 and 373 W/cm^2, respectively.

2.2.3 Experimental design and data analysis

The effects of ultrasound processing were evaluated through a 2^3 face-centered central composite experimental design (CCD) with 3 central points. The power intensity, the bagasse:water ratio (g/g) and the processing time were changed from 75 to 373 W/cm^2, 1:2 to 1:4 and 2 to 10 min, respectively. For all treatments, the initial temperature of the mixture was 20°C. After sonication, the temperature was recorded by a digital thermometer. Experiments using ultrasound have been performed and compared with those not using ultrasound (control process).

Control samples for each bagasse:water ratio (1:2, 1:3, 1:4) were prepared. The samples were submitted to only water immersion without sonication, kept in ambient temperature for 10 min. Vitamin C, total phenolic compounds (TPC), β-carotene, ABTS and DPPH were determined.

2.2.4 Extract preparations

The extracts for total phenolic compounds (TPC) and antioxidant activity were prepared according to the procedure developed by LARRAURI, RUPÉREZ and SAURA-CALIXTO(1997). Fresh and sonicated samples were weighed (2g) in centrifuge tubes and extracted sequentially with methanol and acetone aqueous solution. Firstly, 10 mL of methanol/water (50:50, v/v) was added to the sample at 20 °C. The mixture was kept statically for 1 hour at 20 °C and the extraction was allowed for 60 min. The samples were centrifuged at 10733g (15 min/ 4°C) and the supernatant was recovered. Then, 10 mL of acetone/water (70:30, v/v) was added to the solid residue at 20 °C, extracted for 60 min and centrifuged. Methanol and acetone extracts were combined into a 25 mL volumetric flask, which volume was completed with distilled water.

For β-carotene determination, the extracts were prepared according to the method adapted from DIETZ, SRI KANTHA and ERDMAN(1988). The samples were homogenized with acetone at 4°C for 10 min. Then, 5 mL of a hexane-ethyl alcohol mixture (1:1) was added and centrifuged immediately at 2900g for 5 min at 4°C. The supernatants were concentrated in vacuum evaporator at 45^{o}C (MARTIN-CHRIST RVC 2-18 HCl, Osterode, Germany).

All procedures were done at dark ambient to avoid light decomposition of the bioactive compounds.

2.2.5 Determination of vitamin C

After sonication the samples (5g) were centrifuged at 11.806g (10 min/4°C) in a Sigma 6K-15 centrifuge (Sigma Centrifuges, Germany). The supernatant was used for ascorbic acid determination the analytical determinations by high performance liquid chromatography in a Agilent 1260 Infinity system equipped with four high-pressure pumps model Agilent G1311B, UV–VIS detector ProStar model 345, and column oven (Agilent G1316A). Separations were done using a Biorad HPX 87 H (300×7.8 mm) column at 50°C. $H_2SO_4$0.01N at 0.6 mL/min was used as eluent. All samples were analyzed in triplicate. The software Agilent OpenLAB was used to acquire and handle the data. Results were expressed as milligrams per 100 g DW (dry weight).

2.2.6 Determination of Total Phenolic Compounds

Total phenolic compounds were determined using the Folin-Ciocalteau methodology (OBANDA; OWUOR, 1997). The reaction mixture contained: 250 μL of the phenolic extract, 500 μL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), 500 μL of sodium carbonate and 500 μL of distilled water. The mixture was then left in the darkness for 30 min at 25°C. The absorbance of the sample was measured at 700 nm. Gallic acid (HPLC grade, Sigma-Aldrich) was used as standard. Results were expressed as gallic acid equivalent (GAE), milligrams per 100 g DW.

2.2.7 Determination of β-carotene

β-carotene was analyzed by high performance liquid chromatography (HPLC) in an Agilent 1260 Infinity system equipped with four high-pressure pumps model Agilent G1311B, diode array detector model G4212B and column oven (Agilent model G1316A). Separation was achieved using a C18 ACE® column at 30 °C (ACE121-2546; 250 mm x 4.6 mm). Acetonitrile:methanol:chloroform (47:47:6) at 1 $mL.min^{-1}$ was used as eluent. All readings were taken at 453 nm. The identification of β-carotene was made using retention time comparison with commercially available standard (Sigma Chemical Co.,St. Louis, MO). The software Agilent OpenLAB was used to acquire and handle the data. The results were expressed as mg of β-carotene/100g DW.

2.2.8 Expression of results

The residual concentrations of all antioxidant compounds (vitamin C, TPC, β-carotene) were calculated according to Eq. 2. All determinations were done in triplicate and the results were residual mean values ± standard deviations.

$$\text{Residual } (\%) = \frac{C_0 - C_S}{C_0}.100 \qquad \textbf{Eq. 2}$$

Where the sub indices *0* and *s* in Eq. (2) mean the control sample (non-treated) and sonicated one, respectively. The results of the three control experiments at different bagasse:water ratio 1:2; 1:3 and 1:4 were also evaluated and the residual concentration was calculated for each bagasse:water ratio.

2.2.9 Total antioxidant activity determinations

For ABTS assay, the procedure followed the method of RUFINO *et al.* (2010). This method measures the ability of lipophilic and hydrophilic antioxidants to quench a 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid radical cation. The stock solutions included 7 mM ABTS solution (solution A) (Sigma®) and 140 mM potassium persulfate solution (solution B) (Sigma®). The working solution was prepared by mixing 5000 µL of the solution A and 88

μL of solution B and allowing them to react for 16 h at 20 °C in the dark. The solution was then diluted by mixing $ABTS^{\bullet+}$ solution with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. The samples extracts (30μL) were allowed to react with 3000μL of the ABTS solution for 6 min in a dark condition. The level of radical scavenging was calculated according to the following equation:

$$\text{Scavenging rate}(\%) = (1 - A_i/A_s) \times 100 \qquad \textbf{Eq. 3}$$

Where A_s is the absorbance of pure ABTS, A*i* is the absorbance of ABTS in the presence of sample.

The DPPH scavenging activity was done according to the method of BRAND-WILLIAMS; CUVELIER; BERSET(1995) with some modifications. Attaching an H• atom, removed from the antioxidant, it is possible to observe a decrease in absorbance, which allows to calculate, after the establishment of reaction equilibrium, the amount of antioxidant spent to reduce 50% of DPPH radical. The stock solution was prepared by dissolving 2.4 mg of DPPH (Sigma®) with 100 mL of methanol. Sample extracts (50 μL) were allowed to react with 150 μL of the DPPH solution for 30 min in the dark. Then the absorbance was determined at 515 nm. The decrease in optical density values of the samples was correlated to the control and set the percentage of discoloration of DPPH radical. The scavenging of free radicals was expressed as scavenging rate as done for ABTS (Eq. 3).

2.2.10 Temperature effect

To evaluate the temperature effect on responses evaluated without the ultrasound interference, an assay was carried out at 51 °C, which was the highest temperature, recorded after the cashew apple bagasse sonication at the optimized (226 W/cm^2 for 6 min). The samples were kept in a water bath at the desired temperature for 10 min. Vitamin C, TPC, β-carotene, ABTS and DPPH were determined as described earlier.

2.2.11 Statistical analysis

All experimental assays were carried out in triplicate. Results were expressed as mean±SD. Statistical analysis of the experimental data was carried out using the software.

Statistica 7.5 (Statsoft). F-test and ANOVA analysis were used as significant criteria for the fitted models.

2.3 Results and discussion

2.3.1 *Effect of ultrasound process on bioactive compounds*

Figure 1 shows the cashew apple peduncle, the bagasse (raw material) and the sonicated cashew apple bagasse.

Fig.1 Cashew apple peduncle and nut (1A); cashew apple bagasse (1B) and sonicated cashew apple bagasse (1C).

Table 1 shows the initial concentrations of antioxidant compounds (vitamin C, phenolics and β-carotene) at different bagasse:water ratios evaluated. The results obtained with the control samples, were used as reference to calculate the residual values depicted in Tables 2, 3 and 4.

Table 1 Vitamin C, total phenolic compounds (TPC), β-carotene, DPPH and ABTS of control samples.

Bagasse:water ratio	Vitamin C mg/100g	TPC mg/100g	β-carotene mg/100g	DPPH scavenging rate %	ABTS scavenging rate %
1:2	109.11 ± 0.13	768.51 ± 0.98	12.8 ± 0.01	71.42 ± 0.00	25.89 ± 0.01
1:3	118.25 ± 0.03	792.68 ± 0.12	12.7 ± 0.04	73.21 ± 0.08	45.78 ± 0.06
1:4	135.78 ± 0.09	817.31 ± 0.02	7.6 ± 0.06	79.00 ± 0.01	43.43 0.02

The enhancement in the concentrations of vitamin C, phenolics, β-carotene, DPPH scavenging rate and ABTS scavenging rate of control samples in Table 1 is attributed to the concentration gradient between bagasse and water and thus, an increase rate of mass transfer is observed.

2.3.1.1 *Vitamin C*

Table 2 shows the residual concentrations of vitamin C after ultrasound processing.

Table 2 Experimental design and responses of the influence of ultrasonic process on vitamin C of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
				Vitamin C
1	75	2	1:2	81.51 ± 0.08
2	75	10	1:2	78.99 ± 1.67
3	373	2	1:2	75.27 ± 0.99
4	373	10	1:2	69.12 ± 0.24
5	75	2	1:4	105.97 ± 0.76
6	75	10	1:4	104.80 ± 0.56
7	373	2	1:4	104.26 ± 0.97
8	373	10	1:4	106.63 ± 1.67
9	226	6	1:2	83.50 ± 0.54
10	226	6	1:4	109.18 ± 0.57
11	75	6	1:2	91.80 ± 0.76
12	373	6	1:3	87.78 ± 0.21
13	226	2	1:3	102.75 ± 0.12
14	226	10	1:3	99.15 ± 0.53
15 (C)	226	6	1:3	98.40 ± 0.36
16 (C)	226	6	1:3	95.00 ± 1.04
17 (C)	226	6	1:3	87.43 ± 0.45

The estimated effects of the independent variables showed the significance of bagasse:water ratio on vitamin C concentration after sonication. The proportion of bagasse to water presented the more pronounced effect on vitamin C (confidence level of 95%). A linear increase in water concentration promoted higher vitamin C extraction from cashew apple bagasse (Fig. 2).

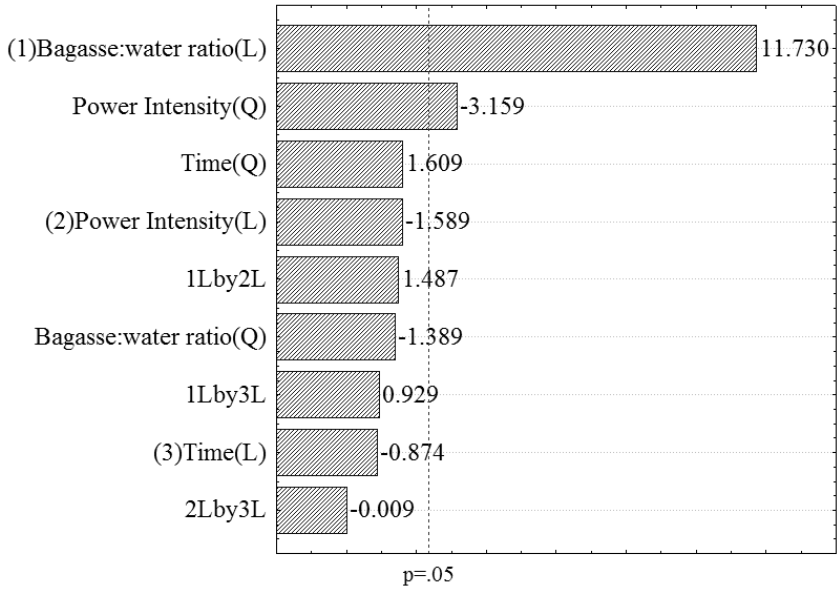

Fig.2 Pareto chart for the effect of sonication of cashew apple bagasse on vitamin C. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

The water addition improves the cavitation and the ultrasound interaction in the solid-fluid interfaces producing microjets, which are formed in the immediate vicinity of the solid surface. The microjets cause the reduction of the diffusion boundary layer thickness (MULET *et al.*, 2003). This phenomenon improved the extraction rates. YE *et al.* (2011) reported that mass transfer speed of solute with ultrasound assistance is faster than that without ultrasound during the extraction of carotenoids from corn.

The quadratic effect of the power intensity was negative on vitamin C extraction ($p<0.05$) because at higher ultrasonic power intensities, the sonication increases vitamin C degradation. In fact, the highest degradation rate (≈30%) of vitamin C was observed at the maximum power intensity and processing time conditions (373W/cm^2, for 10 minutes) (Table 2). This is consistent with the reports from TIWARI *et al.* (2009) where sonication reduced the ascorbic acid content of orange juice by 11% at the maximal treatment conditions (0.61 W/mL for 7 min.). Vitamin C degradation could be due to thermolysis or combustion occurring inside the cavitation bubble or reaction with hydroxyl radicals leading to the formation of oxidation products on the bubble surface (FERNANDES; RODRIGUES, 2012). In addition, matrix disruption due to high intensity energy of ultrasound can facilitate the

contact of vitamin C with enzymes such as ascorbic acid peroxidase, hence facilitating ascorbic acid oxidation.

The experimental data was fitted to a quadratic regression model (Eq. 4). The model was statistically significant at 95% of confidence interval since the calculated F value (16.92) was higher than the listed F value ($F_{9.7}$=3.68). Good coefficient of determination was also obtained (R^2=0.95). Fig. 2A shows the surface response graph built from Eq. 4.

$$Y = 159.71 - 313.33x + 315.77x^2 + 0.17y - 0.0003y^2 - 2.26z + 0.236z^2 - 0.10xy - 2.28xz - 0.00002yz \quad \textbf{Eq. 4}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

Fig. 3 shows the surface plot of the residual vitamin C as a function of bagasse:water ratio and power intensity at a fixed processing time level of 6 minutes.

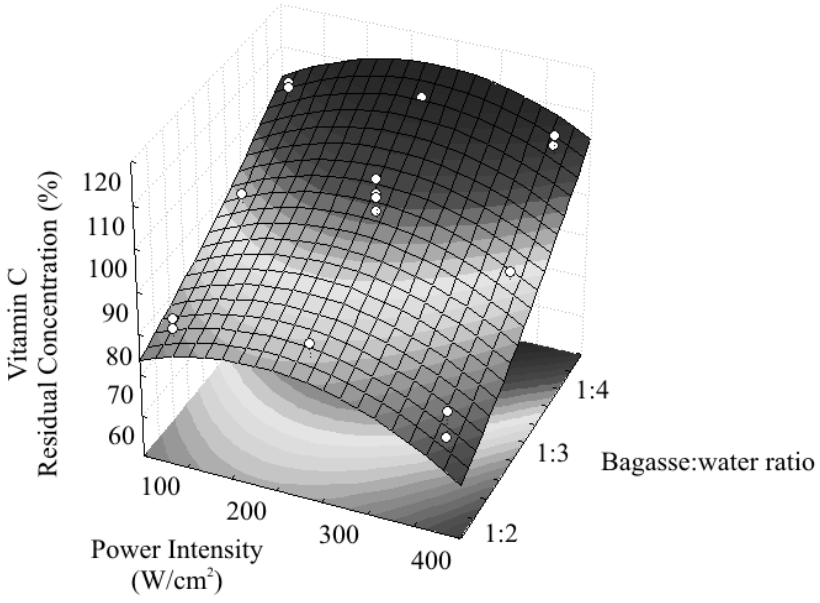

Fig.3 Response surface for the effect of sonication of cashew apple bagasse on vitamin C. The surface was plotted for a constant processing time of 6 min.

The highest level of vitamin C (148.32 mg/100g bagassse DW) was obtained at the following processing conditions: bagasse:water ratio of 1:4, at 226 W/cm^2 for 6 min (assay 10

in Table 1). At this condition an increase of 9% of vitamin C was obtained after the ultrasound processing.

2.3.1.2 *Total Phenolic Compounds*

Phenolic compounds are the most abundant hydrophilic antioxidants in the diet. These compounds have been received increasing interest due to scientific evidences about their effect on the prevention of certain chronic diseases such as cancers, neurological and cardiovascular diseases (INGH *et al.*, 2008). Phenols seem to be resistant to the effect of ultrasound processing in almost all experimental assays (Table 3). The highest yields of phenolics were achieved in assays where higher proportions of water to bagasse were applied (Table 3).

Table 3 Experimental design and responses of the influence of ultrasonic process on total phenolic compounds (TPC) of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
				TPC
1	75	2	1:2	63.04 ± 3.00
2	75	10	1:2	76.93 ± 3.36
3	373	2	1:2	72.18 ± 0.83
4	373	10	1:2	91.43 ± 1.69
5	75	2	1:4	201.06 ± 7.65
6	75	10	1:4	225.17 ± 4.99
7	373	2	1:4	214.94 ± 14.28
8	373	10	1:4	244.40 ± 11.85
9	226	6	1:2	69.00 ± 9.09
10	226	6	1:4	267.47 ± 4.77
11	75	6	1:2	166.61 ± 13.18
12	373	6	1:3	181.26 ± 7.14
13	226	2	1:3	152.39 ± 11.63
14	226	10	1:3	129.60 ± 6.56
15 (C)	226	6	1:3	120.22 ± 0.43
16 (C)	226	6	1:3	123.62 ± 1.22
17 (C)	226	6	1:3	124.24 ± 3.45

In the evaluated range, bagasse:water ratio was the main parameter that positively influenced the extraction of phenolic compounds (Fig. 4).

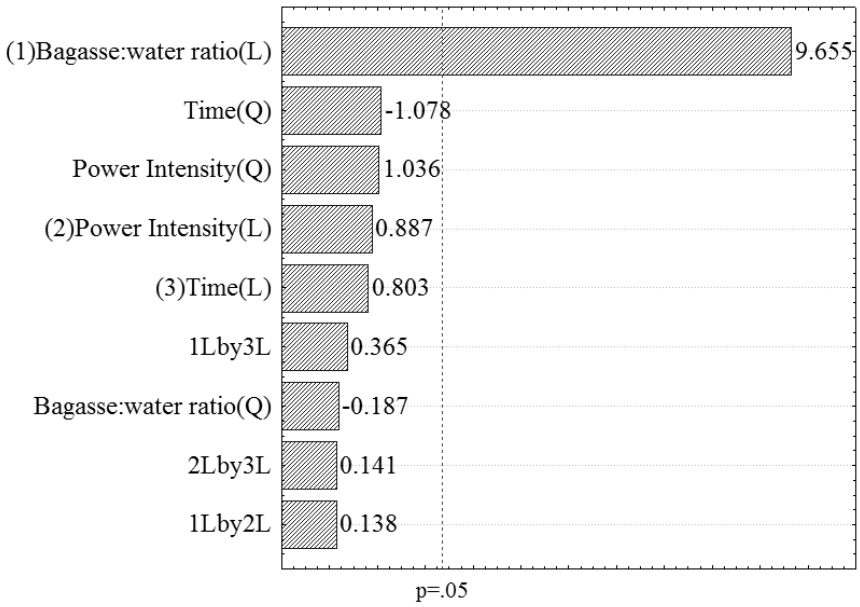

Fig.4 Pareto chart for the effect of sonication of cashew apple bagasse on total phenolic compounds. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

Conversely, marked decreases of TPC were observed when less water was added to bagasse (bagasse:water ratio of 1:2) reaching the highest degradation at experimental assay 1. According to the available reports (ANNEGOWDA *et al.*, 2011; FONTELES *et al.*, 2012) the generation of free radicals like hydroxyl and peroxyl during sonication process might possibly degrade and react with the antioxidants in the extract.

The Eq. 5 shows the relationship between the residual concentration of phenolics and the process parameters bagasse:water ratio, US power intensity and time. The model was statistically significant at 95% of confidence interval since the calculated F value (10.58) was higher than the listed F value ($F_{9,7}$=3.68).The coefficient of determination (R^2) was 0.93, showing that a high correlation was achieved.

$$Y = 712.73 - 2720.89x + 2829.84x^2 - 0.27y + 0.001y^2 + 14.43z - 1.05z^2 - 0.07xy \quad \textbf{Eq. 5}$$
$$1.86xz - 0.002yz$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively

Fig. 5 depicts the response surface plot showing the effect of US power intensity and bagasse:water ratio on the residual yield of total phenolics at the fixed time of 6 minutes.

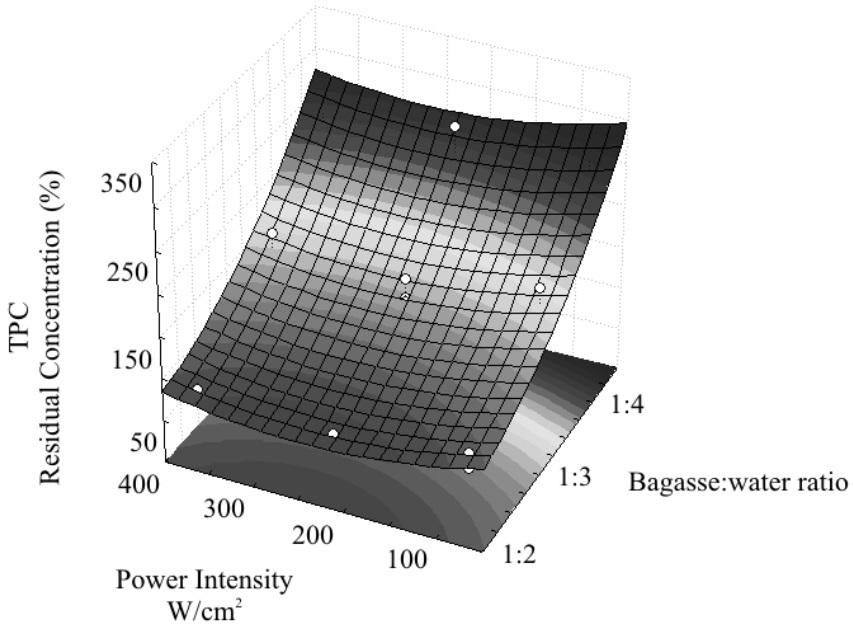

Fig.5 Response surface for the effect of sonication of cashew apple bagasse on total phenolic compounds. The surface was plotted for a constant processing time of 6 min.

