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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS (PPGCTA)

Efeito das etapas do processamento na qualidade do suco de goiaba serrana (*Acca sellowiana*) e aproveitamento da farinha do resíduo

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Porto Alegre
2022

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Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul, como um dos requisitos para a obtenção do grau de Doutor em Ciência e Tecnologia de Alimentos.

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

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RESUMO

O objetivo deste trabalho foi aplicar e avaliar as etapas do processamento para produção de suco de goiaba serrana, como maceração, clarificação, microfiltração, pasteurização e armazenamento, assim como caracterizar a farinha do resíduo proveniente do processamento do suco da fruta. Foram avaliados os rendimentos, pH, acidez, sólidos solúveis, açúcares redutores e parâmetros de cor de 27 preparações enzimáticas, utilizando três enzimas distintas (Pectinex, Lallzyme e Rohapect) sob diferentes concentrações (1, 5 e 10 $U.mL^{-1}$). Os melhores sucos em relação as características físico-químicas, ainda foram avaliados quanto ao perfil de carotenoides e compostos fenólicos, sendo também medida a capacidade antioxidante contra os radicais peroxil ($•ROO^-$) e $ABTS^{•+}$. Para a farinha do resíduo do processamento do suco foram analisados a composição centesimal, assim como a atividade de água, pH, capacidade de absorção de água e óleo, capacidade antioxidante e o perfil de carotenoides por CLAE. A estabilidade do suco controle (*in natura*), do suco clarificado por enzima e pasteurizado, do suco clarificado por enzima e por microfiltração e pasteurizado foi estudada durante o armazenamento em temperatura ambiente (20 °C), sob um fotoperíodo de 12 horas (ciclo claro/escuro) por 3 meses. Os três tratamentos com Pectinex tiveram um aumento no rendimento do suco em 43, 55 e 60% respectivamente, enquanto as enzimas Lallzyme e Rohapect não tiveram aumento no rendimento em relação ao controle. Todas as enzimas testadas, tiveram impacto positivo na cor do suco, melhorando os parâmetros de cor, tornando-os mais translúcidos. Dentre as três enzimas utilizadas, a Pectinex® Ultra Clear apresentou os melhores resultados para os parâmetros físico-químicos, sendo mais eficaz para extração do suco. A concentração adicionada de 10 $U.mL^{-1}$ na etapa anterior ao despolpamento e 5 $U.mL^{-1}$ anterior à filtração (T17) obteve os melhores resultados, principalmente, aumentando o rendimento e intensificando a concentração de açúcares redutores (11,34%) comparado ao controle (8,74%). Além disso, a Pectinex foi eficaz em melhorar a clareza do suco, bem como em diminuir a viscosidade. A microfiltração diminuiu o teor de sólidos solúveis totais e aumentou o teor de acidez do suco, entretanto melhorou a clareza do suco. Além disso, o suco microfiltrado teve um valor de índice de comportamento de 1,091, indicando comportamento newtoniano. O suco controle (*in natura*) apresentou valores abaixo de um (0,2178), indicando um comportamento não newtoniano e tipicamente pseudoplástico. Os valores de pH não mudaram durante o processamento do suco e todas as amostras permaneceram abaixo de 3,0. Os valores L^*, b^*, C^* e ângulo Hue do suco microfiltrado foram muito maiores do que o suco pasteurizado e fresco. Esses resultados mostram que a tonalidade do suco ficou com amarelo

mais intenso e translúcido. O teor de ácido ascórbico foi superior no suco microfiltrado (6,4 mg.100 mL^{-1}), enquanto o suco *in natura* e o suco pasteurizado apresentaram concentrações semelhantes (4,0 mg.100 mL^{-1}). Os sucos pasteurizados e microfiltrados tiveram uma quantidade significativamente maior de compostos fenólicos totais (21.061,30 e 22.198,71 µg.100 mL^{-1}, respectivamente) em comparação ao suco fresco (1591,18 µg.100 mL^{-1}). Para a capacidade antioxidante medida com radical $ABTS^{\bullet+}$, não houve diferença significativa entre os três tratamentos. Em contraste, para o método ORAC, o suco fresco teve uma maior capacidade antioxidante.

A farinha obtida do resíduo da goiaba serrana apresentou baixo teor de lipídios e carboidratos e alto teor de fibra bruta (54,88%). Os compostos bioativos da farinha foram caracterizados principalmente pela presença de zeaxantina (184,76 µg.$100g^{-1}$), β-criptoxantina (136,25 µg.$100g^{-1}$) e β-caroteno (112 µg.$100g^{-1}$). O conteúdo total de carotenoides foi de 586,33 µg.$100g^{-1}$. O teor de ácido ascórbico presente na farinha foi de 27 mg.$100g^{-1}$ e a capacidade antioxidante determinada pelo ensaio ORAC apresentou uma forte potência (1324,30 µM Trolox.g^{-1}).

O uso da enzima Pectinex® Ultra Clear no suco melhorou principalmente o rendimento, parâmetros de cor e o teor de compostos fenólicos, porém, a redução da turbidez foi alcançada de forma mais intensa com a microfiltração. O suco de goiaba serrana, assim como a farinha do resíduo mostraram-se uma boa alternativa de consumo, devido às suas características nutricionais e funcionais.

Palavras-chave: escurecimento enzimático; branqueamento; enzimas; pasteurização; microfiltração; compostos fenólicos; armazenamento.

ABSTRACT

The objective of this work was to apply and evaluate technological aspects of the production of feijoa juice with evaluation of the stages of maceration, clarification, microfiltration, pasteurization and storage, as well as characterizing the residue flour from the processing of fruit juice. Yields and physicochemical parameters were evaluated, such as pH, acidity, soluble solids, reducing sugars and color parameters of 27 enzymatic preparations, using three different enzymes (Pectinex, Lallzyme and Rohapect) under different concentrations (1, 5 and 10 $U.mL^{-1}$). In addition, the best juices were evaluated for the profile of carotenoids and phenolic compounds, and the antioxidant capacity of the juices against the peroxyl radicals (•ROO–) and ABTS was measured. For flour from juice processing residue, proximate composition as well as water activity, pH, WHC, OHC were analyzed. In addition, the carotenoid profile was evaluated by HPLC and the antioxidant capacity of the flour was measured. The stability of the control juice (*in natura*), the juice clarified by enzyme and pasteurized, and the juice clarified by enzyme and by microfiltration and pasteurized was studied during storage at room temperature (20 ° C), under a photoperiod of 12 hours (light/dark cycle) for 3 months. The three treatments with Pectinex had an increase in juice yield by 43, 55 and 60%, while the enzymes Lallzyme and Rohapect had no increase in yield compared to the control. All the enzymes tested had a positive impact on the color of the juice, improving the color parameters. Among the three enzymes used, Pectinex® Ultra Clear showed the best results for the physical-chemical parameters, being more effective for extracting feijoa juice. The added concentration of 10 $U.mL^{-1}$ in the step prior to pulping and 5 $U.mL^{-1}$ prior to filtration (T17) obtained the best results, mainly, increasing the yield and intensifying the concentration of reducing sugars (11.34%) compared control (8.74%). In addition, Pectinex was effective in improving the clarity of the juice, as well as in decreasing the viscosity. Microfiltration decreased the content of total soluble solids and increased the acidity content of the juice; however, it improved the clarity of the juice. In addition, the microfiltered juice had a behavior index value of 1.091, indicating Newtonian behavior. The *in natura* juice showed values below one (0.2178), indicating a non-Newtonian and typically pseudoplastic behavior. The pH values did not change during juice processing and all samples remained below 3.0. The L^*, b^*, C^* and Hue angle values of the microfiltered juice were much higher than the pasteurized and fresh juice. The ascorbic acid content was higher in microfiltered juice (6.4 $mg.100\ mL^{-1}$), while fresh juice and pasteurized juice showed similar concentrations (4.0 $mg.100\ mL^{-1}$). Pasteurized and microfiltered juices had a significantly higher amount of total phenolic compounds

(21,061.30 and 22,198.71 μg.100 mL^{-1}, respectively) than fresh juice (1591.18 μg.100 mL^{-1}). For the antioxidant capacity measured with $ABTS^{\bullet+}$ radical, there was no significant difference between the three treatments. In contrast, for the ORAC method, fresh juice had a greater antioxidant capacity.

The drying of the residue from the processing of feijoa juice was carried out in an air circulation oven to obtain the flour. The feijoa residue flour had a low content of lipids and carbohydrates and a high content of crude fiber (54.88%). The bioactive compounds of feijoa residue were mainly characterized by zeaxanthin (184.76 μg.$100g^{-1}$), β-cryptoxanthin (136.25 μg.$100g^{-1}$) and β-carotene (112 μg.$100g^{-1}$). The total carotenoid content was 586.33 μg.$100g^{-1}$. The ascorbic acid content present in the flour was 27 mg.$100g^{-1}$ and the antioxidant capacity determined by the ORAC assay showed a strong potency (1324.30 μM Trolox.g^{-1}).

The use of Pectinex® Ultra Clear enzyme in the juice mainly improved the yield, color parameters and phenolic compounds, however, the turbidity reduction was achieved more intensely with microfiltration. The mountain guava juice, as well as the residue flour proved to be a good alternative for consumption, due to their nutritional and functional characteristics.

Keywords: enzymatic browning; bleaching; enzymes; pasteurization; microfiltration; phenolic compounds; storage.

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LISTA DE ABREVIATURAS E SIGLAS

ABTS	2,2'- azinobis (3-etilbenzotiazolina-6-ácido sulfônico)
AAPH	2,2' Azobis (2 amidinopropane) dihydrochlorine
CLAE	Cromatografia Líquida de Alta Eficiência
DRI	*Dietary Reference Intakes*
DCNT	Doença Crônica não transmissíveis
H2O2	Peróxido de Hidrogênio
HPLC	High performance liquid chromatography
LOD	Limite de detecção/*Limit of detection*
LOQ	Limite de quantificação/*Limit of quantification*
FW	*Fresh weight*
DW	*Dry weight*
ISO	*International Organization for Standardization*
TTA	*Total titrable acidity*
TSS	*Total soluble solids*
TDF	*Total dietary fiber*
IDF	*Insoluble dietary fiber*
SDF	*Soluble dietary fiber*
MTBE	*Methyl tert-butyl ether*
r^2	Coeficiente de determinação/ determination coeficiente
ORAC	Oxygen Radical Absorbance Capacity

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO

O Brasil possui a flora mais rica do mundo, com mais de 56 mil espécies de plantas, correspondendo a quase 20% da flora mundial. Mesmo com grande diversidade de espécies frutíferas nativas, muitas delas, ainda são pouco conhecidas e estudadas. Entretanto, nos últimos anos, observa-se um maior interesse por essas espécies nativas, à medida que o potencial de utilização tem sido identificado (MORETTO; NODARI; NODARI, 2014; SILVA *et al.*, 2017).

A goiaba serrana (*Acca sellowiana*) é uma fruta nativa da América do Sul. Devido a sua fácil adaptabilidade em regiões subtropicais, é cultivada em muitos países e considerada uma fruta exótica na Europa. É utilizada em maior escala em países como Austrália, Nova Zelândia e Colômbia; entretanto, no Brasil, a espécie ainda é pouco conhecida, apesar do país ser o centro de sua origem. Também chamada mundialmente de feijoa, tem havido nos últimos anos um aumento no interesse mundial pela fruta, devido ao seu sabor único doce-acidulado. A fruta é caracterizada por apresentar 19% de acidez e 13% de sólidos solúveis, além de ser altamente aromática e nutritiva (PASQUARIELLO *et al.*, 2015; WESTON, 2010a).

A goiaba serrana também representa uma fonte de compostos fenólicos, substâncias com capacidade antioxidante, que contribuem para a proteção dos efeitos nocivos do estresse oxidativo na saúde humana. Além de desses compostos a fruta apresenta em menor quantidade carotenoides. As quantidades de compostos bioativos variam de acordo com a localidade que a fruta foi cultivada. Dentre os carotenoides, já foram identificados luteína e β-caroteno em maior quantidade (111 e 116 $\mu g.100g^{-1}$, respectivamente), e zeaxantina, β-criptoxantina e α-caroteno (8, 32 e 14 $\mu g.100g^{-1}$, respectivamente). A concentração de compostos fenólicos totais encontrado na goiaba serrana foi de 11.447,71 $\mu g.g^{-1}$. Os ácidos fenólicos majoritários identificados foram: isômero de pedunculagin e galato de epicatequina. Além desses compostos bioativos, apresenta excelente conteúdo de fibras, minerais e vitaminas (BEYHAN; ELMASTAŞ; GEDIKLI, 2010; MORETTO; NODARI; NODARI, 2014; PASQUARIELLO *et al.*, 2015).

O processamento da goiaba serrana, muitas vezes torna-se necessário, pois estudos relatam um pequeno período de conservação *in natura* após a colheita, com o aparecimento de pigmentos marrons na casca e o escurecimento da polpa, o que compromete sua qualidade. Além disso, a fruta possui uma elevada atividade das enzimas

polifenoloxidade, peroxidase e fenilalanina amonialiase (AMARANTE *et al.*, 2017). A atividade enzimática dos tecidos vegetais é uma das principais causas de comprometimento nutricional e organoléptico de frutas e hortaliças. Na oxidação enzimática há duas enzimas principais envolvidas, a polifenoloxidase e a peroxidase cuja atividade pode levar à produção de polímeros marrons (GOYENECHE; SCALA; ROURA, 2013; LATORRE *et al.*, 2012). Na literatura são encontradas pesquisas envolvendo a fruta e o desenvolvimento de novos produtos, como geleias, purês, bebidas, entre outros; os quais de um modo geral podem estimular o seu consumo pela população.

Uma opção de processamento para as frutas pode ser a produção de sucos industrializados. O suco da goiaba serrana ainda é pouco estudado (MONFORTE et *al*., 2014; PENG *et al*., 2020). O mercado de sucos de frutas representa um grande impacto na economia de muitos países e na nutrição da população. De uma perspectiva internacional, por exemplo, nos Estados Unidos, a receita é de US $ 9.943 milhões e um consumo médio per capita de 17,1 litros de suco no ano de 2017 (CÁSEDAS *et al.*, 2018).

O suco integral se destaca por manter propriedades sensoriais e nutricionais semelhantes às encontradas nas frutas. Essa característica, aliada à demanda por alimentos sem adição de conservantes e açúcares e com características semelhantes à de alimentos não processados, faz com que o suco integral seja preferido pela maioria dos consumidores (ROMANO; ROSENTHAL; DELIZA, 2015). Esse tipo de suco geralmente é composto por água, sólidos solúveis, vitaminas e minerais, compostos aromáticos e em menor quantidade proteínas e lipídeos. Além disso, podem representar uma fonte importante de compostos bioativos (CÁSEDAS *et al.*, 2018; KUMAR, 2015).

Na produção industrial de sucos alguns aspectos tecnológicos são fundamentais para a obtenção de um produto de qualidade. Etapas como a inativação de enzimas, maceração, extração enzimática, pasteurização, embalagem e armazenamento devem ser avaliadas e apresentam parâmetros distintos de acordo com a matéria prima.

O branqueamento é o tratamento térmico aplicado de forma rápida ao material vegetal, sendo necessário para a inativação das enzimas deteriorantes, para evitar assim o escurecimento e demais alterações indesejáveis, com manutenção da qualidade nutricional e sensorial, o que possibilita fornecer produtos de alta qualidade ao mercado e consequentemente ao consumidor (GOYENECHE; SCALA; ROURA, 2013; SCHWEIGGERT; SCHIEBER; CARLE, 2005).

O uso de enzimas na extração de suco tem sido pesquisado extensivamente na indústria alimentícia. As enzimas pectinolíticas comerciais são utilizadas como auxiliares

de processamento para a degradação da pectina, que sedimenta partículas orgânicas em suspensão. A mistura de enzimas pectinolíticas, celulases e hemicelulases mostram-se eficazes para aumento do rendimento, diminuição da viscosidade e da turbidez, melhora da filtração, maior clarificação e estabilização de sucos, aumento da concentração de açúcares, eliminação de compostos indesejáveis. Além disso, a aplicação do tratamento enzimático pode ser uma alternativa para aumentar o teor de compostos fenólicos em sucos, visto que estes apresentam potenciais benefícios para a saúde humana (DAL MAGRO *et al.*, 2016; KUMAR, 2015; SIDDIQ *et al.*, 2018a; TAPRE; JAIN, 2014).

A pasteurização no suco também consiste em um tratamento térmico, entretanto, diferente do branqueamento da fruta, esta etapa, é realizada no final do processamento, com aplicação de elevadas temperaturas por um tempo determinado, com a finalidade de inibir a carga microbiana; assim como, inativar as enzimas deteriorantes, o que proporciona, um aumento da vida útil dos sucos (WIBOWO *et al.*, 2015a). Após a pasteurização, os sucos são embalados e armazenados.

Assim, devido a fatores como o potencial do mercado de sucos de fruta integral, a composição nutricional da goiaba serrana, sua vida útil pós-colheita limitada e problemas de escurecimento enzimático e alta acidez, torna-se necessário avaliar os diferentes processos tecnológicos que podem ser aplicados no desenvolvimento de um suco integral a partir da goiaba serrana, com otimização de todas as etapas.

CAPÍTULO 2

2 OBJETIVOS

2.1 OBJETIVO GERAL

Aplicar e avaliar o efeito das etapas de processamento (maceração, clarificação, pasteurização e armazenamento) para produção de suco da goiaba serrana (*Acca sellowiana*), assim como caracterizar a farinha do resíduo proveniente do processamento do suco da fruta.

2.2 OBJETIVOS ESPECÍFICOS

a) Avaliar o uso de enzimas para a otimização da extração do suco na qualidade físico-química do produto final;
b) Produzir suco *in natura*, pasteurizado e microfiltrado a partir da polpa de goiaba serrana;
c) Caracterizar os sucos de goiaba serrana obtido por diferentes tipos de processamento em relação aos parâmetros físico-químicos (pH, acidez titulável, º Brix, cor e açúcares redutores);
d) Identificar e quantificar os principais açúcares do suco por cromatografia líquida de alta eficiência (CLAE);
e) Identificar e quantificar os carotenoides e os compostos fenólicos presentes nos sucos, sob os diferentes tipos de tratamentos por cromatografia líquida de alta eficiência (CLAE) e espectrometria de massas;
f) Avaliar a capacidade antioxidante dos sucos;
g) Avaliar a estabilidade dos sucos processado sob armazenamento;
h) Caracterizar o resíduo obtido do processamento da fruta mediante análises físico-química, identificação e quantificação de compostos bioativos e capacidade antioxidante.

CAPÍTULO 3

3 REVISÃO BIBLIOGRÁFICA

3.1 Goiaba Serrana (*Acca sellowiana* (Berg) Burret)

Esta espécie pertencente à família Myrtaceae é conhecida popularmente por goiabeira-do-mato, goiabeira-da-serra, goiabeira-serrana ou feijoa. É nativa da Região Sul do Brasil e do Nordeste do Uruguai. Devido à sua fácil adaptabilidade nas regiões subtropicais, é extensivamente cultivada em vários países como Austrália, França, Israel, Itália, Turquia, Irã, Rússia, Colômbia, Paraguai, Argentina. Estados Unidos e principalmente Nova Zelândia. Há cerca de 20 variedades de goiaba serrana (POODI *et al.*, 2018; THORP; BIELESKI, 2002; WESTON, 2010a).

No Brasil, ainda é pouco conhecida, e a produção comercial é incipiente. Essa fruta é encontrada principalmente nos estados de Santa Catarina e Rio Grande do Sul e há quatro variedades principais estudadas: Alcântara, Helena, Mattos e Nonante (AMARANTE *et al.*, 2013; DEGENHARDT *et al.*, 2003; MORETTO; NODARI; NODARI, 2014). Está contemplada na publicação Plantas para o Futuro do Ministério do Meio Ambiente (CORADIN; SIMINSKI; REIS, 2011) que visa o incentivo de sua produção e consumo. Atualmente, a Colômbia lidera o *ranking* de exportação da goiaba serrana, e existem empresas especializadas na venda da fruta para diferentes regiões do mundo, em especial para a Europa (MORETTO; NODARI; NODARI, 2014).

A planta é um arbusto de folhas persistentes que raramente ultrapassa seis metros de altura, com fuste ramificado, especialmente em campo aberto. Apresenta crescimento lento e potencial ornamental devido a sua folhagem discolor com vistosa floração. Suas flores brancas e vermelhas são desprovidas de nectários e apresentam quatro pétalas vistosas, carnosas e adocicadas; que são os principais recursos florais para os polinizadores. A maturação da fruta ocorre de fevereiro a maio. Nos locais onde a goiaba serrana não possui condições climáticas para produzir frutas, foi introduzida como uma planta ornamental em função de suas flores (Figura 1). (DEGENHARDT *et al.*, 2003; MORETTO; NODARI; NODARI, 2014; SOUZA, *et al.*, 2011).