For the same level of power intensity, an increase in residual phenolics concentration was observed due to water addition. ASHOKKUMAR *et al.,* (2008) reported that the antioxidant activity of components such as phenolics may increase because of the increase in the degree of hydroxylation of molecules due to radicals OH• formed in ultrasound processing. The extract obtained from sonication of cashew apple bagasse can be considered a rich source of phenolics (total phenolics = 2186.05 mg/100g DW) compared to other fruit residues such as marc grape (total phenolics = 4190 mg/100g DW) and banana peel (total phenolics = 380 mg/100g DW) and orange peel (total phenolics = 275.8 mg/100g DW) (BABBAR *et al.*, 2011; KHAN *et al.*, 2010).

It is possible that the phenolic acids contributed to the TPC in cashew apple bagasse. BROINIZI *et al.*, (2007) identified and quantified nine phenolic acids (gallic, ferulic, caffeic, protocatechuic, quinic, cinnamic, gentisic, p-coumaric and salicylic acids), from cashew apple bagasse which exhibited free-radical-scavenging activity against DPPH radical.

2.3.1.3 *β-carotene*

The carotenoids are one of the most important groups of natural pigments in fruits and vegetables and several epidemiological studies have correlated their antioxidant properties to prevention of certain diseases. The response surface methodology was not applied to evaluate the effect of sonication on β-carotene of cashew apple bagasse because the evaluated parameters effects on β-carotene content were not statistically significant at the considered confidence level (95%). Thus, response surface analysis cannot be applied. In some experimental assays, it was observed β-carotene degradation, reaching a minimal residual concentration of 42.22% at the following conditions: power intensity of 75 W/cm^2, 1:2 bagasse:water ratio during 10 min (Assay 2; Table 4).

Table 4 Experimental design and responses of the influence of ultrasonic process on β-carotene of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
				β-carotene
1	75	2	1:2	79.99 ± 8.85
2	75	10	1:2	42.22 ± 5.79
3	373	2	1:2	62.39 ± 2.89
4	373	10	1:2	87.76 ± 5.63
5	75	2	1:4	92.85 ± 8.61
6	75	10	1:4	83.69 ± 9.64
7	373	2	1:4	90.52 ± 5.99
8	373	10	1:4	76.27 ± 5.06
9	226	6	1:2	104.35 ± 3.05
10	226	6	1:4	162.96 ± 0.05
11	75	6	1:2	89.14 ± 0.45
12	373	6	1:3	100.07 ± 0.45
13	226	2	1:3	95.06 ± 2.05
14	226	10	1:3	117.00 ± 8.43
15 (C)	226	6	1:3	94.71 ± 5.41
16 (C)	226	6	1:3	92.09 ± 0.56
17 (C)	226	6	1:3	92.89 ± 0.99

Nevertheless, controversial results for β-carotene were also found as observed in Table 4. There was an increment of almost 60% in β-carotene when power intensity of 226 W/cm^2, 1:4 bagasse:water ratio during 6 min was applied (Assay 10; Table 1) reaching a final

concentration of 12.38 mg/100g DW. The same processing conditions that enhanced vitamin C content and TPC in the sonicated bagasse also increased the β-carotene content.

β-carotene is located in the chromoplast, where it is accumulated in the lipid globules (DAVIES; HOBSON, 1981). Any change in the microstructure of cashew apple bagasse during processing that involves changes in the exposition of hydrophilic structures, or by cell decompartmentation influencing the disposition of internal membranes, could induce changes in the solvent accessibility to carotenoids located in the chromoplast (FERNANDEZ GARCIA; BUTZ; TAUSCH, 2001). ABID *et al.* (2014) attributed the increase in total carotenoids of apple juice after sonication to the mechanical disruption of cell walls, enhancing free carotenoids.

YE *et al.*(2011) found that corn sonication during 30 minutes shortened the extraction time and increased the extraction rate of carotenoids compared to magnetic stirring in 300 min. This effect was attributed to the macro turbulent flow in the liquid that collides at a high speed with the solid phase and enhances the eddy diffusion and the internal diffusion speed of solvent and solute.

2.3.2 *Effect of sonication on temperature*

The response surface methodology was not applied to evaluate the effect of sonication on temperature increase of cashew apple bagasse because the effects were not statistically significant. Thus, response surface analysis cannot be applied. The maximal temperature increase was observed at 226 W/cm^2, 1:4 of bagasse:water ratio at 6 min, the optimum operating condition for vitamin C and phenolics extraction.

Table 5 Experimental design and responses of temperature increase due of cashew apple bagasse sonication

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	ΔT (°C)
1	75	2	1:2	2.7 ± 0.09
2	75	10	1:2	5.4 ± 0.10
3	373	2	1:2	2.4 ± 0.05
4	373	10	1:2	12.3 ± 0.02
5	75	2	1:4	3.1 ± 0.01
6	75	10	1:4	11.7 ± 0.01
7	373	2	1:4	7.6 ± 0.00
8	373	10	1:4	21.8 ± 0.80
9	226	6	1:2	8.0 ± 0.06
10	226	6	1:4	30.9 ± 0.00
11	75	6	1:2	5.9 ± 0.03
12	373	6	1:3	4.4 ± 0.05
13	226	2	1:3	2.5 ± 0.00
14	226	10	1:3	28.5 ± 0.01
15 (C)	226	6	1:3	22.8 ± 0.10
16 (C)	226	6	1:3	23.2 ± 0.08
17 (C)	226	6	1:3	21.7 ± 0.06

The energy liberated by the sonication may have caused a synergistic effect on extraction of antioxidants compounds. AYBASTIER *et al.*(2013) suggested that high temperatures increase the diffusion and solubility rate of many compounds resulting in antioxidant compounds being extracted at a higher rate.

TABARAKI; HEIDARIZADI and BENVIDI (2012)reported that the mild heating caused by implosion of cavitation bubbles during US processing soften the plant tissue and weak the cell wall integrity causing the releasing of the bounded phenolic compounds from other biomolecules (proteins or carbohydrates) and, consequently, enhancing their solubility. WANG*et al.* (2008) observed a significant increase of the total phenolic content over the extraction temperature range (25-75°C), and the total phenolic content reached a maximum of around 2.80 mg GAE/g of wheat bran at 65 °C. The authors attributed the efficiency of ultrasonication to the fact that sonication simultaneously enhanced the hydration and fragmentation process while facilitating mass transfer of solutes to the extraction solvent.

2.3.3 Measurement of total antioxidant activity

High-intensity ultrasound is known as a physical elicitor with a variety of biological effects on the cells including the increase of ROS (COMARELLA *et al.*, 2012; SAFARI *et al.*, 2013). Table 6 presents the experimental results of antioxidant activity by DPPH and ABTS methods.

Table 6 Experimental design and responses of the influence of ultrasonic process on antioxidant activity of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Scavenging rate (%)	
				DPPH	ABTS
1	75	2	1:2	84.00 ± 0.00*	76.92 ± 0.04
2	75	10	1:2	81.57 ± 2.22*	69.78 ± 1.22
3	373	2	1:2	83.42 ± 0.00*	79.64 ± 0.99
4	373	10	1:2	84.00 ± 0.40*	69.78 ± 0.78
5	75	2	1:4	83.42 ± 0.00*	66.92 ± 0.34
6	75	10	1:4	83.28 ± 0.60*	82.28 ± 1.99
7	373	2	1:4	83.57 ± 0.20*	97.00 ± 0.33*
8	373	10	1:4	79.57 ± 3.83*	70.00 ± 0.06
9	226	6	1:2	83.57 ± 0.20*	89.08 ± 0.99
10	226	6	1:4	87.14 ± 0.00*	100.03 ± 0.11*
11	75	6	1:2	81.00 ± 1.81*	87.89 ± 0.88*
12	373	6	1:3	82.71 ± 0.20*	94.54 ± 1.45
13	226	2	1:3	83.71 ± 0.80*	90.16 ± 0.01*
14	226	10	1:3	83.00 ± 1.41*	85.90 ± 0.88*
15 (C)	226	6	1:3	83.42 ± 0.00*	95.77 ± 0.09*
16 (C)	226	6	1:3	83.71 ± 0.40*	93.34 ± 0.00*
17 (C)	226	6	1:3	83.28 ± 0.20*	91.08 ± 0.01*

* $p<0.05$, denotes significant increase from the DPPH/$ABTS^{\bullet+}$ percentage of scavenging rate [ANOVA, Tukey's test (Compared to each control)]. Values represent mean±standard error of the means of three independent experiments (n=3).

The surface response methodology was not applied to evaluate the effect of DPPH inhibition after sonication processing because the effects of the evaluated parameters were not statistically significant. Thus, response surface analysis cannot be applied. However, the results indicated that US processing had different effects on extraction of antioxidant compounds but not much effect on DPPH antioxidant activity. In all assays, there was a slightly but significant increase of DPPH scavenging activity compared to control ($p<0.05$) (Table 6). When the experimental process conditions were 226W/cm^2 US power intensity, 1:4

bagasse:water ratio at extraction time of 6 minutes, the highest percentage of DPPH inhibition was achieved (87.14%).

As no single method is sufficient to evaluate the antioxidant activity, the ABTS antioxidant capacity measurement was done to take into account the various modes of action of antioxidants.

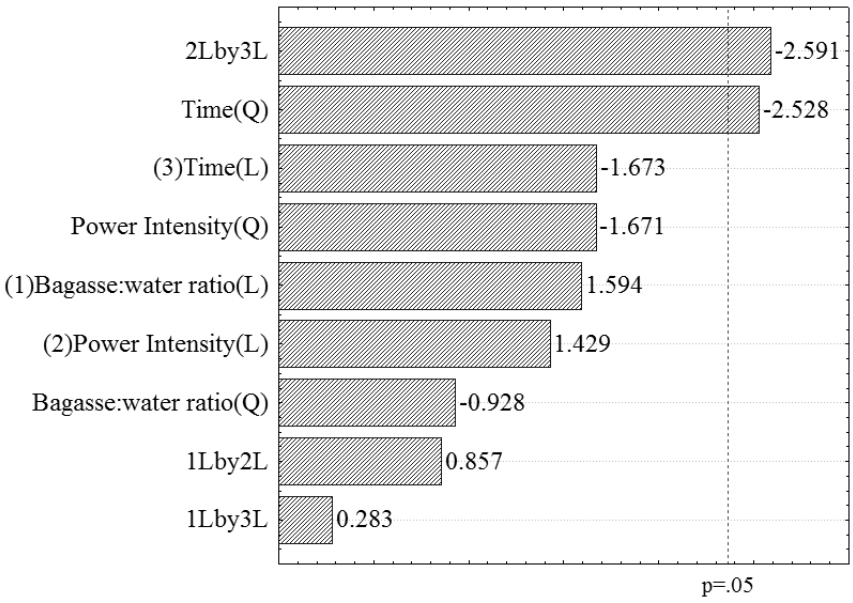

Fig. 6 Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

The fitted model for ABTS inhibition as a function of water content, US power intensity and processing time was expressed on Eq. 6.The model was statistically significant at 95% of confidence interval since the calculated F value (4.73) was higher than the listed F value ($F_{9.7}$=3.68). The coefficient of multiple determination (R^2) was 0.85. Fig. 5 shows the surface response graph built from Eq. 6.

$$Y = 25.95 + 109.08x - 135.92x^2 + 0.24y - 0.0003y^2 + 8.97z - 0.59z^2 - 0.10xy - 1.52xz - 0.009yz \quad \textbf{Eq. 6}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively

The bagasse:water ratio effect was not significant on ABTS. Fig. 7 shows the effects of the significant processing parameters (power intensity and time) on ABTS inhibition. It can be observed that increasing on processing time and the interactive effect of time and power intensity had negative effects on ABTS inhibition.

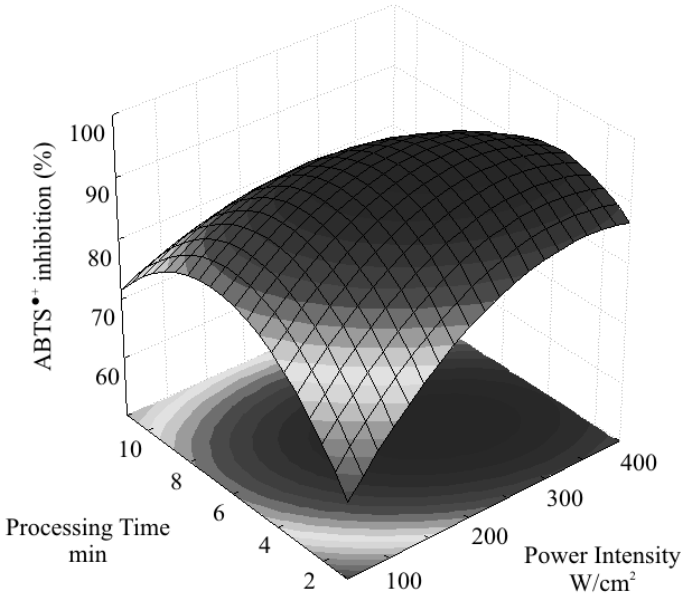

Fig. 7 Response surface for the effect of sonication on ABTS inhibition after sonication of cashew apple bagasse extracts plotted for a constant power intensity of 226 W/cm^2.

As the obtained response surface graphs presented a well-defined region for maximum ABTS inhibition (Fig.7), the optimal process parameters were determined by the critical point (zero derivative) of Eq. 6, calculated by the Statistica software. The optimal parameters for 100% inhibition of ABTS radical were predicted to be power intensity 300 W/cm^2, 1:4 of bagasse:water ratio during 4.9 minutes.

An increase in the antioxidant capacity of sonicated cashew apple bagasse (higher DPPH and ABTS inhibition) was observed when compared to control (Table 6). Probably, the effective scavenging of free radicals is correlated to the high amounts of bioactive compounds, specifically, phenolic compounds with antioxidant activity present in cashew apple bagasse after ultrasonic processing.

2.3.4 *Effect of thermal processing*

Temperature is the most important factor in the extraction of heat sensitive compounds. Along with the increase of temperature, the solvent diffusion rate and the mass transfer intensification result in the dissolution of components (JOVANOVIC-MALINOVSKA; KUZMANOVA; WINKELHAUSEN, 2014).

Table 7 shows the residual concentrations of the evaluated responses obtained keeping the untreated sample (cashew apple bagasse + water) at 51 °C for 10 min. As can be seen, samples treated by the conventional thermal process (51°C/10 min) showed an overall significant decrease ($p<0.05$) of vitamin C when compared to the control samples (Table 7). No significant differences ($p<0.05$) were detected on phenolic concentration after thermal treatment (Table 7).

Table 7 Effect of thermal processing (51 °C/10 min) on bioactive compounds of cashew apple bagasse on different bagasse:water ratio

Response	**Residual Concentration (%)**		
	1:2	**1:3**	**1:4**
Vitamin C	60.23 ± 0.02	51.45 ± 0.10	45.98 ± 0.08
TPC	89.00 ± 0.09	98.04 ± 0.04	99.56 ± 0.02
β-carotene	97.99 ± 0.01	98.67 ± 0.29	80.00 ± 0.10
ABTS (% inhibition)	58.96 ± 0.02	55.34 ± 0.03	55.55 ± 0.03
DPPH (% inhibition)	70.56 ± 0.04	69.02 ± 0.00	75.00 ± 0.05

An increase in the antioxidant capacity of sonicated cashew apple bagasse (higher DPPH and ABTS inhibition) was observed when compared to thermal treated samples (Table 7).

2.4 Conclusions

The experimental results showed that all process variables, in different levels, contributed to the extraction of bioactive compounds from cashew apple bagasse. It was found that the water amount (bagasse:water ratio) was the most significant variable among the process variables studied. Compared to conventional thermal treatment, the sonication

provided higher yield of bioactive compounds. By studying the effect of bagasse:water ratio, power intensity of ultrasound and processing time, optimal conditions were established. Sonication time of 6 min, power intensity of 226 W/cm^2 and bagasse:water ratio of 1:4 led to the maximal amount of vitamin C and phenolic compounds. The results suggested that sonicated cashew apple bagasse could be good source of antioxidants which may have healthy benefits for humans. The sonication also changed the product structure from fibers to a puree as seen in Figure 1.

Acknowledgements

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Capítulo 3

Effect of ultrasound processing on the enzymatic antioxidant system of cashew apple bagasse purée

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Abstract

Effects of ultrasound processing on the activity of antioxidative enzymes of cashew apple bagasse purée (*Anacardium occidentale L.*) were investigated. The changes on the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and polyphenol oxidase were evaluated for either sonicated or control samples. Hydrogen peroxide content and color parameters of sonicated cashew apple bagasse were also measured. 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. Sonication resulted in perceptible color changes in cashew apple bagasse. Despite this result, there were no evidences of browning since h° of sonicated samples was close to the characteristic color of cashew apple pulp. The effect of ultrasound in delaying browning could be correlated to the enhanced antioxidant enzyme activity and decrease in guaiacol peroxidase activity.

Keywords: *Anacardium occidentale* L., ultrasound, antioxidative enzyme activity, oxidative stress

3.1 Introduction

High power ultrasound has been applied as a green, inexpensive and easy-to-use method for food processing with good preservation of bioactive compounds (LI *et al.*, 2013). The effects caused by sonication come from two hydrodynamic events: acoustic cavitation and induced microstreaming cavitation (BERMÚDEZ-AGUIRRE; MOBBS; BARBOSA-CÁNOVAS, 2011). Acoustic cavitation is the process of nucleation, growth and collapse of bubbles in liquids exposed to ultrasonic waves at low frequency (20 kHz–100 kHz) and high power (10–1000 W/cm^2). The collapse of the bubbles generates high local temperatures (5000 K) and high pressures (1000 atm) occurring simultaneously, resulting in formation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) (MASON, 1991).

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).

In terms of food quality, polyphenol oxidase (PPO) and peroxidases (POD) are frequently involved in multiple deteriorative changes, such as enzymatic browning, with consequent loss of sensorial and nutritional properties of fruit and vegetables (OMS-OLIU *et al.*, 2008). Polyphenol oxidase is the main agent responsible for enzymatic browning. It is a copper-containing enzyme that catalyses the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones,which, in turn, are polymerized to brown, red, or black pigments (ESPÍN *et al.*, 1997). For most plant tissues, PPO is compartmentalized in plastids, whereas phenolic substrates are located in the vacuoles. PPO activation occurs only when these compartments are disrupted after tissue wounding (QUEIROZ*et al.*, 2011).

Peroxidases are heat resistant hemme-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyse a number of oxidative reactions. These enzymes are associated to the development of off-flavours and browning pigments (O'DONNELL *et al.*, 2010). Some authors suggested the synergistic action of PPO and POD in enzymatic browning through the generation of H_2O_2 during the oxidation of phenolic compounds in PPO-catalysed reactions (TOMÁS-BARBERÁN; ESPÍN, 2001). The activity of these enzymes can also affect the antioxidant capacity of fruits and vegetables by the oxidation of

antioxidants compounds such as phenolic compounds by PPO and POD (ESPÍN, 2000; JIMÉNEZ; ESCRIBANO-CEBRIÁN; GARCÍA-CARMONA, 1998).

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 paper the effect of sonication of cashew apple bagasse on enzymes involved in antioxidative system and in general quality was investigated.

3.2 Materials and methods

3.2.1 *Sample preparation*

Red cashew apples were harvested at commercial maturity stage in Ceará State, Brazil, from September to December 2013. 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. The peduncles were not protected from light to simulate the conditions that the fruit may be exposed in the postharvest period. The solid residues (bagasse) were packaged in polyethylene bags, vacuum sealed and stored at −18°C until use. Prior to the experiment, the cashew apple bagasse was thawed at 4°C.

3.2.2 *Ultrasound processing*

The processing was carried out with a 500W ultrasound processor (Unique® DES500, São Paulo, Brazil) with a 1.3 cm diameter probe tip, changing the bagasse to water ratio, the ultrasound power and the processing time. The experiments were carried out in separate 600 mL Becker flasks (80mm diameter x 165mm height). The ultrasound probe was submerged to a depth of 25 mm in the sample (200 mL). The intensity of ultrasound power dissipated from the probe tip was calculated by Eq. 1(LI; PORDESIMO; WEISS, 2004).

$$I = \frac{P}{\pi r^2} \qquad \textbf{Eq. 1}$$

Where r is the radius of the titanium tip (cm) and P is the input power level (W). The input power was controlled through the amplitude setting and the power levels were adjusted to

20%, 60%, and 100% of total input power (500 W), which was equal to 100, 300 and 500 W. The calculated intensities were 75, 226 and 373 W/cm^2, respectively.

3.2.3 *Experimental design and data analysis*

The effects of ultrasound processing were evaluated through a 2^3 face-centered central composite experimental design (CCD) with 3 central points. The power intensity, the bagasse:water ratio (g/g) and the processing time were changed from 75 to 373 W/cm^2, 1:2 to 1:4 and 2 to 10 min, respectively. For all treatments, the initial temperature of the mixture was 20°C. After sonication, the temperature was recorded by a digital thermometer. Experiments using ultrasound have been performed and compared with those not using ultrasound (control process).

Control samples for each bagasse:water ratio (1:2, 1:3, 1:4) were prepared. The samples were submitted to only water immersion without sonication, kept in ambient temperature for 10 min. The maximum temperature variation observed in the samples was 20 °C.

3.2.4 Activity of Antioxidant Enzymes

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). Two grams of sonicated cashew apple bagasse were homogenized in 15 mL of 0.1M potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA for 1 min, followed by centrifugation at 3248g for 40 min at 4 °C. The supernatant fraction was used as a crude extract for the enzyme activity assays, and all procedures were performed at 4 °C.

For peroxidase (POD, EC 1.11.1.7) and polyphenol oxydase (PPO, EC 1.10.3.1) determinations, ten milliliters of sonicated cashew apple bagasse were mixed with the same volume (10 mL) of 0.05M potassium phosphate buffer (pH 7.0) containing 1% (w/v) of polyvinylpyrrolidone (PVP). The mixture was centrifuged in a Sigma® 6K15 centrifuge (10.733g for 30 min at 4 °C).The supernatant was used as the enzyme source.