A fruta possui uma alta produção de etileno, quando comparada com outras frutas climatéricas e também elevada taxa de respiração, ou seja, à medida que o amadurecimento avança, ocorrem alterações físico-químicas, como perda de peso, redução da acidez titulável e teor de sólidos solúveis, rápido escurecimento da polpa, e

perdas na qualidade sensorial, o que resulta em sérias limitações para atender às demandas de mercado. A goiaba serrana possui período pós-colheita curto, de três a quatro semanas, sob refrigeração, limitando o tempo de oferta da fruta fresca ao mercado consumidor (AMARANTE *et al.*, 2017b; CASTELLANOS; POLANÍA; HERRERA, 2016; PARRA C; FISCHER, 2013)

A fruta é semelhante à goiaba comum em aparência (Figura 2), tamanho e textura, mas a polpa apresenta cor gelo, possui sabor diferenciado (doce-acidulado) e aromático (DEGENHARDT *et al.*, 2003; PASQUARIELLO *et al.*, 2015). A casca da fruta mantém um tom verde independente do seu grau de maturação, o mesocarpo branco, com polpa suculenta em torno das sementes e pouco arenosa perto da casca. Por apresentar a casca amarga, esta não é geralmente consumida com a polpa (CASTELLANOS; POLANÍA; HERRERA, 2016; TUNCEL; YILMAZ, 2015). A fruta pode variar de 20 a 200 g, com formato variando de redondo a oblongo, apresentar diâmetro de 3 a 5 cm, comprimento de 4 a 10 cm. O rendimento de polpa pode variar de 15 a 50% (ESEMANN-QUADROS *et al.*, 2008; MORETTO; NODARI; NODARI, 2014; ROSSI *et al.*, 2007).

O pH da fruta é em torno de 3,0, possui um teor de sólidos solúveis de 8 a 13 ° Brix e o valor de acidez varia conforme o cultivar entre 19 e 31% (AMARANTE *et al.*, 2013; PASQUARIELLO *et al.*, 2015; ROMERO-RODRIGUEZ *et al.*, 1994). A goiaba serrana apresenta como o principal ácido, o cítrico (13,65 $g.kg^{-1}$), seguido pelo málico (0,55 $g.kg^{-1}$) e oxálico (0,39 $g.kg^{-1}$) (CASTELLANOS; POLANÍA; HERRERA, 2016).

Figura 1. Fotografia da flor da espécie *Acca Sellowiana*

Fonte: http://www.canva.com

A fruta possui em torno de 85% de umidade, 6% de fibra dietética, sendo a maior parte solúvel, baixo teor de lipídeos e valor energético. Assim como a goiaba tradicional, a goiaba serrana é uma boa fonte de vitamina C (60 $mg.100g^{-1}$), com valor superior ao da laranja (54 $mg.100g^{-1}$) e apresenta $5mg.100g^{-1}$ de ácido pantotênico. Al[em disso, apresenta elevado conteúdo de potássio (SCHMIDT, 2018).

Estudos sugerem que as goiabas serranas têm várias atividades biológicas, incluindo atividades antimicrobianas (BEYHAN; ELMASTAŞ; GEDIKLI, 2010; KARAMI; SAEIDNIA; NOSRATI, 2013; PASQUARIELLO *et al.*, 2015; VUOTTO *et al.*, 2000), antitumoral (BONTEMPO *et al.*, 2007), gastropreventiva a anti-infllamatória (MONFORTE, 2014a; MONFORTE, 2014b; ROSSI *et al.*, 2007) e antioxidantes (BEYHAN; ELMASTAŞ; GEDIKLI, 2010; KARAMI; SAEIDNIA; NOSRATI, 2013; PASQUARIELLO *et al.*, 2015).

A capacidade antioxidante da fruta, quantificada pelo método ABTS, é de 618,69 µMol/g. Contudo, essa capacidade pode variar entre as diferentes variedades e localidades (PASQUARIELLO et al., 2015). A atividade antioxidante da polpa da goiaba serrana, se deve principalmente aos polifenois, que habitualmente conferem um gosto adstringente e, por vezes amargo (HAMINIUK *et al.*, 2011; SCHMIDT, 2018; WESTON, 2010a). Quanto aos compostos bioativos, a fruta apresenta compostos fenólicos e em menor quantidade carotenoides, e as quantidades desses compostos variam de acordo com a localidade e condições climáticas que a fruta foi cultivada.

Figura 2. Fotografia da goiaba serrana (*Acca Sellowiana)*

Fonte: http://www.hortresearch.co.nz/

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3.1.1 Compostos fenólicos da goiaba serrana

Compostos fenólicos podem ser divididos em três grupos principais, ácidos hidroxibenzóicos, ácidos hidroxicinâmicos, e flavonoides. Quimicamente, estes compostos têm ao menos um anel aromático, na qual um hidrogênio é substituído por um grupo hidroxil (HELENO *et al.*, 2015). Os ácidos fenólicos são produzidos nas plantas e fungos, e estão presentes em quase todos os alimentos vegetais, representando uma porção significativa da dieta. Eles têm sido amplamente estudados devido aos seus efeitos benéficos para a saúde humana, como possíveis antioxidantes, antimicrobianos e antitumoral (ALVES *et al.*, 2013; CLIFFORD; SCALBERT, 2000; FERREIRA; BARROS; ABREU, 2009; HELENO *et al.*, 2015). Além disso, os compostos fenólicos são encontrados naturalmente em frutas, desempenham função de coloração e *flavor* das frutas, contribuindo para a adstringência, acidez e sabor amargo dos mesmos (CHITARRA, M; CHITARRA, A, 2005). Dentre os vários compostos fenólicos existentes, os mais estudados são: ácido cumárico, ácido caféico, ácido ferúlico, ácido gálico, ácido benzoico, ácido vanílico, ácido cinâmico, ácido hidroxibenzóico, catequinas, quercetina e kaempferol (HELENO *et al.*, 2015).

O teor de compostos fenólicos no suco de frutas é influenciado por fatores ambientais de crescimento aos quais a planta original foi submetida, como tipo de solo, temperatura, quantidade de exposição solar e precipitação (EPHREM *et al.*, 2018a). Os polifenóis são sensíveis ao calor e à luz (MUNIN; EDWARDS-LÉVY, 2011) e são facilmente oxidados, e assim, essa oxidação produz alterações nas propriedades sensoriais do suco de fruta. A concentração dos polifenóis no suco de frutas são altamente influenciadas pelas condições de processamento e armazenamento da bebida, o que pode acarretar um suco turvo devido ao resultado direto da oxidação dos polifenóis causada pela formação de substâncias mais ou menos polimerizadas (QUEIROZ *et al.*, 2008). Sucos de frutas contém uma mistura complexa de fenóis que contribuem para o amargor, adstringência, cor, sabor, odor e estabilidade oxidativa da fruta (EPHREM *et al.*, 2018).

Entre os compostos bioativos encontrados na goiaba serrana, os compostos fenólicos são os principais. A quantidade de compostos fenólicos totais encontrado na goiaba serrana foi de 11.447,71 $\mu g.g^{-1}$. Dentre esses compostos, foi encontrado principalmente ácidos fenólicos das seguintes classes: flavan-3-ols e ácidos hidroxibenzóicos, seguido pelos ácidos hidroxicinâmicos e flavonol. Os principais ácidos fenólicos identificados na goiaba serrana foram isômeros de pedunculagin e galato de

epicatequina. Além desses majoritários, foram encontrados catequina, quercetina, proantocianidinas, derivados do ácido elágico, entre outros (SCHMIDT, 2018). O estudo de Miglio *et al.*, (2014), mostrou que o consumo de suco de frutas que continha altos níveis de flavan-3-ol e concentrações menores de ácidos hidroxicinâmicos e flavonols resultou em um maior efeito antioxidante *in vivo*.

3.1.2 Carotenoides da goiaba serrana

Os carotenoides são compostos lipossolúveis, pigmentos naturais responsáveis pela coloração amarela, laranja e vermelha de frutas, folhas e algumas flores. Mais de 700 compostos já foram identificados, sendo α-caroteno, β-caroteno, licopeno, zeaxantina, luteína e criptoxantina os pigmentos dessa classe mais encontrados em vegetais (VOUTILAINEN et al., 2006; SAINI et al., 2015). Os carotenoides apresentam propriedades funcionais, são excelentes antioxidantes, alguns são precursores da vitamina A, e estudos epidemiológicos têm associado o seu consumo com a redução dos riscos de desenvolver doenças crônicas (RAPOSO et al., 2015).

A localização dos carotenoides nos cloroplastos e a presença de um cromóforo em sua estrutura química os tornam sensíveis à intensidade da luz (DAMODARAN et al., 2010). O processamento e armazenamento de alimentos, à exposição a ácidos e ao calor em excesso são alguns dos fatores que podem provocar a perda de carotenoides (RODRIGUEZ-AMAYA, 2002).

Dentre os carotenoides, já foram identificados na fruta da goiaba serrana, luteína e β-caroteno em maior quantidade (111 e 116 $\mu g.100g^{-1}$, respectivamente), e zeaxantina, β-criptoxantina e α-caroteno (8, 32 e 14 $\mu g.100g^{-1}$, respectivamente).

3.1.3. Processamento da goiaba serrana

Além do consumo *in natura*, as frutas podem ser processadas e utilizadas na produção de alimentos e bebidas. Na Nova Zelândia, existem diversos produtos derivados da fruta como purês, geleias, fruta secas, sorvetes, iogurtes, espumantes, licores e molhos, entre outros produtos (CASTELLANOS; HERRERA, D. R.; HERRERA, A. O., 2016; MORETTO; NODARI; NODARI, 2014). Produtos potenciais a partir goiaba serrana são promissores, já que umestudo sensorial realizado com a goiaba serrana, mostrou que 90% dos participantes consideram o sabor e o aroma da fruta como bons ou ótimos (BARNI *et al.*, 2004).

No processamento da goiaba serrana, o bagaço muitas vezes é descartado, o que origina uma quantidade expressiva de resíduo da fruta. O resíduo da polpa mostra-se uma boa alternativa como ingrediente para alimentos funcionais, devido a suas características nutricionais (SUN-WATERHOUSE *et al.*, 2013).

Um produto potencial a partir da goiaba serrana, é o suco, que ainda é pouco estudado. Foram encontrados apenas dois estudos com o suco da goiaba serrana. No entanto tais pesquisas não avaliaram a extração enzimática do suco ou a caracterização dos parâmetros físico-químicos ao longo do armazenamento (MONFORTE et *al*., 2014; PENG *et al*., 2020).

3.2 Sucos

O mercado global de bebidas está passando por uma mudança, liderada pela exigência do consumidor por produtos saudáveis e de alta qualidade. Existe uma demanda crescente por produtos obtidos a partir de vegetais, que sejam convenientes, saudáveis e prontos para o consumo; como por exemplo: sucos, purês, geleias e assim por diante. Esses produtos devem manter as características originais de sabor, viscosidade, cor, nutriente e aroma das frutas e hortaliças e ainda oferecer um prazo de validade razoavelmente prolongado (LANDL *et al.*, 2010).

Nos últimos anos, os consumidores tornaram-se mais conscientes dos diversos benefícios para a saúde, o que leva a um aumento no consumo de sucos de frutas naturais e outros produtos naturais como uma alternativa às bebidas tradicionais açucaradas e refrigerantes (TAPRE; JAIN, 2014). Além disso, há um mercado em rápido crescimento de sucos de frutas variados, como suco de frutas exóticas e sucos produzidos localmente (PERSIC *et al.*, 2017; RAHIM; RAMAN, 2015). Os sucos de frutas integrais vêm despertando o interesse, por conterem propriedades nutritivas, através da presença de vitaminas, minerais e compostos com atividade antioxidante (GOULAS; MANGANARIS, 2012), particularmente ácido ascórbico e fenóis ((EPHREM *et al.*, 2018b).

Segundo a legislação brasileira (BRASIL, 2009), suco é a bebida não fermentada, não concentrada, e não diluída, destinada ao consumo, obtida da fruta madura e sã, ou parte do vegetal de origem, por processamento tecnológico adequado, submetida a tratamento que assegure a sua apresentação e conservação até o momento do consumo.

O suco integral é o suco sem adição de açúcares e na sua concentração natural, sem conservantes ou aditivos.

Os sucos de frutas são compostos principalmente de água (70-93%), contêm carboidratos como glicose, frutose, amido, celulose e hemicelulose. A celulose, hemicelulose, substâncias pécticas e proteínas contribuem para a textura do suco de frutas (BARRETT; BEAULIEU; SHEWFELT, 2010). Após os açúcares, os ácidos orgânicos são os sólidos solúveis mais abundantes nos sucos de frutas e contribuem para o sabor, pois determinam a doçura e a acidez. (BATES; MORRIS; CRANDALL, 2001). Outros compostos também são encontrados no suco de frutas como, aminoácidos, proteínas, lipídios, vitaminas, minerais, fibras, compostos bioativos como fenólicos, carotenoides e antocianinas. As quantidades destes compostos variam consideravelmente de acordo com o tipo, cultivar e maturidade da fruta, e os mesmos contribuem para o sabor, aroma, cor e viscosidade do suco (EPHREM *et al.*, 2018a).

Os compostos voláteis presentes em sucos de frutas frescos são responsáveis pelo seu sabor e aroma, sendo uma mistura complexa de aldeídos, álcoois, cetonas, ésteres, terpenos, derivados de compostos fenólicos e de outras substâncias como aminoácidos e lipídeos. Esses compostos são liberados pela ação de enzimas, ácidos ou calor (SAFFARIONPOUR; OTTENS, 2018).

Na indústria de sucos, um dos principais desafios consiste em produzir sucos com características próximas das frutas integrais, garantindo aos consumidores um produto de qualidade (YI *et al.*, 2018). Outro ponto a ser considerado é o aumento da vida útil dos sucos, buscando manter os nutrientes, para garantir a segurança dos alimentos (SAEEDUDDIN *et al.*, 2015). Assim torna-se fundamental a investigação dos aspectos tecnológicos que envolvem as etapas de branqueamento, maceração, pasteurização e armazenamento.

3.3 Aspectos tecnológicos fundamentais para produção de sucos

A elaboração de suco integral de frutas, em geral envolve as seguintes etapas: colheita das frutas, recepção, seleção, lavagem, sanitização, prensagem e/ou despolpa, filtragem, inativação enzimática, clarificação, pasteurização, envase, rotulagem e armazenamento.

3.3.1 Recepção, seleção, lavagem e sanitização

Após a colheita das frutas, caso elas não sejam imediatamente submetidas ao processamento, deve-se adotar em adequado método de conservação, como por exemplo, câmaras frigoríferas que podem aumentar o tempo de armazenamento. As frutas são recebidas e selecionadas, assim, eliminam-se as que apresentam podridões, manchas e danos. Em seguida, deve-se realizar a etapa de sanitização das frutas com hipoclorito de sódio, com o objetivo de eliminar as impurezas e matérias estranhas grosseiras, como terra, pedras, folhas e outras sujidades. Assim, a realização de sanitização com água e hipoclorito de sódio garantem um maior controle da qualidade sanitária dos sucos produzidos, evitando, além da contaminação por microrganismos, insetos e roedores, a ocorrência de alterações indesejáveis no produto final (ZANDONÁ, 2017).

3.3.2 Despolpa e filtragem

O despolpamento da fruta consiste em uma operação utilizada para separar da polpa da fruta, o material fibroso, as sementes e as cascas. A fruta pode passar pela despolpadeira inteira ou desintegrada. As despolpadeiras são os equipamentos utilizados nesta etapa e são construídas em aço inox e dispõem de peneiras com diferentes tamanhos, que podem variar para as diferentes frutas (MATTA; FREIRE JUNIOR, 1995). O suco obtido nesta etapa, é direcionado para a filtragem em telas/peneiras de aço inox para uma filtração com intuito de remoção de partículas grosseiras (ZANDONÁ, 2017).

3.3.3 Inativação de enzimas

As enzimas aceleram reações químicas convertendo substratos em produtos. A conformação enzimática é afetada pelo pH, temperatura, pressão, concentração de sal e processos químicos ou físicos, que podem causar desnaturação da proteína. Às vezes a desnaturação pode ser reversível porque a estrutura primária do polipeptídeo é conservada no processo e após o fator desnaturante ser removido, a proteína se regenera e retoma sua função. Outras vezes a desnaturação é irreversível levando à inativação parcial ou total da enzima (SILVA, SULAIMAN, 2018).

Frutas e hortaliças muitas vezes contêm enzimas endógenas, que às vezes são mais resistentes ao calor do que os microrganismos. As enzimas de deterioração devem ser inativadas a um nível mínimo, para evitar reações químicas deteriorantes durante o

armazenamento e permitir uma vida útil mais longa dos alimentos (SILVA, SULAIMAN, 2018)(SILVA; SULAIMAN, 2018). A atividade de enzimas deteriorantes presentes naturalmente em alimentos pode afetar a cor dos alimentos, o sabor e aroma, a textura e a qualidade nutricional. Como exemplos de enzimas envolvidas nessas reações podem ser citadas a polifenoloxidase (PPO), peroxidase (POD), pectinametilesterase (PME), fenilalanina amonialiase (PAL), poligalacturonase (PG), e lipoxigenase (LOX). Em estudos com a goiaba serrana, foram encontrada atividade das enzimas POD, PPO, PAL e PME (BENINCÁ, 2014; ORTUÑO *et al.*, 2013).

É bem conhecido que em frutas e outros vegetais ocorre o escurecimento enzimático e a degradação de cor, devido às lesões mecânicas nos tecidos vegetais, ao descascamento e aos cortes, na presença de oxigênio, o que causam mudanças indesejáveis na qualidade dos produtos durante o manuseio, processamento e armazenamento. O escurecimento enzimático dos tecidos de frutas e hortaliças tem sido uma preocupação constante na indústria, com perdas econômicas devido ao impacto negativo na cor, aparência, sabor, redução do valor nutricional dos alimentos e subsequente a não aceitação pelo consumidor (QUEIROZ *et al.*, 2011; SULAIMAN *et al.*, 2015).

A polifenoloxidase (PPO, EC 1.14.18.1) catalisa a degradação de constituintes fenólicos da fruta, na presença de oxigênio, esta enzima catalisa a hidroxilação de monofenóis à *o*-difenóis e a oxidação dos *o*-difenóis aos seus correspondentes *o*-quinonas. A *o*-quinona resultante irá subsequentemente polimerizar com outras *o*-quinonas, proteínas ou aminoácidos, produzindo compostos castanhos indesejáveis, denominadas melaninas. A PPO, é amplamente distribuída nas plantas, e considerada a principal contribuinte para o escurecimento de frutas e vegetais (BILLAUD *et al.*, 2004; QUEIROZ *et al.*, 2011; YORUK; MARSHALL, 2003). Na goiaba serrana, a atividade da PPO parece ser o fator principal na reação de escurecimento de polpa (BENINCÁ, 2014).

A peroxidase (POD, EC 1.11.1.7), é uma das enzimas mais termoestáveis, e é usada frequentemente como marcador para adequação do processo de branqueamento. Ela é responsável por realizar a oxidação de um único elétron em uma ampla variedade de compostos, na presença de peróxido de hidrogênio. Além disso, a atividade sinérgica de PPO e POD é devida à geração de peróxido de hidrogênio durante a oxidação de compostos fenólicos em reações catalisadas por polifenoloxidase (TOMÁS-BARBERÁN; ESPÍN, 2001).

A pectinametilesterase (PME, EC 3.1.1.11) está entre as enzimas associadas à parede celular. É uma enzima hidrolítica amplamente distribuída em plantas, fungos patogênicos e bactérias. A PME hidrolisa especificamente os grupos metil éster na posição C6 do ácido galacturônico e desempenha um papel importante na degradação de paredes celulares em plantas superiores, visto que, a desmetilação da pectina torna-a altamente polimerizada e suscetível a degradação posterior pela poligalacturonase (CHRISTENSEN *et al.*, 1998).

A fenilalanina amonialiase (PAL, EC 4.3.1.5) catalisa a conversão não oxidativa da l-fenilalanina em ácido *trans*-cinâmico e amônia. Esta reação é o primeiro passo para a síntese dos fenilpropanóides em plantas. As plantas respondem ao estresse com alterações na atividade da PAL e no acúmulo de fenilpropanóides. O dano mecânico no tecido vegetal é um tipo de estresse que eleva a atividade da PAL (EMILIANI *et al.*, 2009). Muitos metabólitos secundários com alto valor biológico (lignina, ácido cumárico, flavonoides, etc) são derivados da via fenilpropanóide em plantas. Além disso, a capacidade natural da PAL de quebrar a l-fenilalanina faz dela um tratamento confiável para a condição genética da fenilcetonúria, um distúrbio hereditário que aumenta os níveis de fenilalanina no sangue (LEVY; SARKISSIAN; SCRIVER, 2018).