SOD activity was determined on the basis of the inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) (GIANNOPOLITIS; RIES, 1977). The reaction

mixture absorbance was measured by a Spectrum SP 2000 UV spectrophotometer at 560 nm, and 1 unit of SOD activity (UA) was defined as the amount of enzyme required to cause a 50% reduction in the NBT photo reduction rate. Thus, results were expressed as UA per milligram of protein.

CAT activity was measured according to the method of BEERS and SIZER (1952). The reaction started by adding the enzyme extract, and then the decrease in hydrogen peroxide (H_2O_2) was monitored through absorbance at 240 nm and quantified by its molar extinction coefficient (36 M/cm). One unit of CAT activity (UA) was defined as the amount of enzyme required to decompose H_2O_2 (μmolH_2O_2/min) and the results were expressed as UA per milligram of protein.

APX activity was assayed according to the method of NAKANO and ASADA (1981). Enzyme activity was measured using the molar extinction coefficient for ascorbate (2.8 $mM.cm^{-1}$) and the results expressed in μmol $H_2O_2.mg^{-1}$ $P.min^{-1}$, taking into account that 1mol of ascorbate is required for a reduction of 1 molH_2O_2.

PPO activity was measured based on the method reported by WISSEMANN and LEE(1980). The reaction mixture contained 0.3 mL of enzyme extract and 1.85 mL of a potassium phosphate buffer solution (0.1 M pH 6.0) containing catechol (0.1 M) and KCl (0.1 M). The reaction mixture was incubated at 30 °C for 30 min. The reaction was interrupted with the addition of 0.8 mL of perchloric acid 2 N. One unit of enzyme activity (1 UA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance (395 nm) per minute.

POD activity was monitored at 470 nm according to the method described by MATSUNO and URITANI (1972). The enzyme activity was measured as follows: 2.75 mL of a phosphate (sodium)-citrate (citric acid) buffer (0.1 M, pH 5.0) containing 1% (v/v) of guaiacol and 0.25 mL of H_2O_2 3% (v/v) were added to 1.5 mL of enzyme extract. The assay mixture was incubated at 30 °C for 5 min. The reaction was interrupted with the addition of 1 mL of sodium bisulfate 30% (w/v). One unit of enzyme activity (1 UEA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

The total protein content was determined according to the method of BRADFORD (1976)using bovine serum albumin (BSA) as a standard.

3.2.5 H_2O_2 content

Hydrogen peroxide (H_2O_2) content was measured by the method of SHAO *et al.*(2008). In brief, 0.2 g of sonicated bagasse was homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid in an ice bath. The homogenate was centrifuged at 12.000g for 15 min, and 0.5 mL of supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7) and 1 mL of 1 M KI. Absorbance of the mixture solution was measured at 390 nm by spectrophotometer. The content of H_2O_2 was expressed based on a standard curve in μmol H_2O_2 g^{-1} dry weight.

3.2.6 Expression of results

The residual concentrations of all antioxidant compounds (vitamin C, TPC, β-carotene) and were calculated according to Eq. 2. All determinations were done in triplicate and the results were residual mean values ± standard deviations.

$$\text{Residual (\%)} = \frac{C_0 - C_S}{C_0}.100 \qquad \textbf{Eq. 2}$$

Where the sub indices*0* and *s* in Eq. (2) mean the control sample (non-treated) and sonicated one, respectively. The results of the three control experiments at different bagasse:water ratio 1:2; 1:3 and 1:4 were also evaluated and the residual concentration was calculated for each bagasse:water ratio.

3.2.7 Color

The color of the cashew apple bagasse after sonication and during drying was determined using a Minolta CR300 colorimeter (Tokyo, Japan). The colorimeter was calibrated using the illuminant D65, and measurements were made through an 8-mm port/viewing area. The reflectance instruments determined the following color parameters: lightness (L^*), redness (a^*), yellowness (b^*), C (chroma) and h°(hue angle). Numerical values

of L^*, a^* and b^* were converted into ΔE (total color difference), according to Eq. 3. The reference value for ΔE was the non-sonicated bagasse.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad \textbf{Eq.3}$$

$$\Delta C = \sqrt{C_0 - C_S} \quad \textbf{Eq.4}$$

The sub indices *0* and *s* in Eq. (3) e (4) mean the control sample (non treated) and sonicated one, respectively.

3.2.8 *Statistical analysis*

Except color determination, which was taken in quintuplicate, all other assays were carried out in triplicate. 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).

3.1 Results and Discussion

3.1.1 Activity of antioxidant enzymes

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 in ambient temperature (20°C) for 10 min.

Table 1 Enzymatic activity and hydrogen peroxide content of control samples

	SOD	CAT	APX	POD	PPO	H_2O_2
Bagasse:water ratio	**UEA. mg^{-1} P**	**µmol H_2O_2 $mg^{-1}P.min^{-1}$**	**µmol H_2O_2 $mg^{-1}P.min^{-1}$**	**UEA $min^{-1}g^{-1}P$**	**UEA $min^{-1}g^{-1}P$**	**µmol g^{-1}**
1:2	4.61 ± 0.09	69.56 ± 0.53	0.39 ± 0.01	164.60 ± 0.97	698.21 ± 0.43	1.90 ± 0.00
1:3	4.63 ± 0.87	65.23 ± 0.00	0.37 ± 0.01	308.94 ± 1.56	947.60 ± 0.01	1.97 ± 0.01
1:4	2.30 ± 0.04	65.78 ± 0.05	0.59 ± 0.00	404.76 ± 5.77	1373.01 ± 0.09	1.91 ± 0.00

UEA=Unity of enzymatic activity

Table 2 presents the effect of sonication on SOD activity of cashew apple bagasse.

Table 2 Experimental design and SOD activity after sonication of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
1	75	2	1:2	171.31 ± 0.08
2	75	10	1:2	83.96 ± 1.67
3	373	2	1:2	72.99 ± 0.99
4	373	10	1:2	126.16 ± 0.24
5	75	2	1:4	249.00 ± 0.76
6	75	10	1:4	376.28 ± 0.56
7	373	2	1:4	162.71 ± 0.97
8	373	10	1:4	300.09 ± 1.67
9	226	6	1:2	172.15 ± 0.54
10	226	6	1:4	345.77 ± 0.57
11	75	6	1:2	124.37 ± 0.76
12	373	6	1:3	44.11± 0.21
13	226	2	1:3	92.01 ± 0.12
14	226	10	1:3	85.29 ± 0.53
15 (C)	226	6	1:3	103.36 ± 0.36
16 (C)	226	6	1:3	105.00 ± 1.04
17 (C)	226	6	1:3	102.07 ± 0.45

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 effects of the variables power intensity, bagasse:water ratio and processing time were presented on Fig. 1.

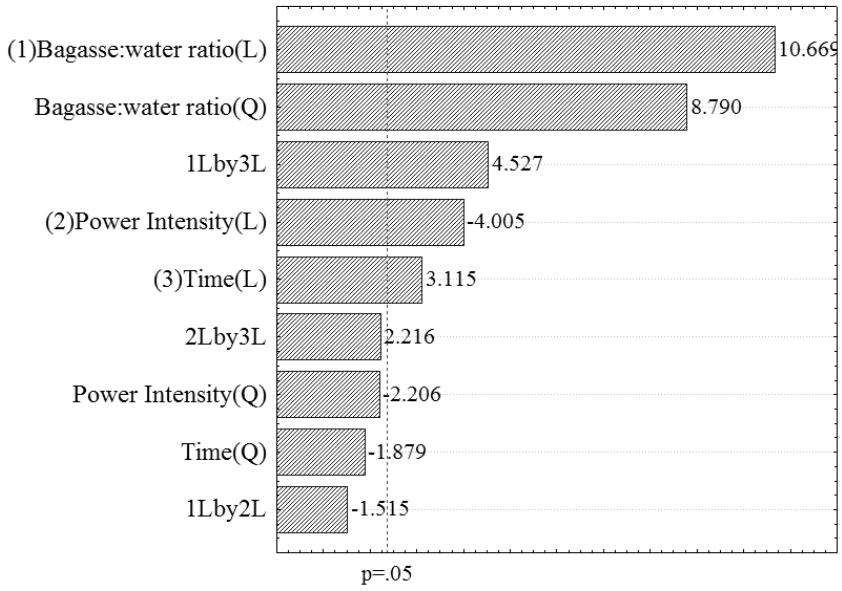


Fig.1 Pareto chart for the effect of sonication on SOD activity of cashew apple bagasse. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

Bagasse:water ratio had the greatest positive effect on SOD residual activity ($p<0.05$) i.e., higher proportions of water added to bagasse increases SOD activity. Also, the interactive effect of bagasse:water ratio and processing time was positive. On the other hand, the linear effect of ultrasound intensity revealed, for the experimental domain evaluated herein, that higher power intensity lead to SOD activity reduction, since this effect was negative (Fig. 1).

The fitted model for residual SOD activity as a function of bagassse:water ratio, US power intensity and processing time is expressed on Eq. (5).The model was statistically significant at 95% of confidence interval since the calculated F value (19.89) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.96. Fig. 2 shows the surface response graph built from Eq. (5). The surface showed a saddle point near to the central point of the experimental domain (Fig. 2).

$$Y = 1906.20 - 9882.23x + 12638x^2 - 0.01y - 0.001y^2 + 44.09z - 1.72z^2 + 0.74xy - 68.21xz + 0.03yz \quad \textbf{Eq. 5}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

Fig. 2 shows the surface for the effect of sonication on SOD activity of cashew apple bagasse.

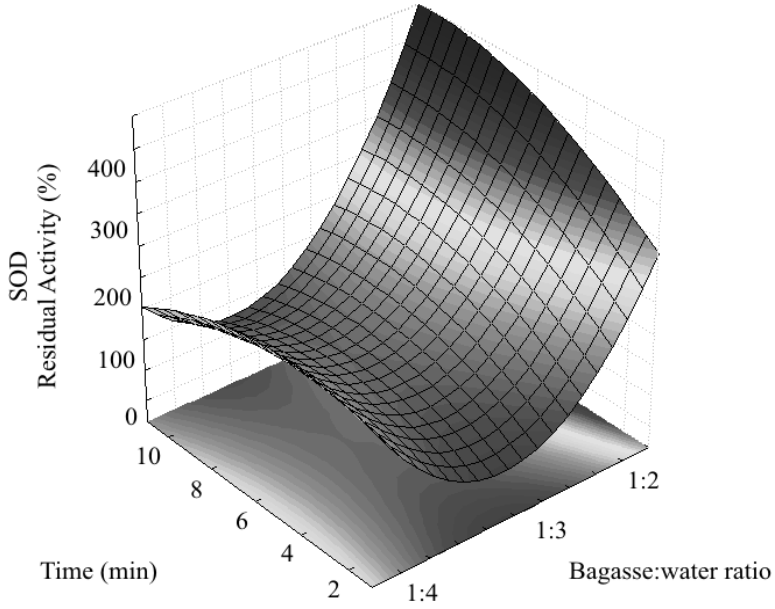


Fig.2 Response surface for the effect of sonication on SOD activity of cashew apple bagasse plotted for a constant power intensity of 226 W/cm^2.

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 for processing time and a local maximum for bagasse:water ratio, showing that when keeping the processing time near to 6min, residual activity increased for bagasse to water ratio higher and lower than 1:3.

The SOD activity is generally increased by a variety of chemical and physical stimuli (CHEN *et al.*, 2008). OLIVEIRA *et al.*(2011) observed an increase of 113% of SOD activity of acerola purée during refrigerated storage. The authors attributed this response to a stress induced by long-term freezing storage.

COMARELLA *et al.*(2012) reported ultrasound as a possible elicitor to induce the defense responses of plant cells and to stimulate secondary metabolic production in plant cell cultures. The mechanical stress caused by acoustic cavitation and microstreaming stimulates the plant defense responses.

YANG *et al.*(2012) reported that the combined effect of salicylic acid and ultrasound enhanced the chilling tolerance of peach fruit by inducing the antioxidant system, such as catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase.

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 sonicatedbagasse which was 19% greater than control sample (Table 3).

Table 3 Experimental design and CAT activity after sonication of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
1	75	2	1:2	100.61 ± 0.01
2	75	10	1:2	112.45 ± 0.01
3	373	2	1:2	95.91 ± 0.06
4	373	10	1:2	85.67 ± 0.04
5	75	2	1:4	108.76 ± 0.00
6	75	10	1:4	110.86 ± 0.08
7	373	2	1:4	119.22 ± 0.08
8	373	10	1:4	108.76 ± 0.00
9	226	6	1:2	104.56 ± 0.02
10	226	6	1:4	112.95 ± 0.64
11	75	6	1:2	87.71 ± 0.00
12	373	6	1:3	98.68 ± 0.00
13	226	2	1:3	104.16 ± 0.09
14	226	10	1:3	97.06 ± 0.08
15 (C)	226	6	1:3	85.78 ± 0.01
16 (C)	226	6	1:3	87.42 ± 0.03
17 (C)	226	6	1:3	85.99 ± 0.05

Regarding the effects of sonication on CAT activity, the quadratic effects of bagasse:water ratio and power intensity presented negative effect on CAT residual activity ($p<0.05$) (Fig. 3).

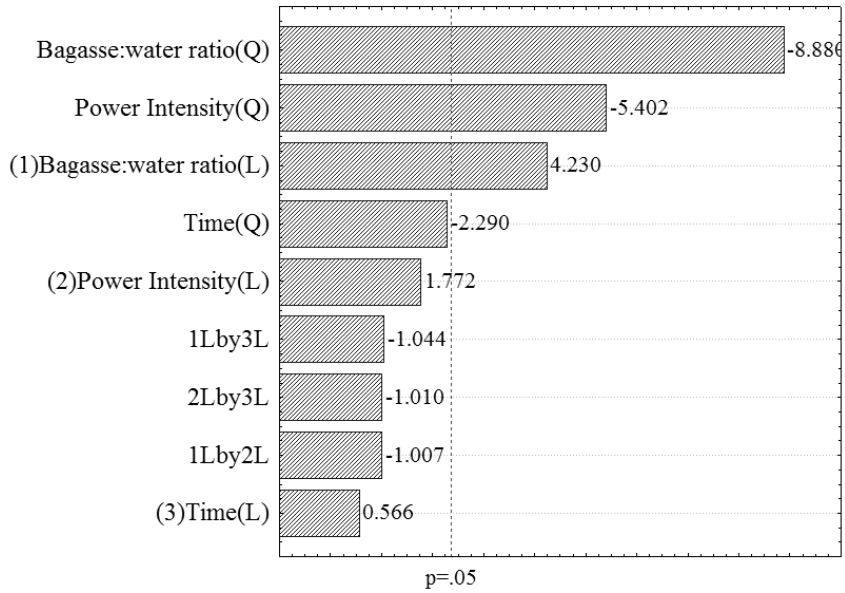


Fig.3 Pareto chart for the effect of sonication on CAT activity of cashew apple bagasse.Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction betweenbagasse:water ratio andprocessing time; 2L by 3L interaction between power intensity and processing time.

The fitted model for residual CAT activity as a function of bagassse:water ratio, US power intensity and processing time is expressed by Eq. (6). The model was statistically significant at 95% of confidence interval since the calculated F value (33.92) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.97. Fig. 4 shows the surface response graph built from Eq. 6.

$$Y = 73.52 + 253.80x - 364.81x^2 + 0.070y - 0.0002y^2 + 1.04z - 0.092z^2 + 0.02xy + 0.678 - 0.001yz \quad \textbf{Eq. 6}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

The response surface graph presented a well-defined region for maximum CAT residual activity, the process parameters for maximal CAT residual activity were determined by the critical point (zero derivative) of Eq. 6, calculated by the Statistica software. The parameters were predicted to be power intensity of 236.12 W/cm^2, 1:3 of bagasse:water ratio during 6.21 minutes.

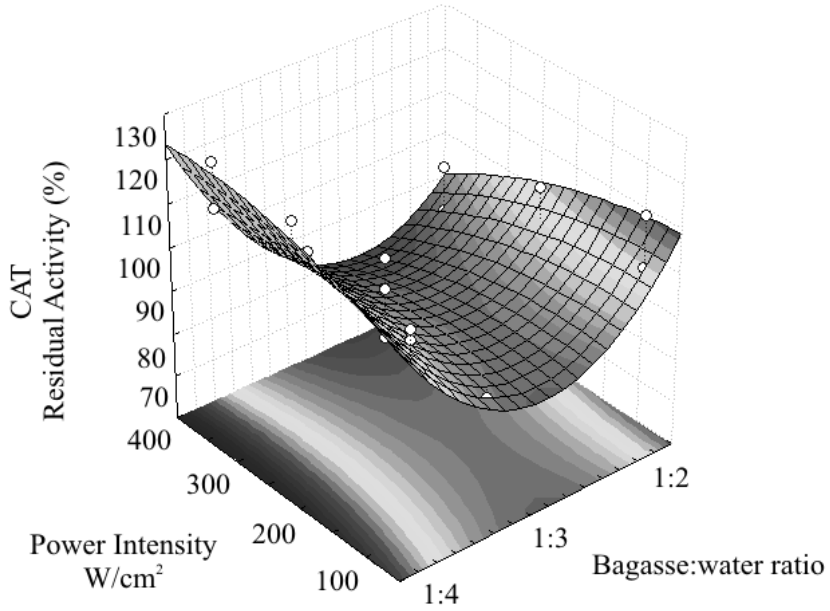


Fig.4 Response surface for the effect of sonication on CAT activity of cashew apple bagasse extracts plotted for a constant processing time of 6 min.

The impact of ultrasound on the APX activity of cashew apple bagasse is summarized in Table 4.

Table 4 Experimental design and APX activity after sonication of cashew apple bagasse

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:waterr atio	Residual (%)
1	75	2	1:2	100.00 ± 0.10
2	75	10	1:2	59.46 ± 0.09
3	373	2	1:2	74.16 ± 0.23
4	373	10	1:2	93.21 ± 0.45
5	75	2	1:4	97.55 ± 0.05
6	75	10	1:4	91.87 ± 0.09
7	373	2	1:4	52.11 ± 0.08
8	373	10	1:4	100.00 ± 0.01
9	226	6	1:2	100.00 ± 0.32
10	226	6	1:4	58.33 ± 0.09
11	75	6	1:2	64.14 ± 0.00
12	373	6	1:3	72.16 ± 0.29
13	226	2	1:3	53.45 ± 0.00
14	226	10	1:3	50.00 ± 0.07
15 (C)	226	6	1:3	51.00 ± 0.15
16 (C)	226	6	1:3	49.77 ± 0.29
17 (C)	226	6	1:3	47.00 ± 0.04

Fig. 5 shows the effect of the independent variables on APX residual activity. At a confidence level of 95%, the variable bagasse:water ratio (quadratic) and the interaction between power intensity and processing time had positive effect on APX residual activity.

Thus, a simultaneous increase of US power intensity and time favored APX activity. A marked decrease (≈50 %) of APX activity was observed (Table 4).

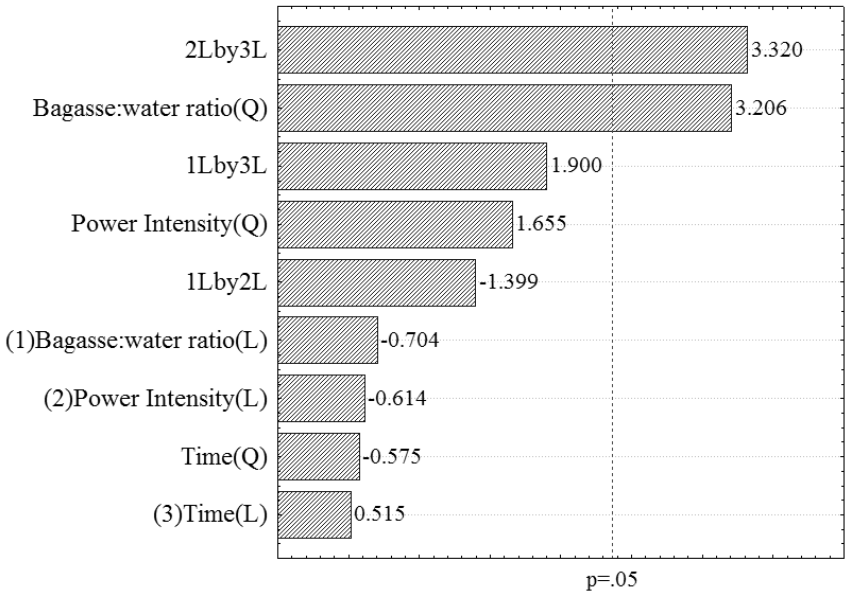


Fig.5 Pareto chart for the effect of sonication on APX activity of cashew apple bagasse. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

The fitted model for residual APX activity as a function of bagassse:water ratio, US power intensity and processing time is expressed by Eq. (7).The model was statistically significant at 95% of confidence interval since the calculated F value (3.94) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.83. Fig.6 shows the surface response graph built from Eq. 7.

$$Y = 312.57 - 1175.60x + 1634.48x^2 - 0.50y + 0.001y^2 + 3.73z - 0.26z^2 + 0.27xy - 14.89xz + 0.02yz \quad \textbf{Eq. 7}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

Fig. 6 shows the surface for APX residual activity of sonicated cashew apple bagasse.