Existem métodos que são utilizados para a conservação de alimentos, a fim de controlar o escurecimento enzimático. Por exemplo, uso de calor (GOYENECHE; SCALA; ROURA, 2013; NDIAYE; XU; WANG, 2009), métodos físicos como microondas (LATORRE *et al.*, 2012; SIGUEMOTO *et al.*, 2018), alta pressão (MARSZAŁEK *et al.*, 2017; ORTUÑO *et al.*, 2013), aquecimento ôhmico (GOMES; SARKIS; MARCZAK, 2018; IÇIER; YILDIZ; BAYSAL, 2008) e adição de muitos agentes químicos, como o sulfitos e ácido ascórbico (LIU *et al.*, 2013; QUEIROZ *et al.*, 2008).

O ácido ascórbico e sua forma de sal são os ácidos orgânicos mais amplamente utilizados na indústria alimentícia para controlar o escurecimento enzimático. No estudo de Queiroz *et al.* (2011), a PPO do caju foi completamente inativada com solução de ácido ascórbico. No entanto, é relatado que a diminuição da atividade da PPO está associada com suas propriedades termodinâmicas. Por isso, muitas vezes, o ácido ascórbico é utilizado em conjunto com o tratamento térmico (LIU *et al.*, 2013). Ndiaye; Xu; Wang, (2009), verificaram que o uso de ácido ascórbico a 1% não inativou a PPO e a POD em purês de mangas. O ácido cítrico em baixas concentrações não teve efeito significativo sobre a atividade da PPO, enquanto o ácido cítrico em altas concentrações

pôde inativar acentuadamente a PPO e quando a maior concentração de ácido cítrico associada com a temperatura de 65 ºC, a inativação da PPO foi elevada. Pode-se concluir que a PPO foi mais suscetível à elevação da temperatura do que o tratamento com ácido cítrico (LIU *et al.*, 2013).

O processamento térmico é o método mais comum usado pela indústria de alimentos, devido à sua capacidade de inativar microrganismos e enzimas de deterioração em frutas, e é frequentemente referido como branqueamento. O principal objetivo do branqueamento é a inativação de enzimas indesejáveis presentes em frutas e hortaliças e a redução da carga microbiana; entretanto, ao mesmo tempo, auxilia na remoção de gases dos tecidos, estabilizando a textura, cor, sabor e qualidade nutricional dos produtos (RAWSON *et al.*, 2011; SILVA; SULAIMAN, 2018).

O branqueamento envolve o contato direto de vapor ou água quente com os pedaços inteiros ou cortados de frutas e hortaliças por um certo período de tempo, seguido de resfriamento. Vegetais branqueados a 60-95 ºC, durante 1 a 10 minutos são necessários para evitar o escurecimento, e assim inativar as enzimas (RAWSON *et al.*, 2011; SILVA; SULAIMAN, 2018) A variabilidade da temperatura e do tempo de branqueamento é grande. Uma revisão de Silva e Sulaiman (2018) mostrou que a aplicação do branqueamento para reduzir atividade enzimática da PPO em frutas e hortaliças, é realizado sob uma variação de temperatura de 60 a 100 ºC e tempos de 0 a 10 minutos, e a variação foi alta também entre a mesma fruta. Entretanto, o calor empregado no branqueamento pode afetar negativamente o sabor da fruta, degradar alguns nutrientes, como vitamina C, compostos fenólicos e outros nutrientes termolábeis (CASTRO *et al.*, 2008; GOYENECHE *et al.*, 2018; QUEIROZ *et al.*, 2008; SULAIMAN *et al.*, 2015; VOLDEN *et al.*, 2008).

Atualmente, existem também tecnologias que utilizam temperaturas brandas para inativar as enzimas deteriorantes como o aquecimento ôhmico, processamento a alta pressão hidrostática, ultrassom, microondas e campo elétrico pulsado, que são alternativas ao tratamento térmico convencional. Entretanto, essas tecnologias emergentes ainda possuem um custo operacional elevados. Estudos têm demonstrado que sob temperatura ambiente essas tecnologias possuem eficácia limitada em relação as enzimas associadas com a degradação dos alimentos, por exemplo, para certas cultivares de frutas e hortaliças, foi visto que não há benefício extra no uso da tecnologia de alta pressão hidrostática (CHENG; ZHANG; ADHIKARI, 2013; SILVA; EVELYN, 2018; TEREFE; BUCKOW; VERSTEEG, 2014).

Estudos mostram que independente da tecnologia emergente utilizada, quando combinadas com temperaturas a partir de 60 ºC ocorre uma maior inativação das enzimas polifenoloxidase e peroxidase (CHENG; ZHANG; ADHIKARI, 2013; SAEEDUDDIN *et al.*, 2015; SULAIMAN *et al.*, 2015).

Devido à importância da preservação da cor da matéria-prima antes de qualquer processamento, a inativação de enzimas que ocorrem naturalmente em tecidos vegetais, como a PPO e a POD, é necessário, e assim minimiza as perdas nutricionais e sensoriais do produto causadas pelo escurecimento.

3.3.4 Maceração

Uso de enzimas

Enquanto muitas enzimas endógenas nativas nos alimentos podem degradar os nutrientes e causar deterioração da qualidade, algumas enzimas comerciais catalisam várias reações envolvidas na preparação de diferentes produtos alimentícios. Em torno de 4000 enzimas são conhecidas e, destas, aproximadamente 200 são utilizadas comercialmente, a maioria delas de origem microbiana. As mesmas podem ser adicionadas em diversos alimentos. Nos sucos, elas promovem reações importantes, como clarificação, remoção de substâncias desagradáveis, aumento da relação sólidos solúveis/acidez e extração de compostos de interesse (DAL MAGRO *et al.*, 2016; KUMAR, 2015; SANDRI *et al.*, 2013).

As frutas tropicais são geralmente muito polpudas e pectináceas para produzir suco por simples prensagem ou centrifugação, que resulta em um rendimento insuficiente de suco. Em frutas como goiaba, manga e maçã a extração do suco é um pouco difícil pelos métodos convencionais e incompleta, visto que alguma quantidade de suco fica retida no resíduo após a extração (TAPRE; JAIN, 2014).

As enzimas pectinolíticas comerciais são uma parte importante da tecnologia de suco de frutas quase desde o início da indústria de processamento de suco. Produtos enzimáticos têm sido utilizados no processo de fabricação de sucos de frutas desde a década de 1930. Elas são usadas para auxiliar na extração, clarificação e estabilidade de sucos de muitas frutas (TAPRE; JAIN, 2014).

Os sucos de frutas são naturalmente turvos, especialmente devido à presença de polissacarídeos (pectina, celulose, hemiceluloses e amido), proteínas, taninos e íons metálicos (VAILLANT *et al.*, 2001). As pectinases são responsáveis pela degradação desses polissacarídeos estruturais da polpa da fruta, e responsáveis pela turbidez. A alta

concentração de pectina forma uma dispersão coloidal, que é um dos principais problemas no processamento de sucos de frutas claras. Embora as partículas de polpa suspensas possam ser removidas por filtração, a presença de pectina pode dificultar o processo (TAPRE; JAIN, 2014).

Um dos principais problemas na produção de suco de fruta é a turbidez. Por isso, a clarificação é uma etapa importante, a qual tem o intuito de retirar as substâncias pécticas, através de tratamento enzimático de despectinização. Além da turbidez, as substâncias pécticas contribuem com o aumento da viscosidade do suco, alteram consistência; e formam complexos com compostos fenólicos oxidados que se depositam, com prejuízos para os aspectos sensoriais dos sucos. Com a adição de pectinases a viscosidade do suco de fruta diminui, a estrutura gelatinosa se desintegra e o suco é facilmente obtido com maiores rendimentos (KASTER, 2009; TAPRE; JAIN, 2014; VENTURINI FILHO, 2010).

Além disso, na indústria de suco de frutas, essas enzimas são usadas para melhorar a qualidade sensorial, proporcionando sucos mais claros, com diminuição de compostos indesejáveis e ainda com diminuição da acidez titulável e aumento de açúcares, o que é visto como um efeito desejável, uma vez que quanto maior a relação acidez/açúcares, maior a percepção do sabor doce pelo consumidor (DAL MAGRO *et al.*, 2016; PERSIC *et al.*, 2017; SANDRI *et al.*, 2013; SIDDIQ *et al.*, 2018a).

Enzimas pécticas são usadas na preparação de suco de maçã e de goiaba para facilitar a extração e auxiliar na separação de um precipitado floculento por sedimentação, filtração ou centrifugação. Pelo tratamento com enzimas pectinase, há aumento no rendimento para suco de goiaba de 25 até 42% e a viscosidade reduziu em até 90% (KAUR *et al.*, 2009; NINGA *et al.*, 2018).

Além de promover uma melhor extração de suco, a adição de enzimas aumenta a liberação de vários compostos fenólicos e outros componentes nutricionalmente importantes. Estudos indicam que vários compostos fenólicos, estão localizados dentro ou pelo menos fortemente associados ao material da parede celular. As pectinases e as celulases degradam a pectina e a parede celular do vegetal, respectivamente, aumentando dessa forma o teor de compostos fenólicos e a atividade antioxidante do suco. No estudo de Magro et al., (2016) os compostos fenólicos aumentaram em até 58% no processo de obtenção do suco de uva com extração enzimática (DAL MAGRO *et al.*, 2016; KUMAR, 2015; LANDBO; MEYER, 2001; SIDDIQ *et al.*, 2018b).

A mistura de enzimas pectinolíticas, celulases e hemicelulases são eficazes não somente no aumento de rendimento e clareza de suco, na diminuição da viscosidade, mas também na preservação de nutrientes, cor original e sabor, resultando em um produto de alta qualidade. Parâmetros como tempo de incubação, concentração utilizada da enzima, temperatura de reação e a espécie/cultivar da fruta escolhida influenciam nos resultados (TAPRE; JAIN, 2014). Esses parâmetros mudam conforme o suco de fruta estudado, como apresentado na Tabela 1. Os autores indicam que a concentração de enzima representa o fator mais importante que afeta as características do suco.

Tabela 1. Concentração enzimática, temperatura e tempo, utilizada em diferentes sucos clarificados

Amostras	**Concentração enzimática, temperatura e tempo**	**Referência**
Suco de banana	0,084%, 43 ºC, 80 minutos	Lee *et al*., 2006
Suco de carambola	0,1%, 30 ºC, 20 minutos	Abdullah *et al*., 2007
Suco de goiaba	600 ppm, 45 ºC, 120 minutos	Brasil *et al*., 1995
	0,70mg.100g^{-1}, 43 ºC, 7 horas	Kaur *et al*., 2009
	75 mg.kg^{-1}, 30 °C, 60 minutos	Rojas-Garbanzo *et al*., 2018
	0,078%, 45 ºC, 40 minutos	Ninga *et al*., 2018
Suco de maçã	10 U.mL^{-1}, 40 ºC, 40 minutos	Sandri *et al*., 2013
Suco de sapoti	0,1%, 40 ºC, 120 minutos	Sin *et al*., 2006
Suco de uva	0,50 U.g^{-1}, 50 ºC, 30 minutos	Dal Magro *et al*., 2016

As enzimas são usadas como componentes no processamento de alimentos na indústria alimentícia, elas são consideradas coadjuvantes de tecnologia. Segundo a resolução RDC Nº 54 (BRASIL 2014) coadjuvante de tecnologia de fabricação é toda substância utilizada na elaboração e/ou conservação de um produto, que não se consome por si só como ingrediente alimentar e que se emprega intencionalmente na elaboração de matérias-primas, alimentos ou seus ingredientes, para obter uma finalidade tecnológica durante o tratamento ou fabricação e que deverá ser eliminada do alimento ou inativada, podendo admitir-se no produto final a presença de traços de substância ou seus derivados (BRASIL, 2014).

3.3.5 Microfiltração

A microfiltração é amplamente utilizada em indústrias de sucos de frutas para garantir a clarificação e a esterilização de muitos sucos de frutas ou para concentrar sua fração de polpa (CASSANO; CONIDI; DRIOLI, 2010; DAHDOUH *et al.*, 2018). A microfiltração constitui uma técnica alternativa aos tratamentos térmicos convencionais que são geralmente usados para melhorar a segurança dos sucos de frutas e para estender seu prazo de validade. Como a microfiltração é uma técnica não térmica, o suco estabilizado e clarificado preserva a frescor, os compostos termo sensíveis e a qualidade nutricional do suco clarificado (CONIDI; CASSANO; DRIOLI, 2012; DAHDOUH *et al.*, 2015a). Assim, a microfiltração tem sido aplicada com sucesso para preservar e clarificar muitos sucos de frutas, como sucos de maçã (YU; LENCKI, 2004), abacaxi (LAORKO; TONGCHITPAKDEE; YOURAVONG, 2013), cereja (WANG; WEI; YU, 2005) e maracujá (DE OLIVEIRA; DOCÊ; DE BARROS, 2012).

No entanto, um dos fatores críticos que limita o desempenho geral desta técnica é a perda significativa da permeabilidade da membrana, devido ao fenômeno de incrustação de membrana. Diversas abordagens e estratégias para melhorar o desempenho de filtração foram sugeridas na literatura; os autores propõem geralmente pré-tratamento ou condicionamento do suco a ser filtrado, como tratamento enzimático (YU; LENCKI, 2004) ou centrifugação (DE OLIVEIRA; DOCÊ; DE BARROS, 2012)).

3.3.6 Pasteurização

Várias técnicas de processamento são desenvolvidas para conseguir um suco de longa duração: um dos métodos mais usados na indústria de alimentos é a pasteurização por calor. A pasteurização consiste em um tratamento térmico, com aplicação de elevadas temperaturas por um determinado tempo, para inibir a carga microbiana composta por microrganismos deteriorantes e patogênicos, como também obter a inativação enzimática, proporcionando assim o aumento da vida útil dos sucos (FELLOWS, 2006; WIBOWO *et al.*, 2015a).

No entanto, semelhante a outros processos de aquecimento, também pode afetar a qualidade nutricional e sensorial do produto levando à insatisfação do consumidor (PASTORIZA *et al.*, 2017). Embora o tratamento térmico possa levar a mudanças no conteúdo de compostos bioativos dos alimentos, há estudos que mostram que o ácido ascórbico e compostos fenólicos permanecem inalterados mesmo em temperatura de 80

a 90 ºC por 1 a 10 minutos (MERTZ *et al.*, 2010; PACHECO-PALENCIA; DUNCAN; TALCOTT, 2009; RAWSON *et al.*, 2011).

Os sucos de baixa acidez (pH> 4,6) são aquecidos em temperatura mais alta que 100°C (esterilização); enquanto, sucos ácidos (pH <4,6) requerem o tratamento térmico abaixo de 100°C (pasteurização). A intensidade do processo de pasteurização (temperatura e tempo) é importante para garantir a segurança, vida útil e qualidade do suco. O processo de pasteurização, dependendo de sua intensidade, destrói certas espécies microbianas vegetativas ou enzimas até o grau desejado. À medida que a temperatura do suco aumenta, os microrganismos e enzimas ficam menos ativos devido a mudanças reversíveis ou irreversíveis suas estruturas (AGHAJANZADEH; ZIAIIFAR, 2018). Dependendo do suco, diferentes combinações de tempo e temperaturas são aplicadas, como mostrado na Tabela 2.

A pasteurização térmica convencional refere-se a aquecer o suco entre 60 °C e 100 °C e mantê-lo por um tempo específico, com base na resistência térmica do microrganismo ou enzima alvo. Em um banho-maria, o mais simples equipamento de pasteurização, o suco embalado é mantido a uma temperatura alta o suficiente para o tempo necessário. Enquanto em um pasteurizador de banho de água contínuo, o produto é submetido a *sprays* de água quente contínua enquanto se move através de um túnel (AGHAJANZADEH; ZIAIIFAR, 2018).

Embora tecnologias não-térmicas, como campos elétricos pulsados, processamento de alta pressão, de ultrassom e irradiação ultravioleta, tenham resultados especialmente promissores para sucos de frutas, a pasteurização térmica ainda é aceita como a técnica mais confiável em termos de obtenção de produtos que são estáveis em prateleira e seguros para consumir desde que foi descoberta. Além da inativação microbiológica, a pasteurização térmica é utilizada com sucesso para inativar as enzimas do suco de frutas como a polifenoloxidase (PPO), peroxidase (POD) e pectinametilesterase (PME), responsáveis pela qualidade da decomposição. Assim, o prazo de validade dos sucos de frutas processados termicamente pode ser estendido por vários meses sem apreensões de segurança ou perdas importantes de qualidade, quando armazenado em baixas temperaturas ou ambiente (AĞÇAM; AKYILDIZ; DÜNDAR, 2017).

Tabela 2. Diferentes combinações de temperatura e tempo na pasteurização de diferentes sucos.

Amostras	Temperatura (ºC)	Tempo	Referências
Suco de abacaxi	80	10 min	Shamsudin *et al*., 2013
Suco de mirtilo	85	1 min	Siddiq *et al*., 2018
Suco de butiá	85	20 min	Jachna *et al*., 2015
Suco de goiaba	60	8,2 min	Rojas-Garbanzo *et al*., 2018
	95	5 min	Ninga *et al*., 2018
Suco de laranja	90	30 seg	Mapelli-Brahm *et al.,* 2018
Suco de maçã	85	15 min	Zandoná, 2017
Suco de manga	92	30 seg	Wibowo *et al*., 2015
Suco misto de frutas	95	20 seg	Bacigalupi *et al*., 2016

3.3.7 Embalagem

A maioria dos produtos de alimentos e bebidas é oferecida aos consumidores em uma ampla gama de alternativas de embalagens feitas de diferentes materiais, em diferentes tamanhos e formatos. Várias questões importantes são consideradas pelos consumidores e empresas na escolha de embalagens para alimentos: a capacidade do pacote para preservar a qualidade do alimento e frescor, imagem e apelo agradáveis, identificação correta do produto, facilidade de armazenamento, distribuição, e impacto ambiental (PASQUALINO; MENESES; CASTELLS, 2011; TU; YANG; MA, 2015).

Os sucos podem ser engarrafados em recipientes de vidro, pelo fato de ser um material impermeável, inerte, reutilizável, reciclável, resistente ao calor e ao empilhamento, e por ser transparente, o que proporciona ao consumidor melhor visibilidade do produto (FELLOWS, 2006). Os recipientes de vidro estão associados com melhor preservação dos atributos sensoriais dos alimentos, como aroma, sabor e textura. Os consumidores atribuem uma percepção de qualidade superior aos produtos contidos em embalagens de vidro do que em embalagens plásticas e uma das vantagens dos recipientes de vidro é a transparência que o material apresenta, o que permite que os consumidores visualizem o real produto antes de comprá-lo (BALZAROTTI *et al.*, 2015; TU; YANG; MA, 2015).

Com relação aos riscos químicos, físicos e microbiológicos, as embalagens de vidro podem fornecer uma barreira eficiente para minimizar as influências ambientais

que podem resultar na deterioração dos alimentos, perda de qualidade ou mesmo na falta de segurança alimentar. A embalagem de vidro suporta altas temperaturas de processamento, o que permite a pasteurização ou esterilização eficiente de muitos produtos (KOBAYASHI, 2016).

Para uma boa conservação das propriedades nutricionais durante o armazenamento, garrafas de vidro são geralmente usadas para embalar os sucos. Do ponto de vista nutricional, o suco embalado em garrafas de vidro contém maior quantidade de antioxidante durante o armazenamento e pode ser uma fonte mais benéfica de compostos bioativos (BACIGALUPI *et al.*, 2015).

Garrafas de vidro retornáveis podem ser consideradas uma das melhores embalagens quando o assunto é sustentabilidade ambiental. A reciclagem de vidro requer cerca de 40% menos energia em comparação com a nova produção de vidro, e por isso, as embalagens de vidro podem ser vantajosas em relação a outros materiais de embalagem. Contudo há preocupações sobre o peso adicional que embalagens de vidro implica no transporte de alimentos e bebidas, comparando com embalagens plásticas, o que resulta em um maior impacto a emissão de gases poluentes e também os custos (IBRAHIM; MEAWAD, 2018; KOBAYASHI, 2016; PASQUALINO; MENESES; CASTELLS, 2011).