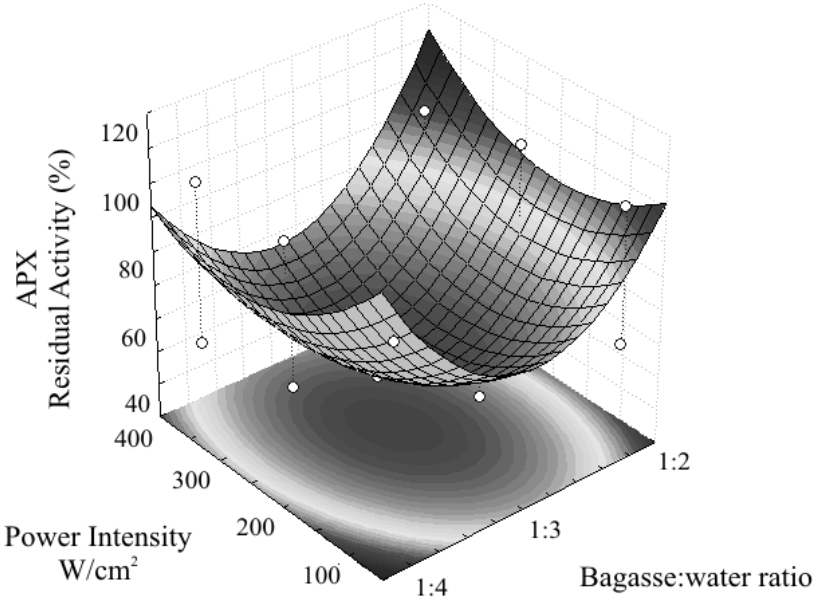


Fig.6 Response surface for the effect of sonication on APX activity of cashew apple bagasse extracts plotted for a constant processing time of 6 min.

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.

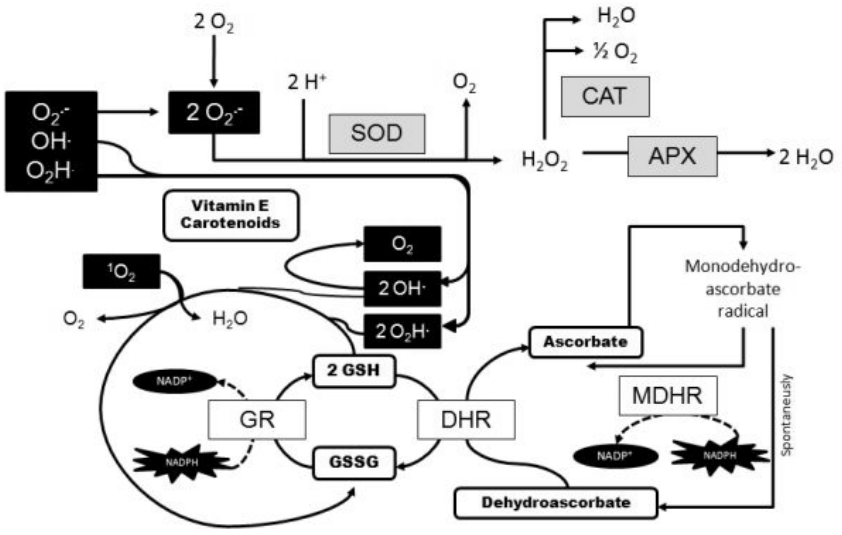


Fig.7 Enzymatic and non-enzymatic antioxidant system in plants. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are the proteins responsible for eliminating ROS. While the elimination of ROS by non-enzymatic processes is carried out by

vitamin E, carotenoids, ascorbate, oxidized glutathione (GSH) and reduced (GSSG). Enzymes that promote the elimination of ROS via the ascorbate-glutathione cycle are monodehydroascorbatereductase (MDHR), dehydroascorbate reductase (DHR) and glutathione reductase (GR)

Fonte: CERÓN-GARCIA *et al.*(2012).

The results for POD residual activity of cashew apple bagasse after sonication were presented in Table 5.

Table 5 Experimental design and POD activity after sonication of cashew apple bagasse

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
1	75	2	1:2	77.32 ± 0.00
2	75	10	1:2	34.97 ± 0.26
3	373	2	1:2	98.46 ± 0.88
4	373	10	1:2	48.18 ± 0.29
5	75	2	1:4	37.05 ± 0.00
6	75	10	1:4	56.47 ± 0.00
7	373	2	1:4	49.41 ± 0.49
8	373	10	1:4	39.11 ± 0.29
9	226	6	1:2	27.61 ± 0.26
10	226	6	1:4	49.41 ± 0.19
11	75	6	1:2	22.92 ± 0.39
12	373	6	1:3	28.14 ± 0.36
13	226	2	1:3	45.31 ± 0.54
14	226	10	1:3	31.07 ± 0.00
15 (C)	226	6	1:3	23.30 ± 0.19
16 (C)	226	6	1:3	22.92 ± 0.67
17 (C)	226	6	1:3	22.92 ± 0.36

Estimated effects of the independent variables on the POD residual activity are presented in Fig. 8.

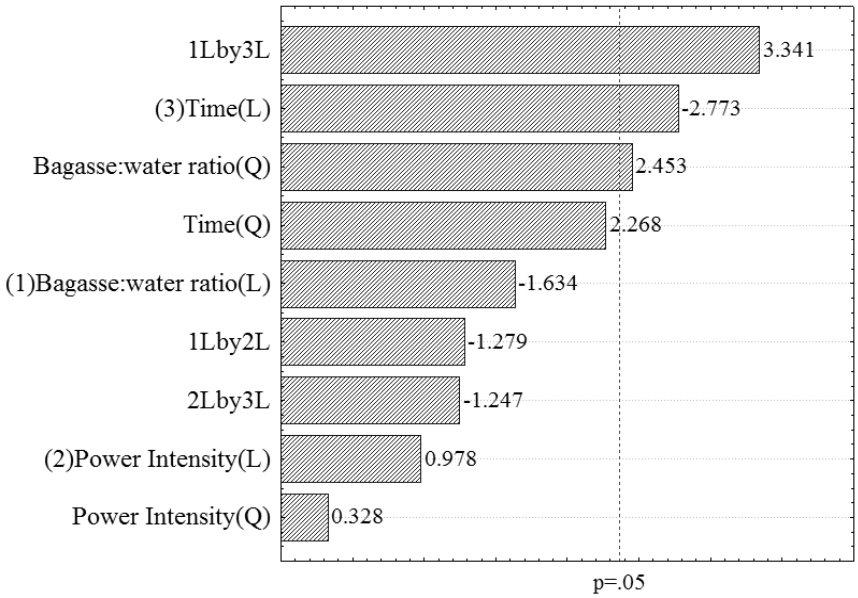


Fig.8 Pareto chart for the effect of sonication on POD activity of cashew apple bagasse. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

Results for POD activity showed that, the effect of processing time (linear) was significant ($p<0.05$) on the POD activity reduction. On the other hand, the effect of power intensity (linear and quadratic) was not significant at the given confidence interval. The effect of ultrasound intensity revealed that, for the experimental domain evaluated herein, higher power intensity at low processing times did not affect POD activity reduction, since this effect was small and not significant. An increase in POD activity was observed for high bagasse:water ratio and processing times.

The fitted model for residual POD activity as a function of bagassse:water ratio, US power intensity and processing time is expressed by Eq. (8). The model was statistically significant at 95% of confidence interval since the calculated F value (6.41) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.89. Fig. 9 shows the surface response graph built from Eq. 8.

$$Y = 144.43 - 582.98x + 960.86x^2 - 0.067y + 0.0001y^2 - 2.487z + 0.922z^2 + 0.258xy - 25.26xz - 0.008yz \quad \textbf{Eq. 8}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

The minimal residual activity was determined by the critical point (zero derivative) of Eq. 8, calculated by the Statistica software. The parameters for 20% of residual activity of POD were predicted to be power intensity 150 W/cm^2, 1:2 of bagasse:water ratio during 6.9 minutes.

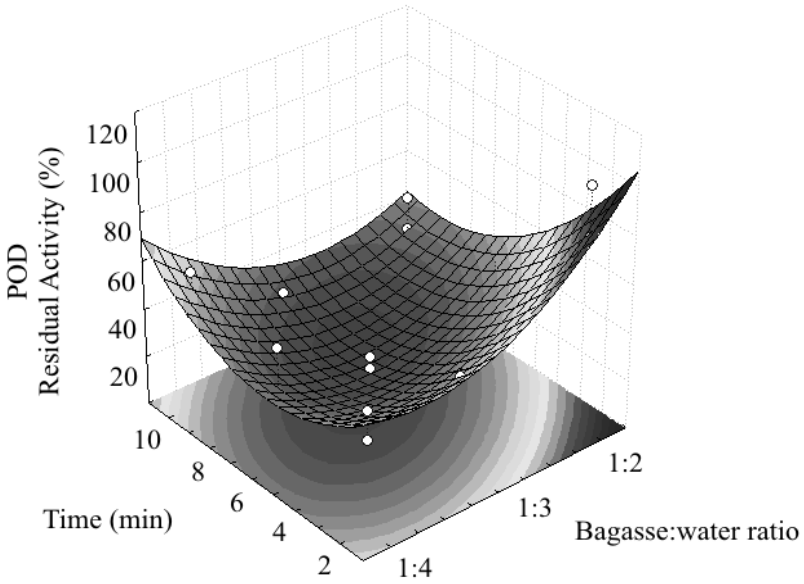


Fig.9 Response surface for the effect of sonication on POD activity of cashew apple bagasse extracts plotted for a constant power intensity of 226W/cm^2.

Table 6 presents the results for PPO residual activity after sonication of cashew apple bagasse.

Table 6 Experimental design and PPO activity after sonication of cashew apple bagasse

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual (%)
1	75	2	1:2	153.20 ± 2.45
2	75	10	1:2	173.96 ± 2.89
3	373	2	1:2	217.13 ± 1.86
4	373	10	1:2	179.02 ± 2.21
5	75	2	1:4	185.52 ± 5.72
6	75	10	1:4	179.30 ± 9.31
7	373	2	1:4	189.97 ± 2.31
8	373	10	1:4	200 ± 3.20
9	226	6	1:2	78.19 ± 0.78
10	226	6	1:4	125.63 ± 4.29
11	75	6	1:2	74.75 ± 3. 10
12	373	6	1:3	97.50 ± 3.13
13	226	2	1:3	127.05 ± 3.28
14	226	10	1:3	129.59 ± 4.23
15 (C)	226	6	1:3	83 ± 1.20
16 (C)	226	6	1:3	84.57 ± 0.88
17 (C)	226	6	1:3	85.54 ± 0.99

Browning reactions have generally been assumed to be a direct consequence of polyphenoloxidase and peroxidase action on polyphenols (MARTINEZ; WHITAKER, 1995; DEGL'INNOCENTI *et al.*, 2005). Figure 10 presents the estimated effects of each independent variable as well as their interaction on PPO residual activity. Pareto chart reveals that increasing processing time and power intensity results in higher PPO activities.

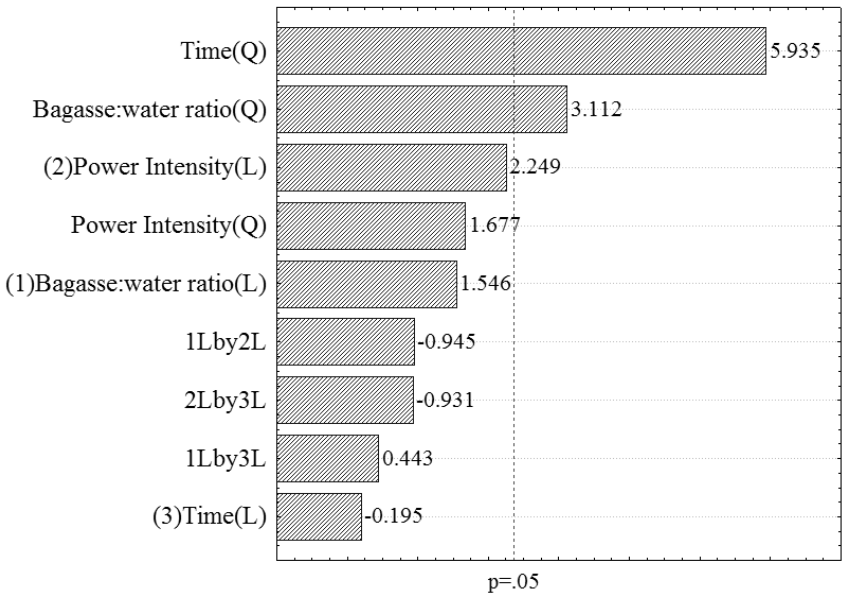


Fig.10 Pareto chart for the effect of sonication on PPO activity of cashew apple bagasse. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

The fitted model for residual PPO activity as a function of bagassse:water ratio, US power intensity and processing time is expressed by Eq. (9).The model was statistically significant at 95% of confidence interval since the calculated F value (15.45) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.95. Fig. 11 shows the surface response graph built from Eq. 9.

$$Y = 347.18 - 628.51x + 756.13x^2 - 0.123y + 0.001y^2 - 43.60z + 3.66z^2 - 0.176xy + 3.082xz - 0.009yz \quad \textbf{Eq. 9}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

Surface response graph, obtained using the fitted model (Eq. 9), is presented in Fig. 11. The minimal activity of PPO was determined by the critical point (zero derivative) of Eq.9, calculated by the Statistica software. The parameters were predicted to be power intensity 168 W/cm^2, 1:2 of bagasse:water ratio during 5.97 minutes reaching 74% of residual activity.

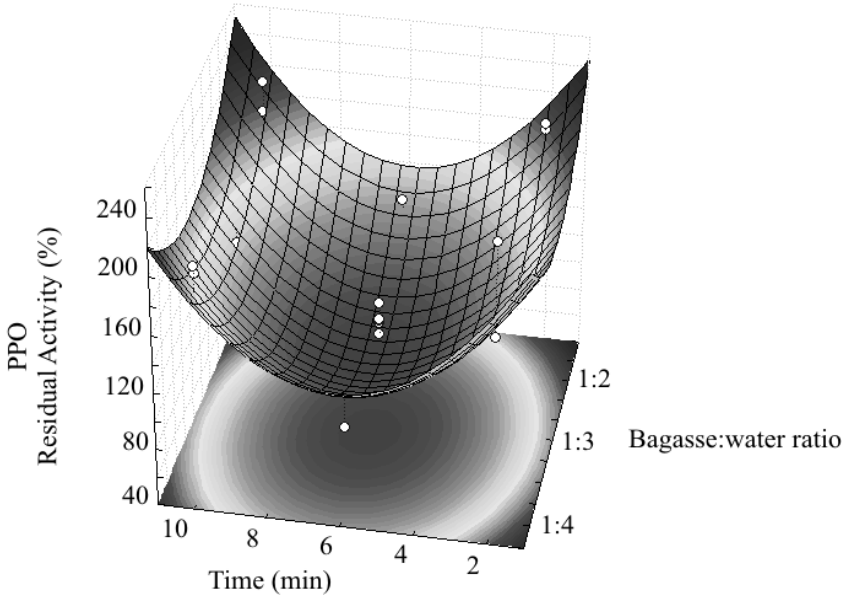


Fig.11 Response surface for the effect of sonication on PPO activity of cashew apple bagasse extracts plotted for a constant power intensity of 226 W/cm^2.

The behavior of PPO after sonication is, at a first, due to US treatment that causes cell disruption of the vegetable tissue. Thus, the intracellular enzyme is released in the liquid medium increasing the enzyme activity. In the central point condition, the rate of enzyme denaturation overcomes the enzyme release.

The ultrasound waves affect the enzyme inactivation through a series of effects including thermal effect, generation of free radicals by water sonolysis, and the mechanical forces which caused by micro-streaming and shock waves (VERCET *et al.*, 2002). These, alone or in combination, can damage the integrity of the enzyme protein structure resulting in reduced enzyme activity (SUSLICK, 1989). VERCET *et al.* (1997) pointed out that at low temperatures the hydroxyl radicals production is favored by ultrasound.

3.1.2 *H_2O_2 content*

The results of the experimental design for H_2O_2 residual concentrations after sonication are presented in Table 7. Ultrasound processing caused significant increase in H_2O_2 content of sonicated cashew apple bagasse, reaching the maximum concentration on assay 4 (373 W/cm^2, 1:2 bagasse:water ratio and 10 minutes).

Table 7 Experimental design and H_2O_2 activity after sonication of cashew apple bagasse puree

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water ratio	Residual %
1	75	2	1:2	84.21 ± 0.03
2	75	10	1:2	175.00 ± 0.03
3	373	2	1:2	140.78 ± 0.03
4	373	10	1:2	486.84 ± 0.03
5	75	2	1:4	248.68 ± 0.03
6	75	10	1:4	164.47 ± 0.03
7	373	2	1:4	109.21 ± 0.03
8	373	10	1:4	161.84 ± 0.03
9	226	6	1:2	109.21 ± 0.03
10	226	6	1:4	98.68 ± 0.03
11	75	6	1:2	119.73 ± 0.03
12	373	6	1:3	307.89 ± 0.03
13	226	2	1:3	115.78 ± 0.03
14	226	10	1:3	156.57 ± 0.03
15 (C)	226	6	1:3	102.63 ± 0.03
16 (C)	226	6	1:3	105.00 ± 0.03
17 (C)	226	6	1:3	102.00 ± 0.03

Figure 10 shows the effects of the evaluated variables on H_2O_2 concentration. The experimental results showed that all process variables, in different levels, contributed to the final concentration of H_2O_2 of sonicated cashew apple bagasse. However, ultrasound intensity exerts higher positive effect.

This result was confirmed by KENTISH and ASHOKKUMAR (2011) who reported that the number of radicals generated is high when the temperature inside the collapsing bubble is at a maximum. This temperature can be increased by increasing the sonication power, increasing the external pressure, or decreasing the external temperature.

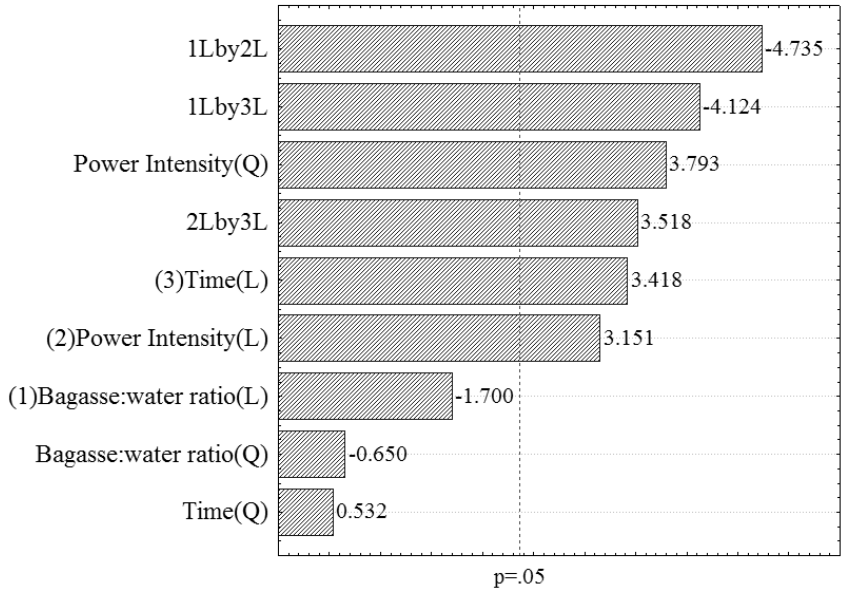


Fig.10 Pareto chart for the effect of sonication on H_2O_2concentration of cashew apple bagasse. Linear (L) and quadratic (Q) responses.1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time.

The fitted model for residual H_2O_2 concentration after sonication as a function of bagassse:water ratio, US power intensity and processing time is expressed by Eq. (10).The model was statistically significant at 95% of confidence interval since the calculated F value (11.06) was higher than the listed F value ($F_{9,7}$=3.68). The coefficient of determination (R^2) was 0.93. Fig. 11 shows the surface response graph built from Eq. 10.

$$Y = -109.16 + 1147.17x - 385.51x^2 - 1.08y + 0.004y^2 + 14.41z + 0.79z^2 - 2.14xy - 69.71xz + 0.082yz \quad \textbf{Eq. 10}$$

Where x, y and z are coded values for bagasse:water ratio, US power intensity and processing time, respectively.

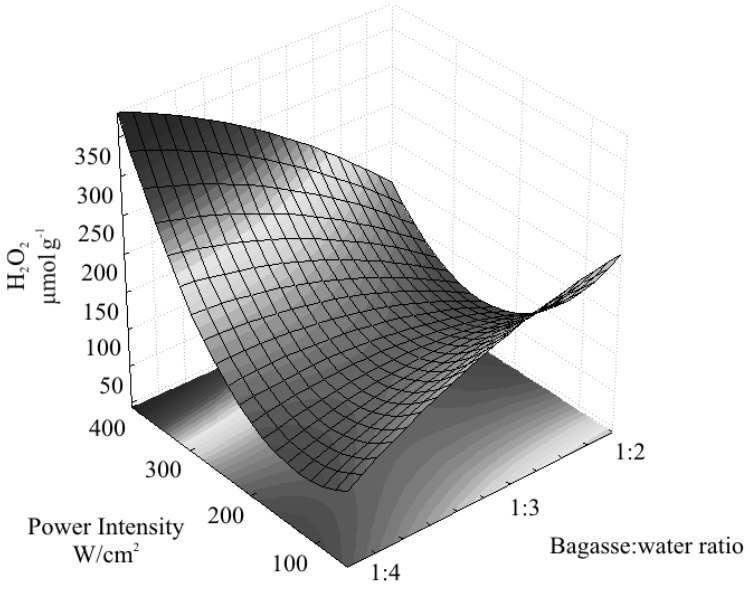


Fig.11 Response surface for H_2O_2 production after sonication of cashew apple bagasse plotted for a constant time of 6 minutes.

Radical formation is considered as a disadvantage for preserving the bioactivity of food components such as phenols (WAN *et al.*, 2005). At the high ultrasound power, the stimulating effect of ultrasound on H_2O_2 production increased steadily with exposure time (Table 7). In the present study, the increase in SOD and CAT activity of sonicated cashew apple bagasse may be suggested by its effective scavenging mechanism to remove H_2O_2, and it indicates the protective role of these enzymes against oxidative stress induced by sonication.

CAT activity was apparently most important in the removal of H_2O_2 of sonicated cashew apple bagasse, because its action was greater than the APX (Tables 3 e 4), and also appears to be associated more clearly the content of H_2O_2 (Table 7).

3.1.3 Effects on color parameters

Color is an important quality attribute in food products as it affects the visual appeal of the finished product as perceived by consumers. In general, discoloration is accompanied by some other deleterious effects such as off-flavour development and loss of nutrients. Therefore, the loss of phytochemicals and other nutrients in food can be closely related to the discoloration rate of the product (ONG; LAW; HII, 2011).

Table 8 presents the results of the experimental design carried out to evaluate the individual and interactive effects of bagasse:water ratio, ultrasound power intensity and processing time on colors parameters of cashew apple bagasse.

Table 8 Effect of ultrasound on color of cashew apple bagasse puree

Assay	L	h°	ΔC	ΔE
1	66.41 ± 0.05	87.77 ± 0.07	2.24 ± 0.00	6.31 ± 0.04
2	67.13 ± 0.00	88.72 ± 0.01	2.26 ± 0.00	7.20 ± 0.03
3	64.40 ± 0.01	87.59 ± 0.02	5.22 ± 0.02	7.08 ± 0.01
4	65.01 ± 0.03	89.09 ± 0.01	5.17 ± 0.09	7.63 ± 0.01
5	63.27 ± 0.00	87.36 ± 0.04	6.21 ± 0.06	7.17 ± 0.00
6	64.64 ± 0.00	88.53 ± 0.11	5.66 ± 0.02	7.56 ± 0.10
7	65.81 ± 0.10	88.79 ± 0.19	6.03 ± 0.00	8.39 ± 0.04
8	71.03 ± 0.03	91.87 ± 0.03	5.15 ± 0.01	12.3 ± 0.12
9	65.27 ± 0.09	89.51 ± 0.00	3.22 ± 0.04	6.70 ± 0.00
10	65.68 ± 0.05	89.67 ± 0.00	3.57 ± 0.00	7.26 ± 0.13
11	64.68 ± 0.01	89.44 ± 0.34	4.28 ± 0.00	7.01 ± 0.05
12	68.60 ± 0.00	91.70 ± 0.01	3.26 ± 0.10	9.89 ± 0.00
13	65.56 ± 0.01	88.07 ± 0.02	0.84 ± 0.03	5.43 ± 0.04
14	66.28 ± 0.02	89.14 ± 0.01	-0.41 ± 0.00	6.68 ± 0.03
15	67.06 ± 0.03	89.28 ± 0.02	0.61 ± 0.00	7.13 ± 0.01
16	67.67 ± 0.01	89.70 ± 0.00	0.62 ± 0.00	8.25 ± 0.01
17	66.59 ± 0.01	89.22 ± 0.00	0.61 ± 0.07	6.72 ± 0.02
		Control		
	L	**h°**	**Chroma**	
1:2	63.10 ± 0.01	84.99 ± 0.09	43.28 ± 0.07	---
1:3	61.90 ± 0.00	84.65 ± 0.05	42.09 ± 0.03	---
1:4	60.79 ± 0.00	83.32 ± 0.00	40.27 ± 0.02	---

Estimated effects of the independent variable on the responses are presented in Fig.12. Fig. 12A depicts the effects of independent variables on the color parameter lightness (L^*). At a confidence level of 95%, ultrasound intensity (linear), processing time (linear) and the effect of the interaction of power intensity and bagasse:water ratio presented significant positive effects on L^* values. Thus, in the experimental domain evaluated herein, sonication improves luminosity of resulting cashew apple puree.

During the US process, there is a disruption of cell membranes forming carotenoid-protein complexes that confer a greater homogenization and subsequent intensification of the red color due to the sonication (FONTELES *et al.*, 2012).

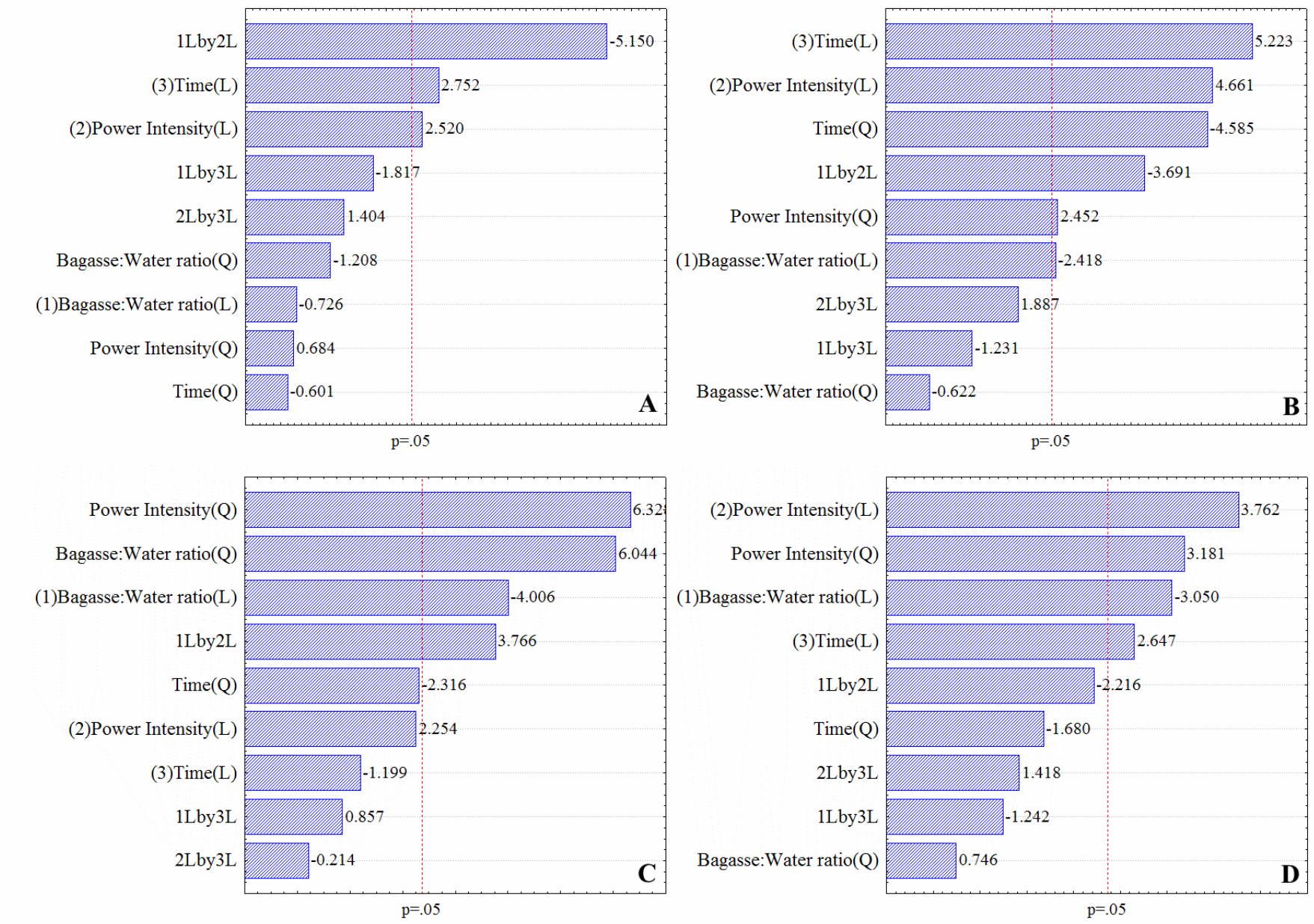
Fig. 12B shows the Pareto chart of the effect of independent variables on hue angle (h°). The processing time (linear) strongly influenced this parameter. However, a quadratic increase of time results in decrease of h° angle.

Despite some changes, the hue angle observed for sonicated cashew apple bagasse was an average 65°, which represents the characteristic color of cashew apple bagasse. This value is between 0° (red) and 90° (yellow) (BASTOS, 2004).

Fig. 12C shows that quadratic effects of ultrasound intensity and bagasse:water ratio significantly influenced the variation of chroma values. The ΔC indicates the degree of variation in the intensity of the chroma of the sonicated sample with relation to control. The lower the value of ΔC, the less the variation.

The effects of power intensity (linear and quadratic), as well as linear effects of bagasse:water ratio and processing time showed positive effects on ΔE at 95% of confidence level. CHOI *et al.* (2002) suggested that a ΔE>2 corresponds to visually perceptible differences in various products.

Fig. 12 Pareto chart for color parameters L^*(A), h°(B), ΔChroma (C) and ΔE (D) after sonication of cashew apple bagasse. Linear (L) and quadratic (Q) responses; 1L by 2L interaction between processing time and power intensity; 1L by 3L interaction between bagasse:water ratio and processing time; 2L by 3L interaction between power intensity and processing time



Thus, the sonication resulted in perceptible color changes in cashew apple bagasse. Despite this result, there were no evidences of browning since h° of sonicated samples was close to the characteristic color of cashew apple pulp.

Data presented in Table 3 was fitted to the quadratic models given in Eqs. 11-14. F test and ANOVA analysis were used as significance criteria for the fitted models. All models were statistically significant at 95% of confidence interval since the calculated F values (5.86 for Eq. (11); 11.90 for Eq. (12); 18.00 for Eq. (13) and 5.86 for Eq. (14)) were higher than the listed F value ($F_{9.7}$=3.68). Good correlation coefficients were also obtained (R^2=0.88 for Eq. (11); R^2=0.93 for Eq. (12); R^2=0.95 for Eq. (13) and R^2=0.88 for Eq. (14)). Fig. 6 shows the surface response graphs built from Eqs. 11-14.

$$\boldsymbol{L} = 48.51 + 64.58x - 51.83x^2 + 0.02y + 0.00002y^2 + 0.73z - 0.02z^2 - 0.09xy - 1.19xz + 0.0008yz \quad \textbf{Eq.11}$$

$$\boldsymbol{h}° = 82.42 + 16.47x - 13.06x^2 - 0.0005y + 0.00003y^2 + 1.18z - 0.08z^2 - 0.03xy - 0.39xz + 0.0005yz \quad \textbf{Eq.12}$$

$$\Delta\boldsymbol{C} = 37.15 - 159.41x + 186.02x^2 - 0.06y + 0.0001y^2 + 0.51z - 0.06z^2 + 0.04xy + 0.403xz - 0.0001yz \quad \textbf{Eq.13}$$

$$\Delta\boldsymbol{E} = 8.70 - 15.25x - 27.69x^2 - 0.01y + 0.0001y^2 + 0.90z - 0.05z^2 - 0.03xy - 0.70xz + 0.0007yz \quad \textbf{Eq.14}$$

Where:

x: Bagasse:water ratio;

y: Power intensity (W/cm^2);

z: Processing time (min).

The response surface plots showing the effect of power intensity and bagasse:water ratio on color parameters were illustrated in Fig. 14.

As shown in Fig. 14, power intensity of ultrasound was the parameter that most influenced the color change. The increase of processing time and power intensity increased L^*, h°, ΔC and consequently, ΔE (Figs. 14A, 14B, 14C, respectively).

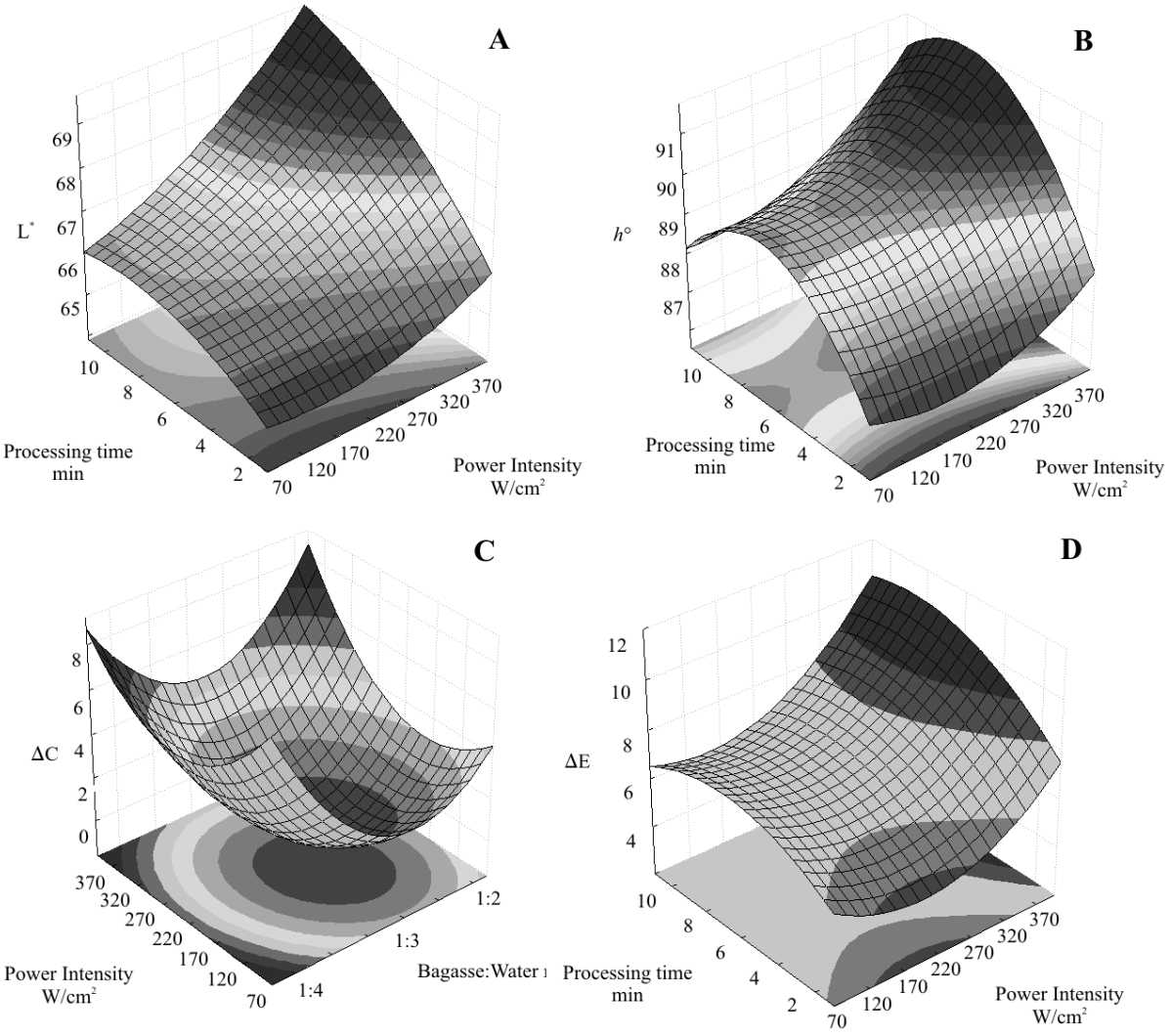


Fig.14 Response surfaces showing the effects of bagasse:water ratio, ultrasound power intensity, process time and their interactions on the color parameters L^*(A), h°(B), ΔChroma (C) and ΔE (D) for a constant bagasse:water ratio of 1:3 (14A, 14B, 14D) and processing time of 6 min (14C).

According to XU *et al.* (2013), there is a positive correlation between the accumulation of ROS and enzymatic browning. In this study, the effect of ultrasound in delaying browning could be correlated to the enhanced antioxidant enzyme activity and to decrease in guaiacol peroxidase activity.

3.4 Conclusions

The results collectively indicate that the the physical stimulus from sonication ultrasound induced the increase in hydrogen peroxide concentration and the activities of SOD, CAT and APX were responsible to neutralize the toxic effect of H_2O_2,converting it into water. Sonication also reduced the activities of deteriorative enzyme POD with preservation of color.

Acknowledgements

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Capítulo 4

Ultrasound processing to enhance drying of cashew apple bagasse: Influence on antioxidant properties and in vitro bioaccessibility

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Abstract

The effects of ultrasound on air-drying process and bioactive compounds of cashew apple bagasse were evaluated. Sonication induced disruption of cashew bagasse parenchyma, which resulted in lower resistance to water diffusion, less hysteresis effect and increased rehydration rate. Sonication did not affect the lignocellulosic fibers, or eschlerenquima cells. For sonicated samples, in the first 2 hours of drying, water activity reached values below 0.4, considered appropriated in order to prevent bacterial and fungi growth. The increase of the mass transfer due to ultrasound processing can be attributed to the reduction on the boundary layer thickness produced by pressure variations, oscillating velocities and microstreaming. The sorption isotherms of cashew apple bagasse had sigmoid-shape for all samples and followed the type II of BET classification. Sonicated cashew apple bagasse presents high antioxidant activity, and high total phenolic compounds (TPC) and vitamin C values. The increase in phenolic compounds and the high final amount of vitamin C may lead to enhance the antioxidant activity. The ultrasound processing caused some reduction of bioaccessibility of vitamin C but increase on TPC bioaccessibility, compared to control. Sonication reduces the severity of conventional drying treatments, therefore, improved the quality of the dried product.

Keywords: Sonication, drying, sorption, bioactive compounds, bioaccessibility.

4.1 Introduction

One of the main purposes in modern food technology is to maximize the retention of nutrients during processing and storage. Drying is a technology that promotes important modifications in physical and chemical properties of food affecting the bioactive compounds and antioxidant capacity. To avoid these undesired changes, drying can be combined with pre-treatments in order to improve process parameters in addition to obtain high quality products.

It has been demonstrated that ultrasound can improve mass and/or heat transfer phenomena (OLIVEIRA *et al.*, 2011). In treatments with a solid immersed in a fluid, ultrasound could accelerate the internal transport making the entry of fluids in the solid matrix and/or their exit easier and also facilitating the exchanges between the solid surface and the surrounding fluid (CÁRCEL; BENEDITO; MULET, 2012). The effect of ultrasound on fruit tissue depends on the tissue structure and composition, and ultrasound might be beneficial to improve air-drying efficiency, with consequent reduction in process costs (RODRIGUES *et al.*, 2009).

Cashew apple is an important crop of Brazil, representing 1.1% of world production of exotic fruits (RAWSON *et al.*, 2011). The cashew apple bagasse is the industrial waste processing of cashew apple peduncle. The residue has a dark yellow color, a fibrous aspect, and a typical adstringent aroma due to the presence of tannins. More recently, many studies were done to develop new cashew products and to make a better use of this source (ROCHA *et al.*, 2014; SANTOS *et al.*, 2007; MACEDO *et al.*, 2014). Thus, the objective of this study was to evaluate the effect of ultrasonic processing on convective drying and antioxidant compounds of cashew apple bagasse. The in vitro bioaccessibility of the phenolic compounds and vitamin C from cashew apple bagasse, using an in vitro model that simulated some chemical and biological gastrointestinal conditions were also investigated.

4.2 Materials and Methods

4.2.1 Sample preparation

Red cashew apples were harvested at commercial maturity stage in Ceará State, Brazil, from September to December 2012. 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. The peduncles were not protected from light to simulate the conditions that the fruit may be exposed in the postharvest period. The solid residues (bagasse) were packaged in polyethylene bags, vacuum sealed and stored at −18°C until use. Prior to the experiment, the cashew apple bagasse was thawed at 4°C.

4.2.2 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 (Unique® DES500, São Paulo, Brazil) with a 1.3 cm diameter probe tip without mechanical agitation or temperature control. The ultrasound frequency was 20 kHz.

The ultrasound probe was submerged to a depth of 25 mm in the sample. The treatment was carried out in triplicate. The intensity of ultrasound power which dissipated from the probe tip was calculated by Eq. (1) (LI; PORDESIMO; WEISS, 2004).

$$I = \frac{P}{\pi r^2} \qquad \textbf{Eq.1}$$

Where r is the radius of the titanium tip (cm) and P is the input power level (W). The input power was controlled through amplitude setting and the power level was adjusted to 20, 60 and 100% of total input power (500 W), which was equal to 300 W. The calculated intensity were 75, 226 and 373 W/cm^2, respectively.

4.2.3 *Experimental design and data analysis*

The effects of ultrasound processing were evaluated through a 2^3 face-centered central composite experimental design (CCD) with 3 central points. The power intensity, the bagasse:water ratio (g/g) and the processing time were changed from 75 to 373 W/cm^2, 1:2 to 1:4 and 2 to 10 min, respectively.

Control assay was carried out at 51 °C, which was the temperature recorded after the cashew apple bagasse sonication. Samples of 200 mL of the non sonicated bagasse were kept in a water bath at the desired bagasse:water ratio and time, according to experimental design, at 51°C.

4.2.4 Air-Drying

The samples were air-dried in a forced circulating air-drying oven (Marconi model MA-085, Brazil) after the ultrasound processing. The forced circulating air-drying oven was set at 60°C with air moisture content at 18% (determined by psychrometry). The sonicated cashew apple bagasse was set in a single-layer and transferred to the forced circulating air-drying. The fruit moisture (water content) during the air-drying period was measured weighting the samples every hour until constant weight.

The experimental data was used to calculate the effective water diffusivity in cashew apple bagasse during air-drying according to Fick's law of diffusion. The equation used for the falling-rate period of the drying process was based on the simplification of Fick's second law considering long processing period (PERRY; GREEN, 1999).The effective water diffusivity was adjusted using Eq. 2 with a parameter estimation procedure based on the minimization of the error sum of squares.

$$\frac{\mathrm{d}H}{\mathrm{d}t} = -\frac{2\pi}{\delta^2}.D.(H - H_{\mathrm{eq}}) \qquad \textbf{Eq.2}$$

Where D is the effective water diffusivity (m^2/min), H is the moisture content (g of water/g of dry matter), H*eq* equilibrium moisture content (g of water/g of dry matter), t is time (min), e δis the bed height (or thickness) of the fruit (mm).

4.2.4.1 *Water activity*

Water activity was determined with a water activity meter (AquaLab, Decagon CX-2, Pullman, Washington, USA).

4.2.4.2 *Determination of sorption isotherms*

Vapor sorption isotherms of dried cashew apple bagasse were generatedusing a Vapor Sorption Analyzer (VSA) (Aqualab, Decagon Devices, Pullman., USA) at 25 °C.

The experimental data of adsorption were fitted to the mathematical models of Brunauer, Emmet e Teller (BET); Guggenheim- Anderson-De Boer (GAB) and Double Log Polynomial (DLP) as follow:

GAB **Eq.3**

$$Xeq = \frac{X_m C k a_w}{(1-k a_w)(1-k a_w)+C k a_w}$$

BET **Eq.4**

$$Xeq = \frac{X_m C a_w}{((1-a_w)(1-(C.\ln(1-a_w))))}$$

DLP **Eq.5**

$$Xeq = b3.chi^3 + b2.chi^2 + b1.chi + b0$$

Where:

Xeq = equilibrium moisture content on dry basis (kg water/kg dry solid);

Xm = moisture content in molecular monolayer on dry basis (kg water/kg dry solid);

Aw = water activity (dimensionless);

C, k, a, b, b0, b1, b2 e b3 =empirical coefficients*;*

$Chi = \ln(\ln(a_w))$.