3.3.8 Armazenamento

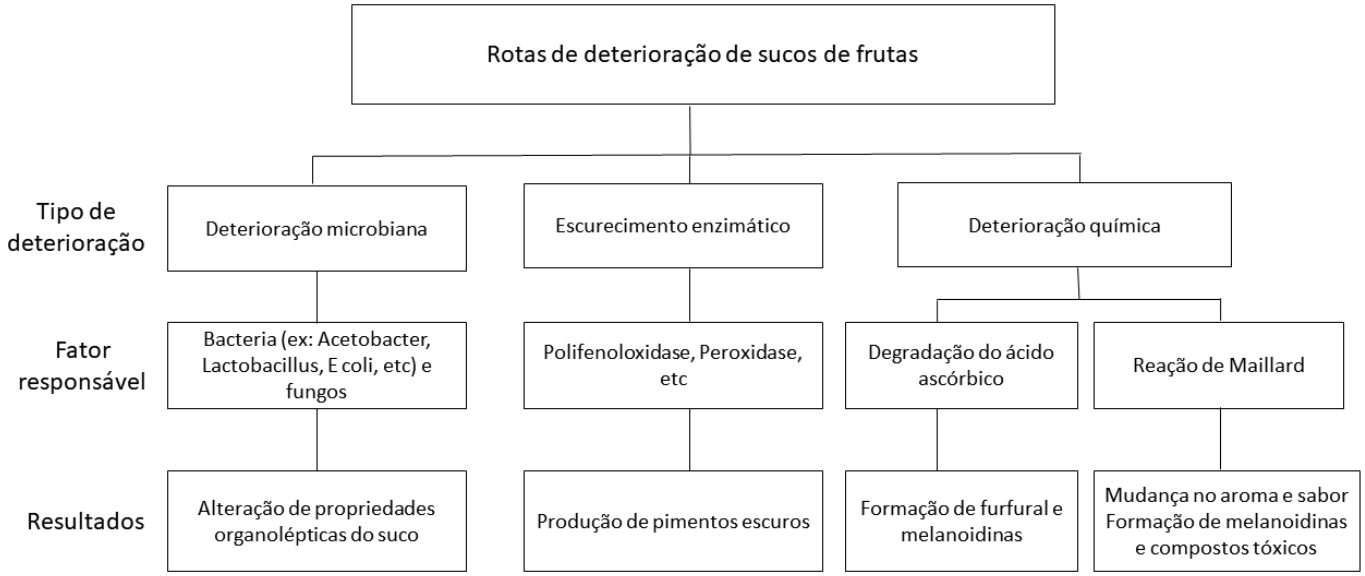
Vida útil pode ser definida como um período finito de tempo após a fabricação e embalagem, durante o qual o produto alimentício mantém um nível de qualidade aceitável para o consumo (NICOLI, 2012). Além disso, o prazo de validade pode ser influenciado por fatores intrínsecos, relacionado a mudança nas propriedades organolépticas (por exemplo características do produto: acidez, cor, sabor, perda de nutrientes) e extrínsecos (por exemplo características do meio ambiente: temperatura, luz e aeração). A curta vida de prateleira do suco de fruta natural é causada por uma combinação de fatores microbianos, enzimáticos e químicos, como mostrado na Figura 3 (EPHREM *et al.*, 2018)

Durante o armazenamento, podem ocorrer nos sucos de frutas, importantes mudanças químicas relacionadas à qualidade, como mudanças de cor (por exemplo, escurecimento), degradação do sabor e perdas de nutrientes. Consequentemente, essas mudanças podem afetar o grau de aceitação pelos consumidores. A deterioração do suco

de frutas é expressa principalmente como uma mudança nas propriedades sensoriais (sabor, aroma e cor) do suco (WIBOWO *et al.*, 2015a).

A estabilidade do suco durante o armazenamento depende de parâmetros internos, incluindo pH, potencial redox, teor de água, composição química e microflora natural do suco. O alto teor de água em qualquer produto alimentício estimula a deterioração microbiana, enzimática e química (ANEJA *et al.*, 2014; EPHREM *et al.*, 2018).

Figura 3. Fluxograma com as principais rotas de deterioração do suco de fruta fresco durante a estocagem.



Fonte: Adaptado de Ephram (2018)

Sucos de frutas não são tradicionalmente reconhecidos como veículos para doenças transmitidas por alimentos devido à sua acidez inerente, que inibe a multiplicação da maioria dos patógenos (SIGUEMOTO *et al.*, 2018). Entretanto, a proliferação de microrganismos tolerantes a ácido pode ser aumentada em sucos de frutas com valores de pH moderadamente ácidos (2,5 a 4,8), bem como com a disponibilidade de uma quantidade substancial de açúcares (KIMBALL, 1999). Os microrganismos que podem ocorrer no suco de frutas e causar deterioração são bactérias tolerantes ao ácido (Acetobacter, Alicyclobacillus, Bacillus, Gluconobacter, Lactobacillus, Leuconostoc, Zymomonas e Zymobacter) e fungos (leveduras e bolores). O armazenamento a frio diminui a taxa de proliferação microbiana nos sucos de frutas, prolongando assim seu tempo de prateleira (ANEJA *et al.*, 2014)..

A vida útil do suco de frutas também é influenciada por parâmetros extrínsecos, como temperatura, luz e aeração. A temperatura do suco e a aeração controlam sua colonização por microrganismos (RAYBAUDI-MASSILIA *et al.*, 2009). Além disso, a ocorrência de reações de escurecimento não enzimáticas é promovida pela presença de oxigênio no suco. O escurecimento não enzimático é um processo químico que leva à perda da qualidade do suco e do valor nutricional. Pode ser causada pela degradação do ácido ascórbico, ou pela reação de Maillard. Esses processos levam à formação de diferentes produtos, responsáveis pela cor marrom que pode aparecer durante o armazenamento (BATES; MORRIS; CRANDALL, 2001; EPHREM *et al.*, 2018).

O armazenamento do suco pode ser feito sob diferentes temperaturas, a depender do tipo de processamento térmico que este foi submetido. Portanto, todas as etapas do processamento do suco, irão influenciar significativamente no produto final e consequentemente, na sua vida de prateleira (RAYBAUDI-MASSILIA *et al.*, 2009; WIBOWO *et al.*, 2015a).

Atualmente, a pasteurização convencional, combinada com outros métodos de preservação, garante segurança alimentar microbiológica e vida de prateleira estável (SIGUEMOTO *et al.*, 2018).

3.4 Resíduo do processamento da goiaba serrana

A Organização das Nações Unidas para Agricultura e Alimentação (FAO) estimou que aproximadamente um terço dos alimentos produzidos globalmente para consumo humano é perdido ou desperdiçado, representando uma perda significativa dos recursos gastos na produção, processamento e transporte desses alimentos e uma ameaça à segurança alimentar (GUSTAVSSON; CEDERBERG; SONESSON, 2011). Estima-se que 161 kg por ano de alimentos/por pessoa são desperdiçados anualmente na União Europeia. Considera-se o desperdício de alimentos gerado desde o estágio de fabricação até o consumo (EUROPEAN COMMISSION, 2018). De muitos fatores que contribuem para a sobrecarga ambiental global nos últimos anos, o efeito do desperdício de frutas foi identificado como uma grande preocupação (BANERJEE *et al.*, 2017).

As frutas contêm uma ampla gama de componentes promotores da saúde, como fibra alimentar, vitaminas, minerais e compostos bioativos. Assim, os resíduos derivados de frutas representam uma boa fonte de ingredientes alimentares funcionais. A utilização de resíduos de frutas também é economicamente viável, criando valor e resolvendo

problemas de descarte. Resíduos do processamento de frutas consistem de 20% a 50% do total de resíduos produzidos nas indústrias. As aplicações de alimentos e na saúde desses compostos bioativos extraídos de resíduos alimentares são um importante campo de pesquisa em andamento (BANERJEE *et al.*, 2017; SCOTT; DUNCAN; FLINT, 2008; SUN-WATERHOUSE *et al.*, 2013).

O bagaço apresenta um conteúdo significativamente maior de compostos bioativos quando comparado com o suco. Assim, o bagaço pode ser desidratado, e, portanto, tornar-se um ingrediente de longa duração para aplicação em uma variedade de produtos alimentares para proporcionar benefícios adicionais à saúde (SIDDIQ *et al.*, 2018b).

Aproximadamente metade das goiabas serranas cultivadas comercialmente na Nova Zelândia estão sujeitas a processamento adicional em produtos como sucos, bebidas alcoólicas e conservas, com a outra metade indo para o mercado de frutas frescas. Processamento industrial e frutas frescas inteiras resultam em grandes quantidades de material residual. O descarte de resíduos de frutas (principalmente casca e bagaço) remanescentes após o processamento da goiaba serrana, afeta em custos, que reduzem as margens de lucro dos produtos processados. Esses resíduos de frutas ainda contêm quantidades elevadas de nutrientes e compostos bioativos importantes, que são benéficos para a saúde humana, e que poderiam ser isolados e usados para produzir ingredientes e materiais de valor agregado para aplicações alimentícias e farmacêuticas (SUN-WATERHOUSE *et al.*, 2013).

A casca da goiaba serrana, que representa cerca de 50% do peso da fruta, é azeda e amarga, sendo descartada durante o processamento industrial da fruta (SANTOS et al., 2019). Valente *et al.* (2011) relataram que o conteúdo de vitamina C na casca da goiaba serrana é duas vezes maior do que na polpa. A utilização do resíduo da fruta também permitiria que as pessoas consumissem produtos à base de goiaba serrana fora da estação através de alimentos ricos em bioativos da fruta. A utilização de resíduos de goiaba serrana para a produção de ingredientes funcionais é uma abordagem viável e econômica (SUN-WATERHOUSE *et al.*, 2013).

CAPÍTULO 4

4 MATERIAL E MÉTODOS

4.1 Material

4.1.1 Matéria-prima

A goiaba serrana (*Acca sellowiana* (O. Berg) Burret) foi coletada na Região Sul do Brasil, no estado de Santa Catarina, na cidade de São Joaquim (latitude 28º16'34.47"S, longitude 49º56'9.57"W). Aproximadamente 100 Kg de fruta foi adquirida, em estágio de maturação completa, quando as frutas podem ser facilmente separadas da planta segundo Amarante *et al.* (2017), nos meses de fevereiro a abril de 2019 e 2020. As frutas foram transportadas sob refrigeração até o Laboratório de Compostos Bioativos do Instituto de Ciência e Tecnologia de Alimentos (ICTA) da Universidade Federal do Rio Grande do Sul (UFRGS) e mantidas em câmara fria (-17 °C) até o momento do processamento.