The parameters used to evaluate the model fittings were the coefficient of determination (R^2) and the estimated error calculated by the Software VSA Downloader 1.0.967.

4.2.4 *Microscopic analysis*

4.2.5.1 *Light microscopy*

After sonication processing, small tissue fragments were fixed for four hours in a solution of 2.5% glutaraldehyde, 4.0% formaldehyde, freshly prepared in 0.05 M cacodylate buffer, pH 7.2. The material was then dehydrated in a graded ethanol series and embedded in Historesin embedding kit (Leica Historesin, Germany). The tissue blocks were sectioned on a Leica microtome (RM2255- Leica, Germany). Thin sections (aproximatelly 3μm) were stained with 1% toluidine blue pH 4.0. Photomicrographs of the cell structure were taken using an Olympus BX51 (Olympus, Japan) light microscope with digital image capture system.

4.2.5.2 *Scanning electron microscopy*

After sonication processing, small tissue fragments were fixed for four hours in a solution of 2.5% glutaraldehyde, 4.0% formaldehyde, freshly prepared in 0.05 M cacodylate buffer, pH 7.2. Subsequently, the samples were rinsed in the same buffer and post-fixed for one hour at room temperature (20 °C) with 1.0% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.2. The post-fixed samples were dehydrated in an ascendant acetones series. Acetone was partially dried on absorbent paper and the fragments were fixed on stubs with carbon conductive tape. Afterwards, the samples were air dried, sputtered coated with 10nm gold and observed with a scanning electron microscope (Inspect F50-FEI).

4.2.6 *Rehydration ability*

Dried cashew apple bagasse samples were rehydrated by immersion in distilled water (solid-to-liquid ratio 1:50) at 20°C for 2 h, as described by (GAMBOA-SANTOS *et al.*, 2014). Excessive water was removed from samples before weighting the samples. The rehydration ratio was calculated according to the following equation was calculated as follows:

$$\text{Rehydration ratio} = \frac{m_r}{m_d} \qquad \textbf{Eq.6}$$

Where m_r is the mass of the rehydrated sample and m_d is the mass of dehydrated sample.

4.2.7 Color

The color of the cashew apple bagasse during drying was determined using a Minolta CR300 colorimeter (Tokyo, Japan). The colorimeter was calibrated using the illuminant D65, and measurements were made through an 8-mm port/viewing area. The reflectance instruments determined the following color parameters: lightness (L^*), C (chroma) and hue angle (h°). Color measurements were taken in quintuplicate.

4.2.8 Enzymatic assay

For peroxidase (POD, EC 1.11.1.7) and polyphenoloxidase (PPO,EC 1.14.18.1) determination, enzyme extraction was done according to the methodology described by WISSEMANN and LEE (1980). Samples (10 g) were mixed with 10 mL of potassium phosphate buffer (0.05 M pH 7.0) containing1% (w/v) of polyvinylpyrrolidone (PVP). The mixture was centrifuged in a Sigma® 6K15 centrifuge (10,733 g for 30 min at 4 °C).The supernatant was used as the enzyme source.

POD activity was monitored at 470 nm in a spectrophotometer(Spectrum® SP200UV) according to the method described by MATSUNO and URITANI (1972). The enzyme activity was measured as follows: 2.75 mL of a phosphate (sodium)-citrate (citric acid) buffer(0.1 M, pH 5.0) containing 1% (v/v) of guaiacol and 0.25 mL of hydrogen peroxide 3% (v/v) were added to 1.5 mL of enzyme extract. The assay mixture was incubated at 30 °C for 5 min. The reaction was interrupted with the addition of 1 mL of sodium bisulfite30% (w/v). One unit of enzyme activity (1 UEA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

PPO activity was measured based on the method reported by WISSEMANN and LEE (1980). The reaction mixture contained 0.3 mL of enzyme extract and 1.85 mL of a potassium phosphate buffer solution (0.1 M pH 6.0) containing catechol (0.1 M) and KCl (0.1 M).The reaction mixture was incubated at 30 °C for 30 min. The reaction was interrupted with the addition of 0.8 mL of perchloric acid 2 N. 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.

4.2.9 Quantification of bioactive compounds

4.2.9.1 Extract preparations

Extracts for ascorbic acid analysis were obtained by submitting the samples to centrifugation at 11.806×*g* for10 min in a Sigma 6K-15 centrifuge (Sigma Centrifuges, Germany).The supernatants were recovered as ascorbic acid source.

The extracts for total phenolic compounds (TPC) and antioxidant activity were prepared according to the procedure developed by LARRAURI; RUPÉREZ and SAURA-

CALIXTO (1997). Samples were weighed (2g) in centrifuge tubes and extracted sequentially with methanol and acetone aqueous solution. Firstly, 10 mL of methanol/water (50:50, v/v) was added to the sample at room temperature (20 °C). The mixture was kept statically for 1 hour at room temperature and the extraction was allowed for 1h.The samples were centrifuged at 10733g (15 min/ 4°C) and the supernatant was recovered. Then, 10 mL of acetone/water (70:30, v/v) was added to the solid residue at room temperature, extracted for 60 min and centrifuged. Methanol and acetone extracts were combined into a 25 mL volumetric flask, which volume was completed with distilled water.

4.2.9.2 Antioxidant determinations

Ascorbic acid content was analyzed by high performance liquid chromatography in a Agilent 1260 Infinity system equipped with four high-pressure pumps model Agilent G1311B, UV–VIS detector ProStar model 345, and column oven(Agilent G1316A). Separations were done using a Biorad HPX 87 H (300×7.8 mm) column at 50°C. H_2SO_4 0.01N at 0.6 mL/min was used as eluent. All samples were analyzed in triplicate. The software Agilent OpenLAB was used to acquire and handle the data.

Total phenolic compounds were determined using the Folin-Ciocalteau methodology (OBANDA; OWUOR, 1997). The reaction mixture contained: 250 μL of the phenolic extract, 500μL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), 500 μL of sodium carbonate and 500 μL of distilled water. The mixture was then left in the darkness for 30 min at 25 °C. The absorbance of the sample was measured at 700 nm. Gallic acid (HPLC grade, Sigma-Aldrich) was used as standard. Results were expressed as mg/100g.

4.2.10 *Total antioxidant activity determinations*

For $ABTS^{\bullet+}$ assay, the procedure followed the method of RUFINO *et al.* (2010). The stock solutions included 7 mM $ABTS^{\bullet+}$solution (solution A) (Sigma®) and 140 mM potassium persulfate solution (solution B) (Sigma®). The working solution was then prepared by mixing 5000 μL of the solution A and 88 μL of solution B and allowing them to react for 16 h at room temperature (20 °C) in the dark. The solution was then diluted by mixing $ABTS^{\bullet+}$ solution with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. The

extracts (30μL) were allowed to react with 3000μL of the $ABTS^{\bullet+}$ solution for 6min in a dark condition. The level of radical scavenging was calculated according to the following equation:

$$\text{Scavenging rate}(\%) = \left(1 - \frac{A_i}{A_s}\right) \times 100 \qquad \textbf{Eq.7}$$

Where *As* is the absorbance of pure $ABTS^{\bullet+}$, *Ai* is the absorbance of $ABTS^{\bullet+}$ in the presence of sample.

4.2.11 *In vitro digestion*

The evaluation of phenolics and vitamin C submitted to gastric and enteric simulated conditions was performed according to the method described by (CHEN *et al.*, 2014) with modifications. The analysis consisted of two sequential phases: gastric and enteric digestion. In gastric phase, the pH of samples (10g) was adjusted to 2.0 with 6 M HCl in the sequence pepsin solution 300U/mL (10mL) was added. The mixture was incubated at 37 °C, with agitation of approximately 100 rpm during 2 h. In the enteric phase, the pH of samples was increased to 6.0 using an alkaline solution (1M NaOH). Bile and pancreatin were added to reach a concentration of 10g/L and of 1g/L, respectively. Samples were incubated again at 37 °C for 2 h under agitation (enteric phase 1). In the last step, the pH was increased to 6.7–7.5 using the same alkaline solution, bile and pancreatin concentrations were adjusted (10 g/L and 1 g/L, respectively), and samples were incubated again at 37 °C for 2 h under agitation (enteric phase 2), achieving 6 h of assay. Immediately after the enteric phase, 2 mL of each sample was extracted and analyzed for TPC and vitamin C concentrations as described above. All reagents were purchased from Sigma-Aldrich©.

4.2.12 *Statistical analysis*

Except color determination, which was taken in quintuplicate, all other assays were carried out in triplicate. 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 ($p<0.05$). Pearson's correlation coefficients were calculated at 5% of probability using the Student's t test for all variables. Statistical analysis of the experimental data was carried out using the software Statistica 7.0 (Statsoft).

4.3 Results and discussion

4.3.1 Drying

Table 1shows the data for water diffusivity and the mean values of initial moisture content of the cashew apple bagasse for the experimental design.

Table 1 Experimental design and responses of the influence of ultrasonic processing on water activity and water diffusivity of cashew apple bagasse

Assay	Power Intensity (W/cm^2)	Time (min)	Bagasse:water Ratio	Water diffusivity ($10^{-10} m^2/s$)
1	75	2	1:2	1.06 ± 0.00
2	75	10	1:2	1.07 ± 0.01
3	373	2	1:2	1.18 ± 0.00
4	373	10	1:2	1.04 ± 0.04
5	75	2	1:4	2.14 ± 0.01
6	75	10	1:4	2.16 ± 0.02
7	373	2	1:4	1.50 ± 0.01
8	373	10	1:4	1.13 ± 0.03
9	226	6	1:2	1.04 ± 0.03
10	226	6	1:4	1.59± 0.00
11	75	6	1:2	1.18 ± 0.00
12	373	6	1:3	1.11 ± 0.02
13	226	2	1:3	1.20 ± 0.04
14	226	10	1:3	1.18 ± 0.01
15 (C)	226	6	1:3	1.66 ± 0.01
16 (C)	226	6	1:3	1.11 ± 0.02
17 (C)	226	6	1:3	1.24 ± 0.00
			Control	
			1:2	1.06 ± 0.00
			1:3	1.18 ± 0.01
			1:4	1.66± 0.01

The application of ultrasound in different levels affected the effective water diffusivity of cashew apple bagasse during the air-drying process (Table 1). The maximum diffusivities observed were2.16 x 10^{-10} in the assay 6 (75W/cm^2; 10 min; 1:4 bagasse:water ratio) and 2.14 x 10^{-10} in the assay 5 (75W/cm^2; 2 min; 1:4 bagasse:water ratio).The increase of the mass transfer due to ultrasound processing can be attributed to the reduction on the boundary layer thickness produced by pressure variations, oscillating velocities and microstreaming (OZUNA *et al.* 2011). FUENTE-BLANCO *et al.* (2006) also reported that cavitation produced by ultrasound is beneficial for the removal of moisture strongly attached.

Fig. 1 depicts the estimated effects of the independent variables on water diffusivity.

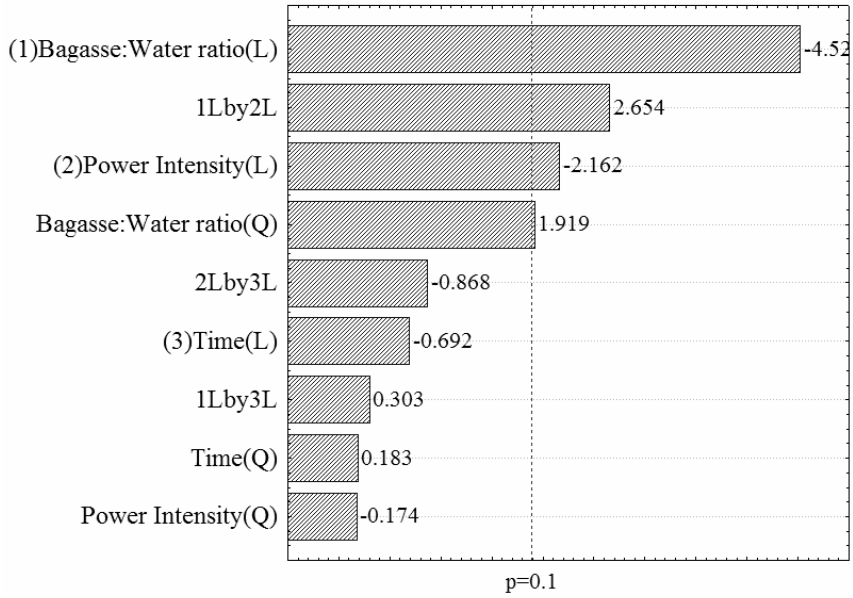

Fig. 1 Pareto chart for water diffusivity of cashew apple bagasse in air-drying process after sonication. Linear (L) and quadratic (Q) responses; 1L by 2L interaction between processing time and power intensity; 1L by 3L interaction betweenbagasse:water ratio andprocessing time; 2L by 3L interaction between power intensityand processing time.

The Pareto chart reveals that a linear increase of bagasse:water ratio and power intensity results in decrease of water diffusivity. However, the effect of the interaction of bagasse:water ratio and power intensity was significant on the water diffusivity increase ($p<0.1$).Meaning that, a simultaneous increase of US power intensity and bagasse:water ratio favor the increase of water diffusivity.

Water diffusivity data was fitted to the quadratic model given in Eq. 8. F test and ANOVA analysis were used as significance criteria for the fitted models. The model was statistically significant at 90% of confidence interval since the calculated F value was higher than the listed F value ($F_{9.7}=2.72$).Good correlation coefficients was obtained ($R^2=0.83$). Fig. 6 shows the surface response graph built from Eq. 8.

$$\boldsymbol{D} = 5.90 - 19.65x + 19.22x^2 - 0.003y - 0.000001y^2 - 0.02z - 0.0015z^2 + 0.01xy + 0.04xz - 0.0001yz \quad \textbf{Eq.8}$$

The response surface plot showing the effect of power intensity and bagasse:water ratio on water diffusivity was illustrated in Fig. 2.

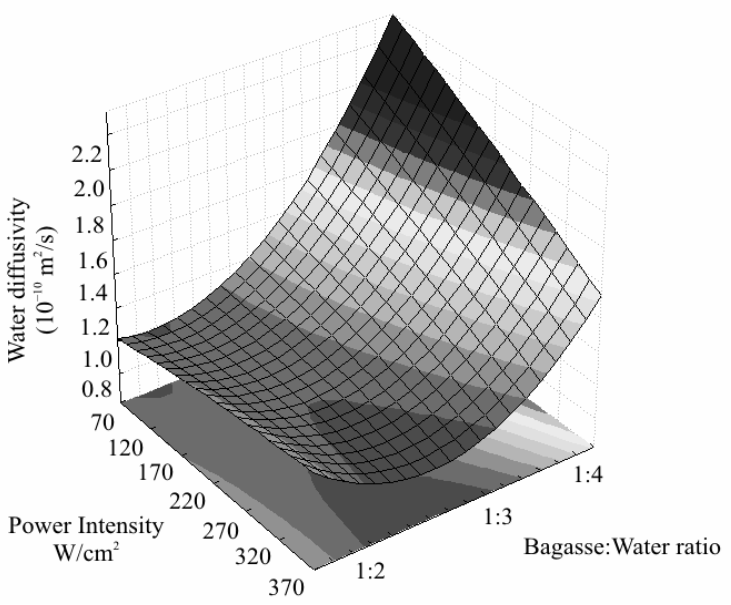

Fig.2 Response surface plot showing the effects of bagasse:water ratio and ultrasound power intensity on water diffusivity plotted for a constant processing time of 6 min.

Generally, water diffusivity was increased with increasing bagasse:water ratio and power intensity. The highest water diffusivity was observed at higher bagasse:water ratios (Table 1). OZUNA *et al.* (2011) reported that the ultrasonic effects were dependent on the applied power, the higher the ultrasonic power, the higher the identified effective diffusivity values. The increase in water diffusivity implies in reduced processing times which can represent an economy of energy, since air drying is expensive. The authors also pointed out that to achieve an improvement of effective diffusivity just by heating, an increase of the air drying temperature from 40 to 65 °C should be necessary, which could involve the degradation of heat sensitive compounds.

Fig. 3 shows the evolution of water activity along drying. The faster decreases of water activity were observed for experimental assays 6, 8, 10 and 11 (Table 1). However, in assay 5 (1:4 bagasse:water ratio, 75 W/cm^2/ 2min) the decrease in water activity was more intense in the first hours of drying.

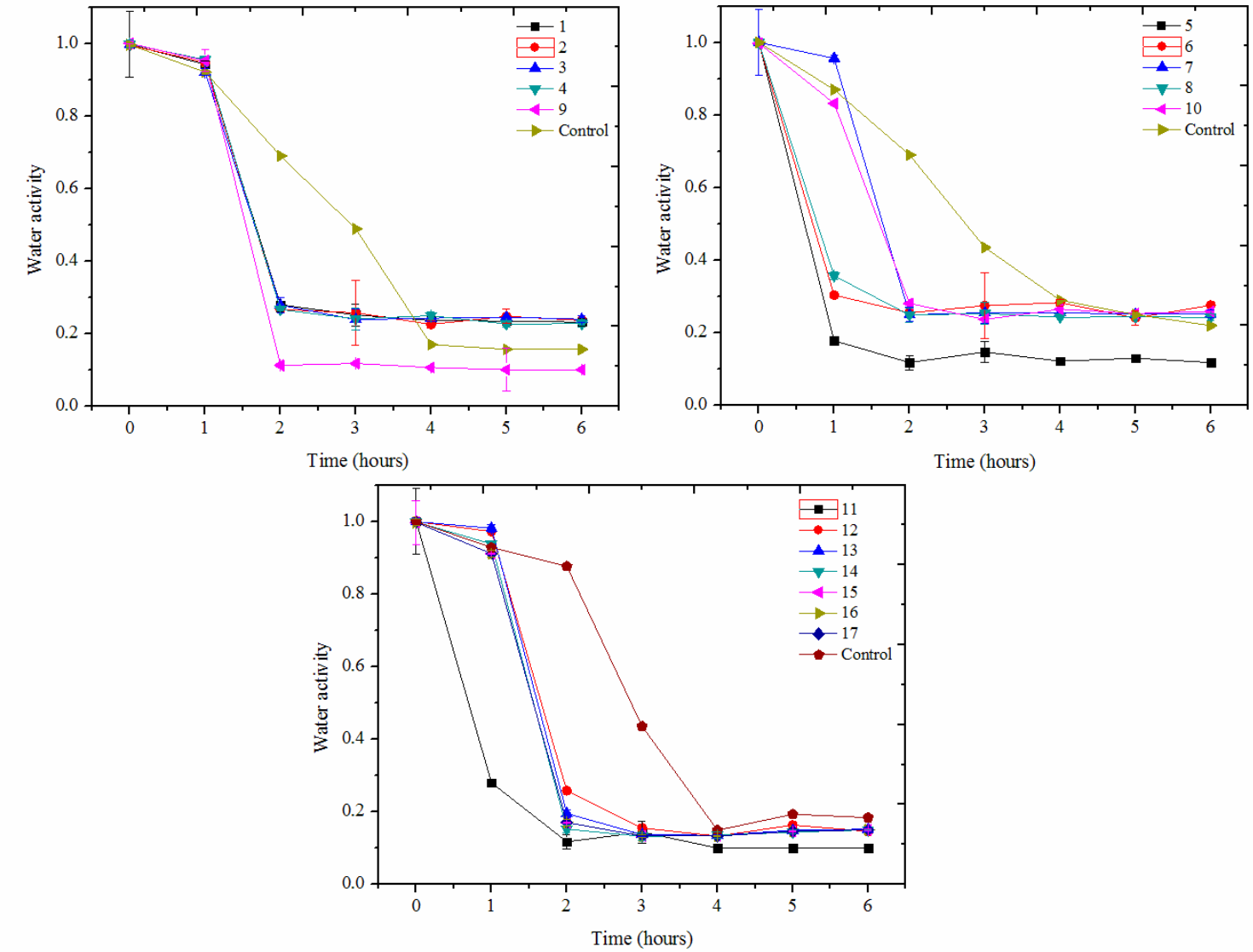

Fig.3 Evolution of water activity during drying of sonicated cashew apple bagasse. Each assay of experimental design was compared to its respective control.

Ultrasound is assumed to act on the moisture removal after cell membrane disintegration by decreased viscosity of the liquid, increased diffusivity, local heating due to cavitation phenomena, improved evaporation due to pressure drops, minimized diffusion boundary layer thickness and compression and rarefaction caused by the sound wave (sponge effect) (SCHÖSSLER; KNORR, 2012).

For sonicated samples, in the first 2 hours of drying, water activity reached values below 0.4, considered appropriated in order to prevent bacterial and fungi growth (JAY *et al.*, 2005).

For the subsequent drying optimization, the experimental assays 5 and 6 (1:4 bagasse:water ratio; 226 W/cm^2/6 min) was chosen due their higher water diffusivity values. The obtained moisture contents were plotted as a function of water activity to yield the sorption isotherms of cashew apple bagasse at 30 °C (Fig.4).

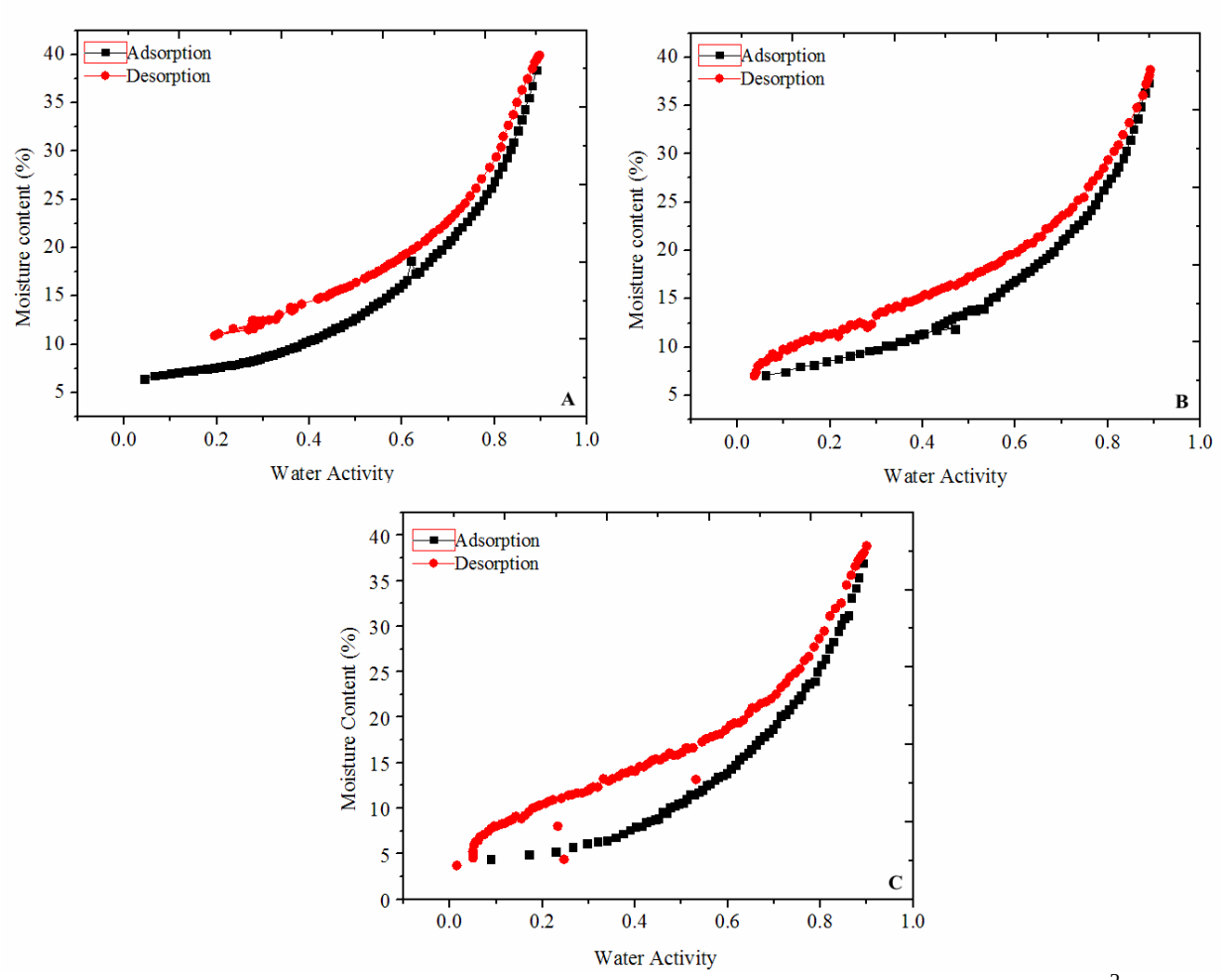

Fig.4 Sorption isotherms of dried cashew apple bagasse at 30°Csonicated at 226 W/cm^2 using bagasse:water ratio of 1:4 during 2 min (4A) and 10 min (4B). Fig. 4C shows the sorption isotherm for control sample.

The sorption isotherms of cashew apple bagasse had sigmoid-shape for all samples and followed the type II of BET classification (BRUNAUER *et al.*, 1938) (Fig.4). ALCÂNTARA *et al.* (2009) also found type II isotherm for dried cashew apple bagasse.

The plots of the desorption and adsorption isotherms of cashew apple bagasse presented in Fig. 4show evidence of the occurrence of moisture sorption hysteresis, which occurs when the adsorption isotherm has lower values than the desorption isotherm. According to CAMPBELL (2008), it results from the fact that, at given water content, more energy is required to remove water from dried material.

The hysteresis at different processing conditions presented distinct profiles. Sonicated samples (4A and 4B) resulted in the less hysteresis effect, reflected on closer adsorption and desorption curves compared to control (Fig. 4C). CAURIE (2007) reported that hysteresis can be an index of food quality since its increase indicates reducing the stability of the food; since its reduction or absence indicates better stability of the stored products.

Tables 2, 3, and 4 shows the parameter values of the models of desorption isotherm of sonicated cashew apple bagasse and control sample.

Table 2 Parameter values of the models of the desorption isotherm of cashew apple bagasse sonicated at 75W/cm^2 during 2 min at 1:4 of bagasse:water ratio (Assay 5) and their coefficients of determination (R^2)

Models	Parameters				R^2	E
GAB	**Xm**	**C1**	**k**			
	9.23	1.06x10^4	0.86		0.998	0.335
BET	**Xm**	**C**				
	12.38	14.97			0.976	0.223
DLP	**b0**	**b1**	**b2**	**b3**		
	13.54	-6.35	3.08	0.19	0.998	0.322

Table 3 Parameter values of the models of the desorption isotherm of cashew apple bagasse sonicated at 75W/cm^2 during 10 min at 1:4 of bagasse:water ratio (Assay 6) and their coefficients of determination (R^2)

Models	Parameters				R^2	E
GAB	**Xm**	**C1**	**k**			
	10.16	82.92	0.82		0.998	0.32
BET	**Xm**	**C**				
	12.01	34.25			0.965	0.52
DLP	**b0**	**b1**	**b2**	**b3**		
	14.45	-7.05	1.45	-0.20	0.999	0.23

Table 4 Parameter values of the models of the desorption isotherm of cashew apple bagasse (control sample) and their coefficients of determination (R^2)

Models	Parameters				R^2	E
GAB	**Xm**	**C1**	**k**			
	8.73	35.10	0.86		0.997	0.57
BET	**Xm**	**C**				
	10.93	17.60			0.972	0.49
DLP	**b0**	**b1**	**b2**	**b3**		
	12.41	-6.94	1.32	-0.54	0.997	0.50

Where: Xeq = equilibrium moisture content on dry basis (kg water/kg dry solid);Xm = moisture content in molecular monolayer on dry basis (kg water/kg dry solid);Aw = water activity (dimensionless);*C, k, b0, b1, b2 e b3* = empirical coefficients.

Analyzing the parameters of BET and GAB models (Tables 2, 3 and 4), sonicated samples showed higher values of monolayer (Xm) compared to control (Table 4). McMINN and MAGEE (1999) attributed an inverse relation of Xm and temperature. In other words, an increase of temperature results in decreased monolayer resulting in reduced ability to absorb water, which may be caused by thermal physical and chemical changes. In this study, these chemical and/or physical changes have been caused by ultrasound, which may have reduced the number of sites (hydroxyl groups) on the bagasse surface available to bond to water molecules (hydrogen bonds).According to GABAS *et al.* (2009), the value of monolayer moisture (Xm) reflects the amount of water that is strongly adsorbed on specific locations of food is considered the best value to assure its stability.

IZIDORO (2011) also found higher monolayer values for sonicated banana starch compared to control. The author observed a significant reducing in hygroscopicity of pre-treated starch.

The GAB constants C1 and K are indicative of the type of isotherm, according to the classification of BRUNAUER *et al.* (1938). Observing the parameters C1 and K in Tables 2, 3, and 4, it is possible to note that in all $k<1$ and $C> 2$ which, according BLAHOVEC (2004) values for these isotherms are type II isotherms as can be observed in Fig. 4.

According to R^2 values, all models have adjusted well to data, but to evaluate the best fit, the lower error (E) was considered. Therefore, the DLP equation was the best fit to all samples evaluated, followed by GAB and BET models, respectively. DLP is a model developed by Decagon Devices©. The developers considered DLP superior to the others modeling complex isotherms because it provides a greater adjustment in a wide range of Aw (DECAGON, 2009).

4.3.2 Tissue structure

Figure 5 shows the scanning electron microscopy of raw and sonicated cashew apple bagasse.

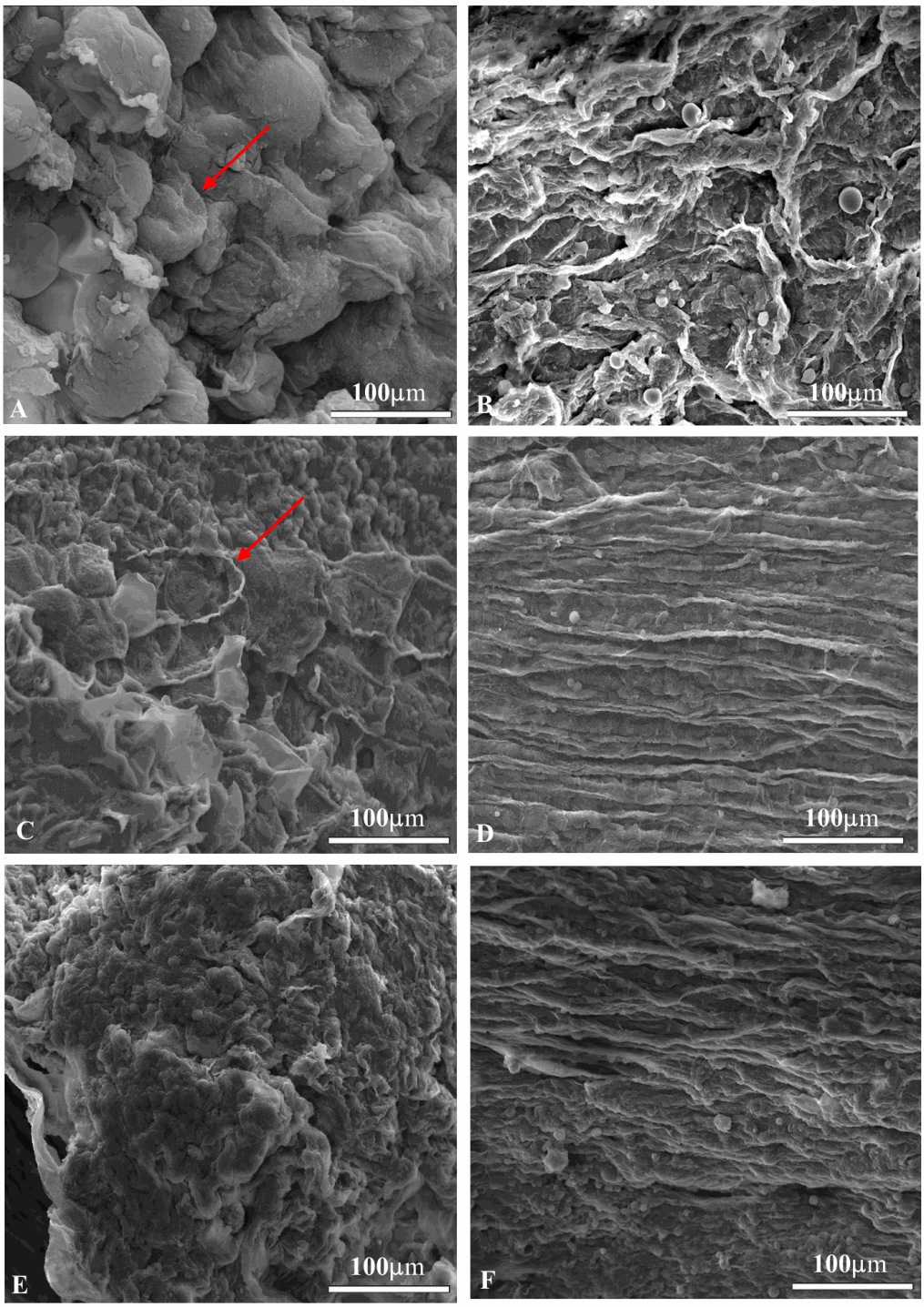

Fig.5 Scanning electron micrographs of cashew apple bagasse after 2 min of sonication at 75 W/cm^2: (5A and 5B); bagasse after 10 min of sonication at 75 W/cm^2 (5C and 5D); and raw bagasse (5E and 5F). 1000X

The application of ultrasonic waves caused severe structural damage to cells with visible rupture of parenchyma cells compared to raw bagasse (Fig. 5E) as seen in the scanning electron micrographs (Fig. 5C; assay 5). Although no observable rupture was found for assay 6 (Fig. 5A), it is possible to note the plasmolysed aspect of cells. Parenchyma cells are composed almost entirely of fibrils of cellulose and play a role in water movement and transport of substances in plants (SUDHEER; INDIRA, 2007; RAVEN *et al.*, 2001).

Sonication did not affect the lignocellulosic fibers, or sclerenchyma cells (Fig. 5B; 5D).The main feature of sclerenchyma cells is the presence of thickened and often lignified secondary walls. Due to the presence of these walls, sclerenchyma cells are important elements in the strength and support of the plant (RAVEN *et al.*, 2001).

This result is consistent with previous findings from RODRIGUES and FERNANDES (2007) that the ultrasonic pretreatment affects the fruit tissue, making it easier for water to diffuse during air-drying, and indicated that the microscopic channels may contribute to the increase in water diffusivity.

RODRIGUES *et al.* (2009) also found disruption and breakdown of cells with elongation of parenchyma cells applying ultrasound for sapota. The ultrasound frequency used was 25 kHz and the intensity was 48.70 W/cm^2 during 10min. In this work, higher ultrasound intensities were applied to samples, which can explain the intensity of damages observed for cashew apple cells. As the authors suggested, the effect of ultrasound on fruit tissue depends on the tissue structure and composition, but also depends on the processing parameters.

Fig. 6 evidenced the formation of microchannels and the collapse of cells due sonication.

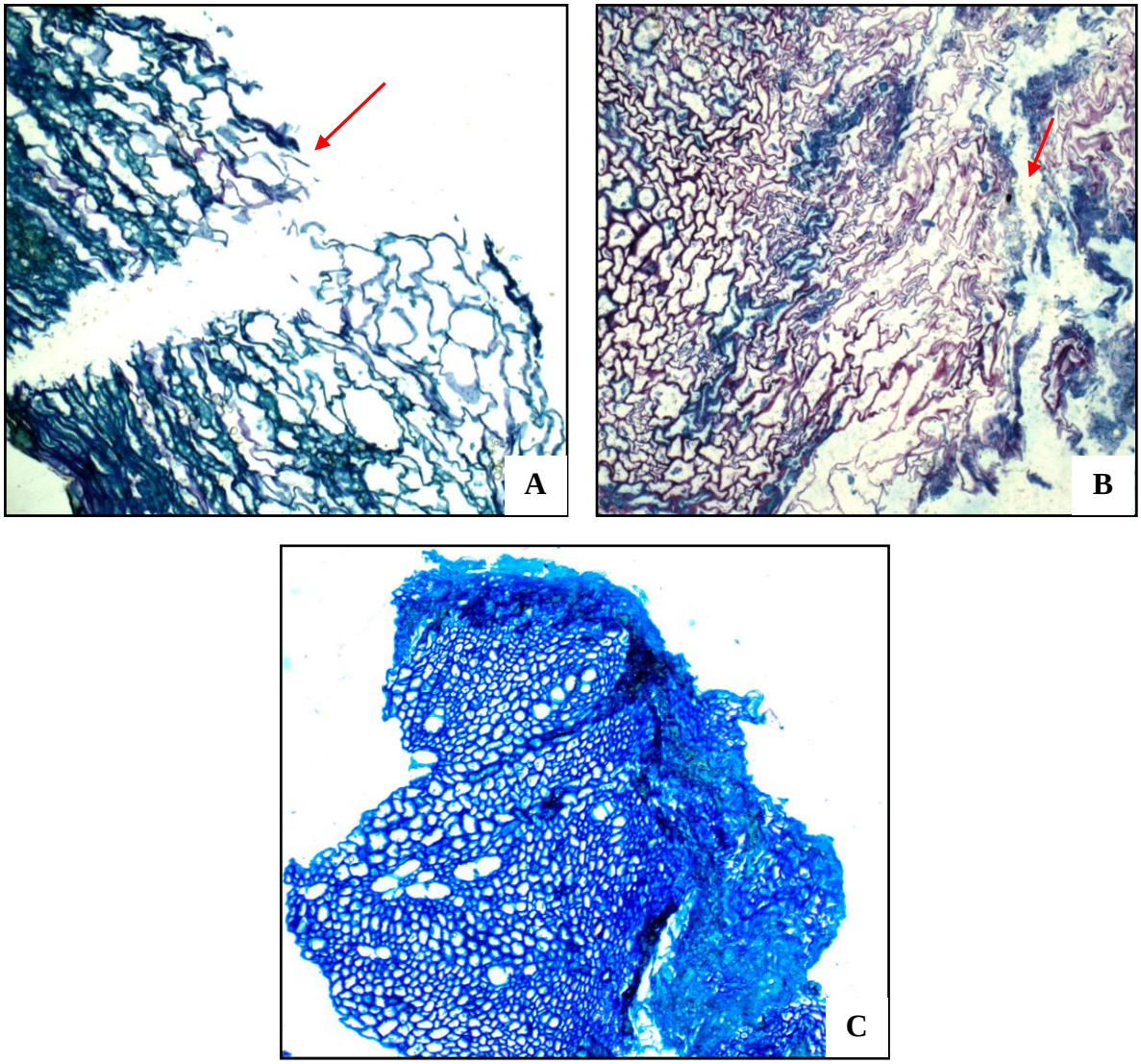

Fig.6 Photomicrographs of cashew apple bagasse after 2 min of sonication at 75 W/cm^2: (A), region with collapsed cells; and bagasse after 10 min of sonication at 75 W/cm^2 (B), region with wide microscopic channels. Control sample (C). Magnification: 20X.

The structural changes observed for sonicated samples significantly affected the rehydration capacity of cashew apple bagasse (Fig.7).

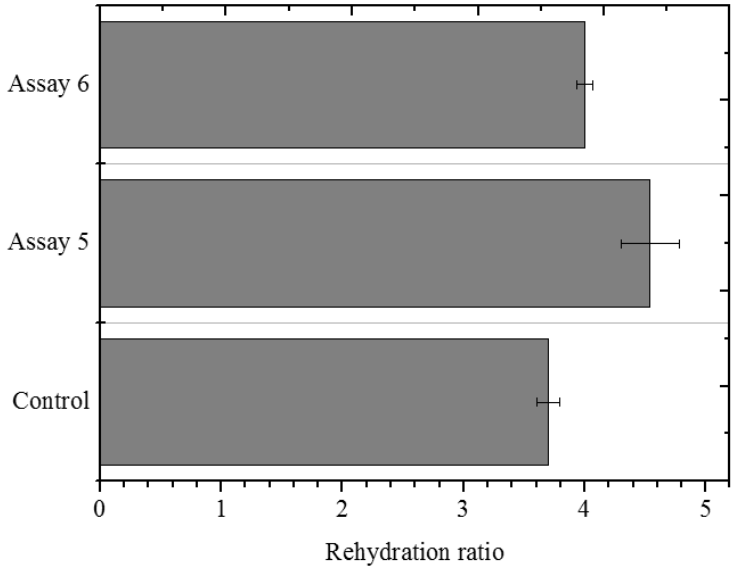

Fig.7 Rehydration ratio of convective dried cashew apple bagasse.

Rehydration rate increased 22% (assay 5) and 8% (assay 6) compared to control. This result may be attributed to the structural changes caused by ultrasound such as the formation of microchannels, loss of cellular adhesion and rupture of the cell walls (HE *et al.*, 2012; GAMBOA-SANTOS *et al.*, 2014).

4.3.3 Effect on enzymes

The most important factors that determine the rate of the enzymatic browning of fruits and vegetables are the concentrations of enzyme polyphenoloxidase, phenolic content, pH, temperature, and oxygen availability (COSTA *et al.*, 2011). Fig. 8 depicts the evolution of PPO activity during drying.

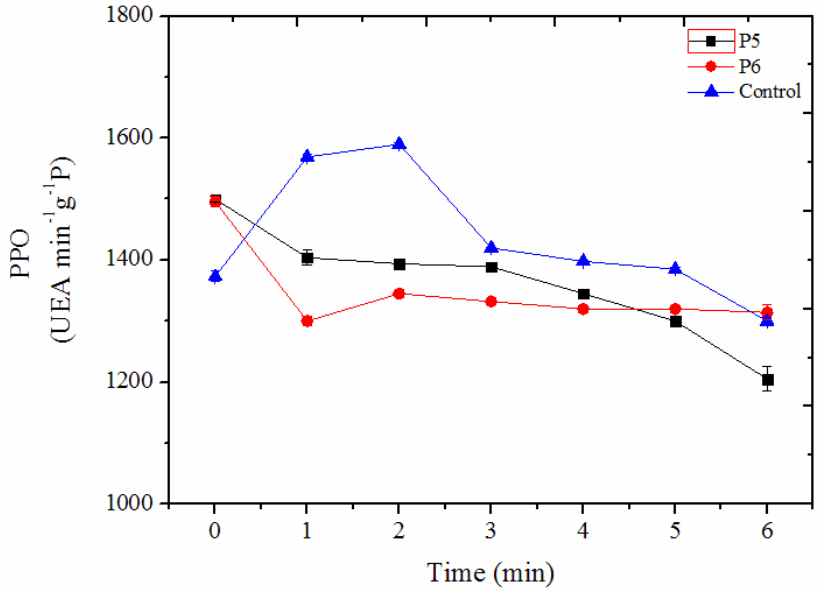

Fig.8 PPO activity of cashew apple bagasse sonicated at 75 W/cm^2 during 2 min (Assay 5) and 10 min (Assay 6) using a bagasse:water ratio of 1:4 along drying.

Drying reduced the PPO activity in sonicated cashew apple bagasse resulting in about 20 and 12% loss for assays 5 and 6, respectively (Fig. 8). However, PPO activity increased ≈20% for control sample after 2h of drying. PIGA *et al.* (2003) reported that during the dehydration process PPO activity remains high for long periods when the drying temperature is around 55 °C, whereas only moderate activity is observed at temperatures higher than 75 °C. Meanwhile, TEREFE *et al.* (2010) reported that the thermostability of PPO depends on the species as well as plant cultivar.

Although the high activity of PPO, there were not observed noticeable browning in sonicated samples (Fig.9). Non-enzymatic browning reactions were typically found after extended drying to low product water activities (POTT *et al.* 2005).

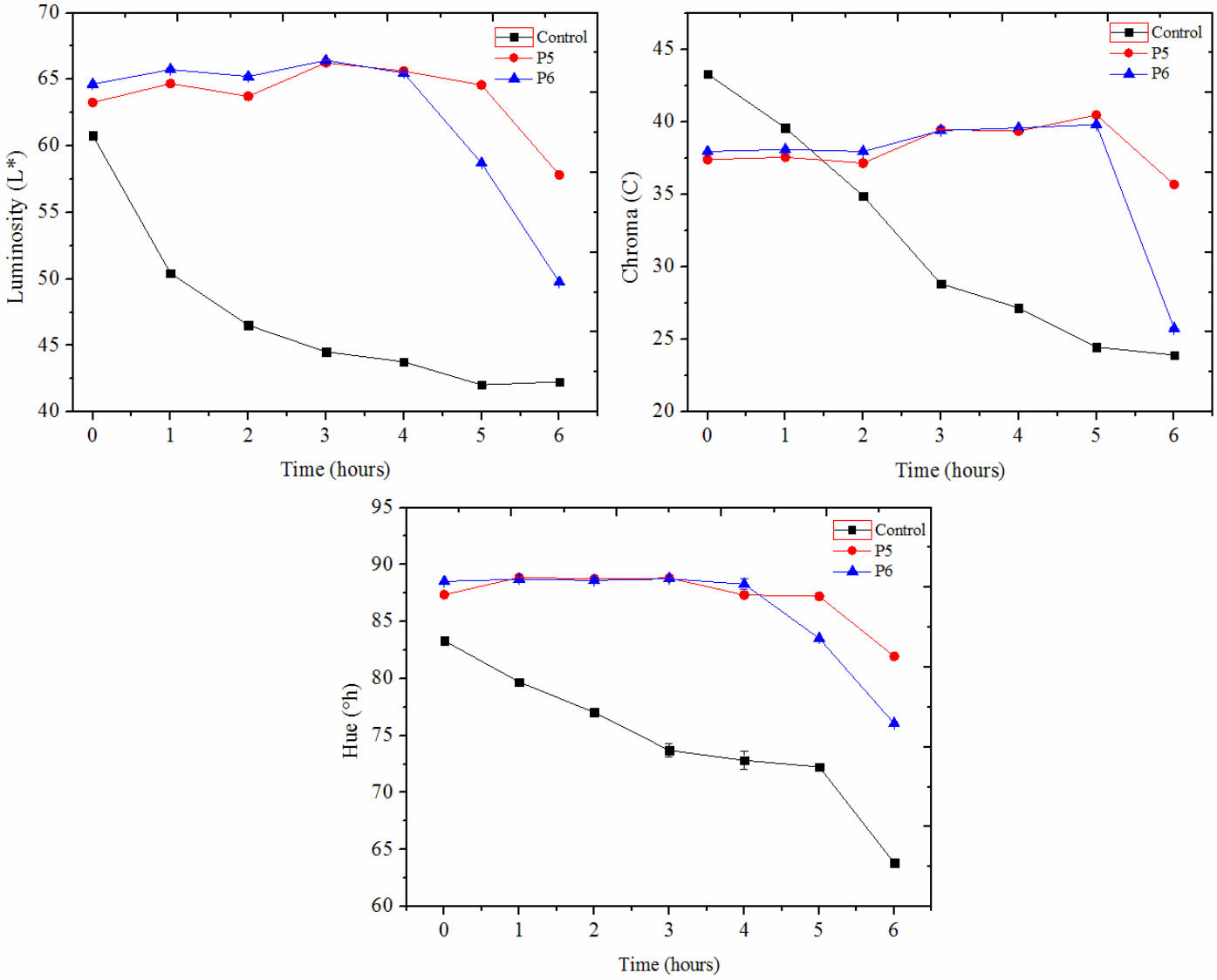

Fig. 9 Evolution of color parameters L^*(A), h° (B) and chroma (C) along drying of cashew apple bagasse sonicated at 75 W/cm^2 during 2 min (Assay 5) and 10 min (Assay 6) using a bagasse:water ratio of 1:4.

A higher decrease in all parameters evaluated was observed for control sample whichdenotes a more intense browning of the product surface. The results clearly reveal that sonication could effectively minimize browning in samples during drying.

Fig. 10 shows the activity of POD from sonicated cashew apple bagasse during drying.

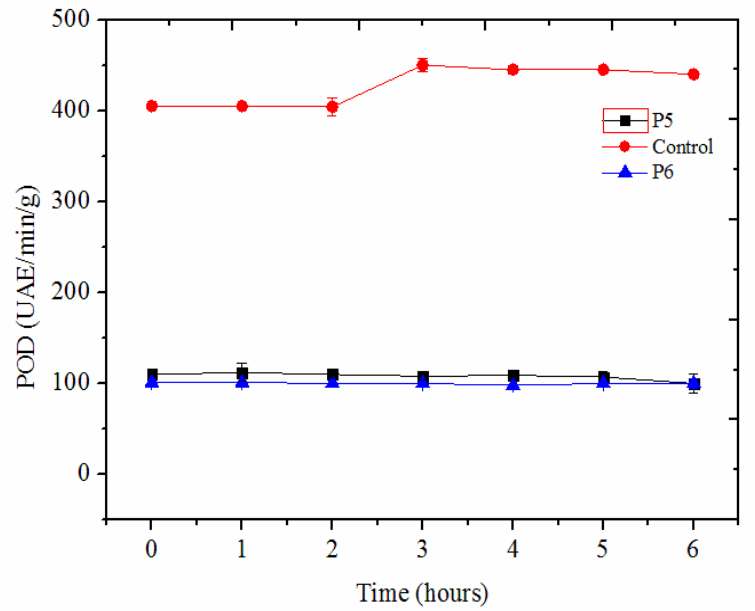

Fig.10 POD activity of cashew apple bagasse sonicated at 75 W/cm^2 during 2 min (Assay 5) and 10 min (Assay 6) using a bagasse:water ratio of 1:4.

During drying, POD activity did not increase, which shows that ultrasound irreversibly inactivated the enzyme. The enzyme activity in the control increased about 9% after 6h (Fig.10).

The reduced activity of PPO and POD of sonicated and dried cashew apple bagasse is relevant to the final product quality. This agrees with the findings of TEREFE *et al.* (2010) who reported that the activities of PPO and POD are involved in degradation of anthocyanins and other polyphenols in strawberry products, leading to discoloration and loss of antioxidant activity.

4.3.4 Effects of sonication on the functional compounds of dried cashew apple bagasse

In many studies, ascorbic acid has been taken as an index of nutrient quality of foods. Ultrasound is a technology reported to have a minimal effect on the quality of food that contain heat labile vitamins (GOLMOHAMADI *et al.*, 2013).

Fig. 11 shows that vitamin C content decreased progressively with increasing drying time, as expected, due to the heat liable nature of ascorbic acid. The initial vitamin C content

was 200 mg.100g^{-1} and 189 mg.100g^{-1} for samples sonicated according to assays 5 and 6, respectively, and 135 mg.100g^{-1} for control sample.

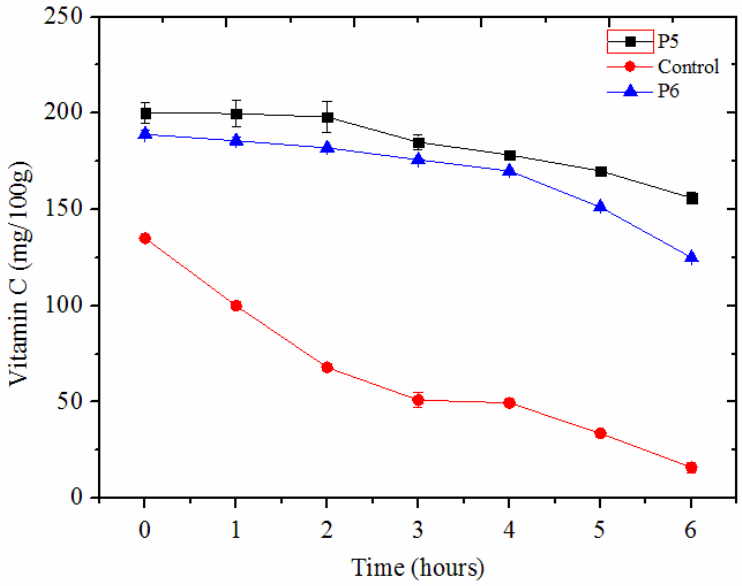

Fig. 11 Vitamin C of cashew apple bagasse sonicated at 75 W/cm^2 during 2 min (Assay 5) and 10 min (Assay 6) using a bagasse:water ratio of 1:4.

MRAD *et al.* (2012) reported that stability of vitamin C during drying is affected by several variables, not only drying conditions, but also the sample moisture content. As shown in Fig. 11, for sonicated samples there was an initial slow rate of vitamin C loss at relatively higher moisture contents (beginning of drying), followed by a period of faster degradation rates as the moisture content decreased. The maximum losses observed after 6h of air-drying were 20% and 34% for sonicated samples (assays 5 and 6, respectively) and 88% for control samples (Fig. 11).

A similar trend was observed by GAMBOA-SANTOS *et al.* (2014) who evaluated the effect of dehydration by convection assisted by power ultrasound of strawberries. The authors showed that sonicated samples, even under most severe processing conditions (70°C and 60W), presented higher vitamin C retention compared to thermal treated samples.

Fig. 12 shows the concentration of TPC during the drying process. Sonicated samples showed a significant increase ($p<0.05$) in TPC along drying, ranging from 1576 to 2244 mg GAE/100g for assay 5 and 1600 to 1984 mg GAE/100 g for assay 6. For control samples, it was observed a gradual decrease in TPC concentration (30%) (Fig.12).

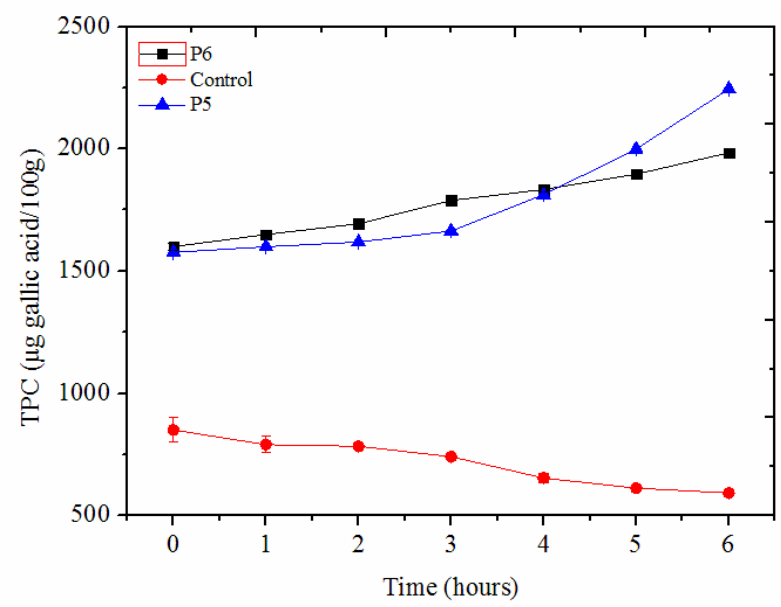

Fig.12 Total phenolic compounds of cashew apple bagasse sonicated at 75 W/cm^2 during 2 min (Assay 5) and 10 min (Assay 6) using a bagasse:water ratio of 1:4.

The higher concentrations of vitamin C and phenolics for sonicated samples can be related to the reduction in the activities of oxiredutases, peroxidase and ascorbate peroxidase (See chapter 3). These results have been confirmed by TOMÁS-BARBERÁN and ESPÍN, (2001)that reported the reduction of antioxidant activity of fruits and vegetables by direct oxidation of phenolics catalyzed by both PPO and POD. Vitamin C acts as substrate for ascorbate peroxidase.

Cashew fruit presents a high antioxidant activity, and high TPC and vitamin C values (RUFINO *et al.*,2010).As can be seen in Figure 13, the antioxidant capacity tended to increase ($p<0.05$) during drying. The increase in phenolic compounds and the high final amount of vitamin C may lead to enhance the antioxidant activity.

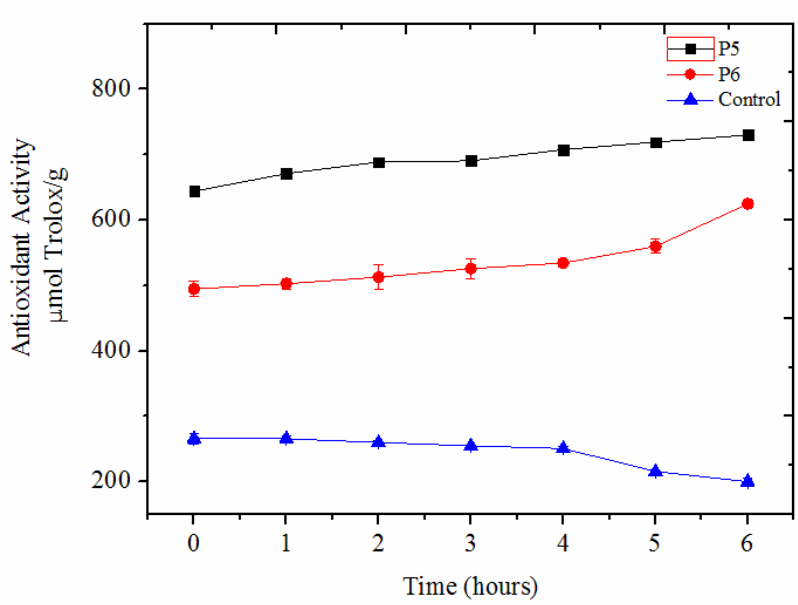

Fig.13 Changes in antioxidant activity of cashew apple bagasse sonicated at 226 W/cm^2 during 6 min using a bagasse:water ratio of 1:4 during drying.

PIGA *et al.* (2003) studied the influence of drying parameters on the phenolic compounds and antioxidant activity of prunes and also found a higher antioxidant activity after drying. The authors attributed the result to the formation of new compounds with higher antioxidant activity, for example, in Maillard reaction, which creates various products with markedly higher antioxidant power.

The total phenolic contents showed a positive correlation with antioxidant activity (R= 0.87; assay 5 and R=0.93; assay 6) (Table 5) indicating that phenolic compounds are the major contributors to the antioxidant properties of this product.

Table 5 Pearson's correlation coefficients (R) between antioxidant capacity and antioxidant compounds of dried sonicated bagasse

Assay 5			Assay 6		
R	**TPC**	**Vitamin C**	**R**	**TPC**	**Vitamin C**
ABTS	0,87*	-0,91*	**ABTS**	0,93*	-0,96*
Vitamin C	-0,96*		**Vitamin C**	-0,98*	

* Significant at $p < 0.05$.

A significant and positive correlation has also been obtained by other authors (DUDONNÉ *et al.* 2009; RUFINO *et al.*, 2010; CONTRERAS-CALDERÓN *et al.*, 2011).

4.3.5 *In vitro bioaccessibility of vitamin C and phenolic compounds*

Bioaccessibility is defined as the amount of an ingested nutrient that is available for absorption in the gut after digestion (HEDREN *et al.*, 2002). KAMILOGLU *et al.* (2014) emphasized the importance to evaluate the availability of antioxidants after digestion due to evidences of poor bioavailability of certain antioxidants, which would in turn have a limited effect on health. Fig. 14 depicts the effect of sonication on bioaccessibility of vitamin C of dried cashew apple bagasse.

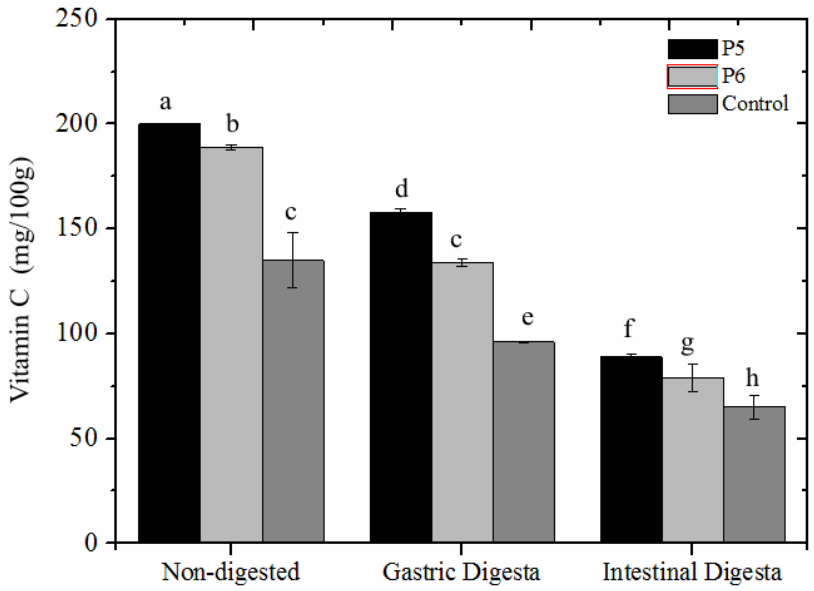

Fig. 14 Vitamin C concentration during in vitro gastrointestinal digestion of sonicated and dried (6h) cashew apple bagasse. Different lower case letters indicate significant differences ($p < 0.05$).

As can be seen in Fig. 14, vitamin C concentration of sonicated samples was reduced 21% (assay 5) and 29% (assay 6) in the gastric digesta, compared to non digested sonicated cashew apple bagasse. After intestinal digestion, greater losses of vitamin C were observed (55% for assay 5 and 58% for assay 6). Similar behavior was observed for control samples (52%). The ultrasound processing caused less reduction of bioaccessibility of vitamin C compared to control. Despite the observed losses, the daily requirement of vitamin C (40 and 45 mg per day according to the FAO/WHO) is achieved in ≈ 50g of dried cashew apple bagasse.

RODRÍGUEZ-ROQUE *et al.* (2014) found similar recovery of vitamin C in gastric digesta (67.7%) and intestinal digesta (47.3%) of blended fruit juice. The authors attributed the vitamin C oxidation in the gastrointestinal tract to the fact that this compound keeps metal ions in reduced state, or regenerate the active form of other dietary constituents by donating an electron.

Fig. 15 shows the effect of sonication in bioaccessibility of total phenolic compounds of cashew apple bagasse.

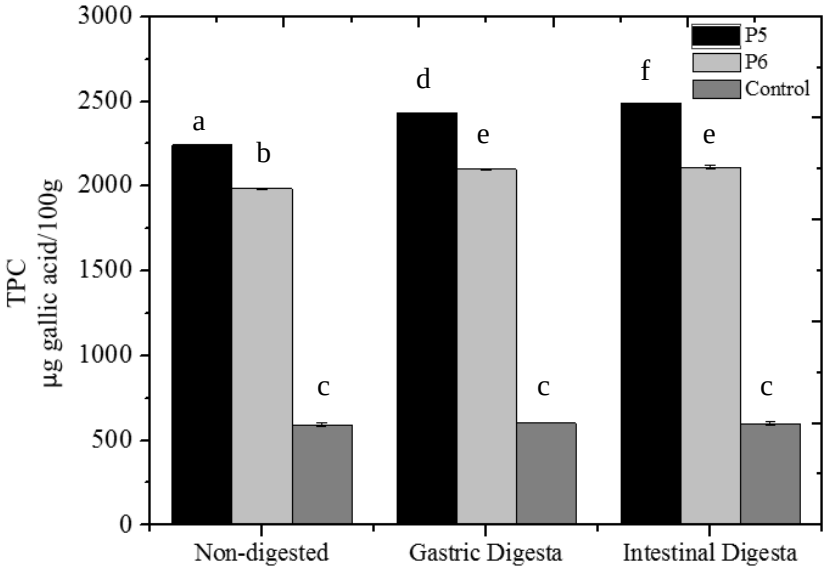

Fig.15 Total phenolic concentration during in vitro gastrointestinal digestion of sonicated and dried (6h) cashew apple bagasse. Different lower case letters indicate significant differences ($p< 0.05$).

The total phenolic concentration increased 8% (assay 5) and 6% (assay 6) in the gastric digesta, with regard to the content in non digested cashew apple bagasse (Figure 15). Likewise, TPC increased 2% (assay 5) and 1% (assay 6) in the intestinal digesta with regard to gastric digesta samples. No significant differences were found for gastric and intestinal digesta of control samples.

During the gastric phase, the extraction of phenolics from bagasse was more efficient suggesting that the release of polyphenols from cashew apple bagasse following simulated gastro-intestinal digestion is mainly achieved during the gastric phase.

These results have been confirmed by PALAFOX-CARLOS; AYALA-ZAVALA; GONZÁLEZ-AGUILAR (2011) who reported that phenolic acids in the aglycone form are

generally absorbed in the upper part of the gastrointestinal tract, explaining the rapid absorption of these compounds ranging from 1 to 2 h after intake of fruits and vegetables.

Overall, too few studies to evaluate the effects of ultrasound on bioaccessibility of bioactive compounds have been done. ANESE *et al.*(2013)showed that sonication caused loss of cell integrity and decrease in the degree of pectin esterification of tomato pulp. The greater gel-like properties resulted in a decrease in lycopene in vitro bioaccessibility. The authors attributed this result to the presence of a stronger network that make lycopene less available to the digestion.

4.4 Conclusions

Sonication increased drying rates of cashew apple bagasse and retained more vitamin C and phenolics compared to conventional drying, therefore, improving the quality of the dried product. The results obtained could be promising in order to fully develop this technology on an industrial scale, taking energy saving into account. The ultrasound processing increased the bioaccessibility of phenolic compounds compared to control samples.

Acknowledgments

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Conclusões Finais

Neste trabalho foi possível verificar as alterações físicas e químicas decorrentes do uso do ultrassom para o processamento do bagaço do pedúnculo do caju. O processamento ultrassônico do bagaço resultou em um purê homogêneo de cor amarela intensa, rico em compostos bioativos com elevado potencial antioxidante. Foi possível verificar ainda a influência do ultrassom sobre as altas taxas de transferência de massa em relação ao processo convencional de secagem. O ultrassom aumentou as taxas de secagem além de melhorar a qualidade nutricional do produto desidratado. Os resultados obtidos podem ser promissores, para o desenvolvimento desta tecnologia em escala industrial, levando em consideração os ganhos na qualidade do produto final e na economia do processo.