As exsicatas também foram coletadas e depositadas no Herbário do Instituto de Biociências/UFRGS - Instituto de Ciências Naturais sob os números ICN 187146 e ICN 187148.

No dia do processamento, as amostras de frutas foram selecionadas visualmente quando ao dano mecânico, e as frutas que estavam danificadas foram descartadas. Após, as frutas foram lavadas em água corrente e sanitizadas por imersão em solução clorada (200 $mg.L^{-1}$ por 15 min). Uma nova lavagem com água corrente foi feita para remoção do cloro residual.

4.1.2 Enzimas

No processamento do suco de goiaba serrana foi testada a adição de três enzimas comerciais, Pectinex Ultra Clear (Novozymes, Spain), Rohapect 10 L (Amazon group, Brasil) e Lallzyme Beta (Lallemand Wine, France). As duas primeiras são enzimas com maior atividade de pectinase, e a terceira, celulase (DAL MAGRO *et al.*, 2016). As enzimas foram testadas em 3 concentrações (1, 5 e 10 U/mL) antes e/ou após o despolpamento, conforme a literatura (DAL MAGRO *et al.*, 2016; ROJAS-GARBANZO *et al.*, 2019; SANDRI *et al.*, 2013).

Na primeira adição de enzima, antes do despolpamento, o objetivo foi aumentar o rendimento da extração do suco. Já na segunda adição, após o despolpamento, a finalidade principal foi clarificar o suco.

4.1.3 Preparo dos sucos

Os sucos foram processados em condições de laboratório, seguindo as etapas industriais. Para a determinação das melhores condições experimentais do processamento do suco, as amostras foram avaliadas por análises físico-químicas. Após realizado os testes com as três enzimas e suas diferentes concentrações antes e após o despolpamento, foi selecionada a melhor combinação (enzima *x* concentração) para a produção do suco e avaliação do período de estocagem de 90 dias. O Fluxograma adotado no processamento dos sucos de goiaba serrana está apresentado na Figura 1.

Figura 1. Fluxograma adotado no processamento dos sucos de goiaba serrana (*Acca sellowiana*)

4.1.3.1 Suco *in natura*

As frutas foram lavadas, sanitizadas com solução clorada, selecionadas, e as frutas danificadas foram retiradas. Após, elas foram pesadas e foi realizada a fragmentação com facas de plástico. Após as frutas foram despolpadas e homogeneizadas em despolpadeira vertical (DES-20, Braesi®, Caxias do Sul, RS, Brasil). A seguir, o suco foi filtrado em tecido de organza. O suco foi transferido para garrafas de vidro transparente de 100 mL (Pilfer 24, Flint, Envidro, Porto Alegre, RS, Brasil), e, em seguida, um fluxo de nitrogênio foi aplicado no gargalo da garrafa, a fim de diminuir a concentração de oxigênio e foram fechadas com tampa rosqueáveis. As garrafas foram armazenadas sob refrigeração a 4 ºC na câmara de Demanda Bioquímica de Oxigênio (DBO) (NL-41-01 A, NewLab, Piracicaba, SP, Brasil) por fotoperíodo de 12 horas (ciclo claro/escuro), com o intuito de simular as condições de condicionamento do mercado. A umidade relativa e a temperatura da câmara foram monitoradas (50,7 ± 3,5% e 4,7 ± 0,5 °C), respectivamente. O período de armazenamento foi de quatro dias e o suco foi avaliado nos dias 1,3 e 4. Em cada dia de análise foram retiradas quatro garrafas de 100 mL contendo o suco e homogeneizados em um béquer. Uma alíquota foi retirada para as análises físico-químicas, e o restante do suco foi acondicionado em tubos falcons de 15 e 50 mL e congelados (-18 ºC) para análises posteriores. Na noite anterior às análises, as amostras de suco foram descongeladas em uma geladeira (4 °C por 12 horas). Para todos os experimentos, um suco controle (suco *in natura*) foi implementado sem enzima.

4.1.3.2 Suco clarificado por enzima e pasteurizado

No suco clarificado, após a higienização e fragmentação das frutas, elas foram acondicionadas em recipientes de vidro. Foi realizado o branqueamento da polpa da fruta, a fim de evitar o escurecimento enzimático, a 80 °C por 2,5 minutos em banho maria (Dubnoff NT 232 – Novatecnica®, Piracicaba, SP, Brasil), seguido pelo resfriamento a 10 °C. O binômio tempo e temperatura utilizado para o branqueamento da polpa, foi definido após testes preliminares. Posteriormente, foi aplicado a enzima Pectinex Ultra Clear (Novozymes, Spain), sob as seguintes condições: concentração de 10 U mL^{-1}, temperatura de 50 °C por 40 minutos, sob agitação, seguido do resfriamento a 20 °C.

As frutas foram despolpadas e homogeneizadas em despolpadeira vertical (DES-20, Braesi®, Caxias do Sul, RS, Brasil). Após, foi adicionada novamente a enzima Pectinex

Ultra Clear na concentração de 5 $U.mL^{-1}$, e deixado em banho-maria a 50 °C por 40 minutos, sob agitação e seguido pelo resfriamento até 20 °C. O suco foi filtrado em tecido de organza, acondicionado em garrafas de vidro transparente de 100 mL, pasteurizado a 90 °C por 1 minuto, seguido pelo resfriamento a 20 °C. Em seguida, um fluxo de nitrogênio foi aplicado no gargalo da garrafa, a fim de diminuir a concentração de oxigênio e foram fechadas com tampa rosqueáveis.

As garrafas foram armazenadas sob refrigeração a 20 ºC na câmara de Demanda Bioquímica de Oxigênio (DBO) (NL-41-01 A, NewLab, Piracicaba, SP, Brasil) e fotoperíodo de 12 horas (ciclo claro/escuro), com o intuito de simular as condições de condicionamento do mercado. A umidade relativa e a temperatura da câmara foram monitoradas (65,3 ± 4,6 % e 20,7 ± 0,3 °C), respectivamente. O período de armazenamento foi de noventa dias e o suco foi avaliado nos dias 1, 7, 14, 21, 30, 60 e 90. Em cada dia de análise foi retirado quatro garrafas de 100 mL contendo o suco e homogeneizados em um béquer. Uma alíquota era retirada para as análises físico-químicas, e o restante do suco foi acondicionado em tubos falcons de 15 e 50 mL e congelados (-18 ºC) para análises posteriores. Na noite anterior às análises, as amostras de suco foram descongeladas em uma geladeira (4 ° C por 12 horas).

4.1.3.3 Suco clarificado por enzima e por microfiltração, e pasteurizado

Neste suco, foi realizado o mesmo procedimento do item anterior, entretanto, com a adição do processo de microfiltração (MF). A clarificação por enzimas foi utilizada como pré-tratamento para remoção dos sólidos em suspensão e clarificação do suco. Após etapa de filtração em tecido de organza, como descrito anteriormente, o suco passou pela etapa de microfiltração. Para a MF, utilizou-se uma membrana de poliamida (PAM Membranas Seletivas, Rio de Janeiro, RJ, Brasil) com um diâmetro de poro médio 0,4 μm e uma área filtrante de 0,7 m^2. Foram utilizadas condições fixas de 20 ± 2 °C e pressão de 3 bar.

Após a MF, o suco foi pasteurizado sob as mesmas condições descritas (90 °C por 1 min, seguido pelo resfriamento a 20 °C) e todas as etapas após a pasteurização e o método de armazenamento foram procedidas da mesma forma que a etapa descrita anteriormente na seção 4.1.3.2.

4.1.4 Resíduo do processamento da goiaba serrana

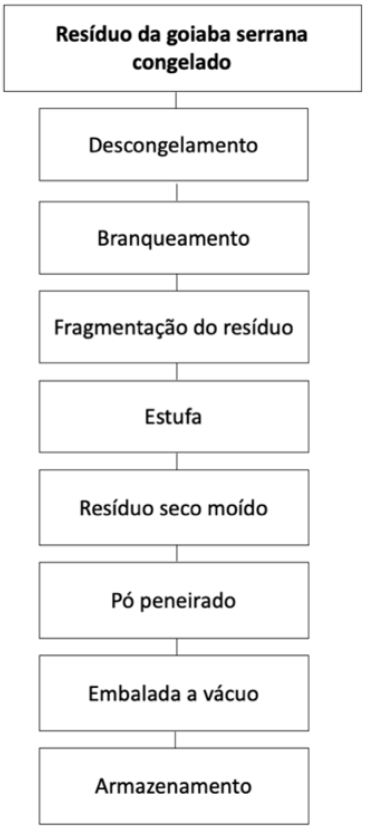
4.1.4.1 Material

O resíduo da goiaba serrana (*Acca sellowiana* (O. Berg) Burret) foi proveniente da produção de suco conforme Schmidt et al. (2021). Após a produção do suco da fruta, o resíduo foi acondicionado em embalagens plásticas a vácuo (FASTVAC®/F200 flash) e armazenadas em freezer a -18 °C até a utilização.

4.1.4.2 Obtenção da farinha

Para a elaboração da farinha, foi seguida a metodologia proposta por Almeida et al. (2020) com algumas modificações, como mostrado na Figura 2. Os resíduos da goiaba serrana foram descongelados sob refrigeração durante a noite. Após, foi realizado o branqueamento a 100 °C por 2 minutos seguido de resfriamento com água e gelo. Os resíduos foram cortados manualmente em pequenos pedaços de 2 x 2 cm e secas em estufa com circulação de ar forçada *(*DeLeo, B4AFD, Brazi*l)* a 55 °C durante 16 horas. A casca depois de seca foi moída em Moinho de facas *(*Solab, modelo SL – 31, Brasil), e o pó obtido foi peneirado em peneira de 35 mesh. A farinha foi então embalada à vácuo em sacos plásticos e armazenada sob refrigeração a 5 °C para análises posteriores.

Figura 2. Fluxograma adotado para obtenção da farinha do resíduo da goiaba serrana (*Acca sellowiana*)



4.1.5 Reagentes e padrões

Acetato de sódio, fosfato de sódio monobásico e bibásico, fosfato de potássio monobásico e bibásico, hidróxido de sódio, citrato de sódio, carbonato de sódio, ferrocianeto de sódio, ferrocianeto de potássio, acetato de zinco, sulfato de cobre, sulfato de sódio e tartarato de sódio e potássio foram obtidos da Neon Comercial® (São Paulo, Brasil). Ácido clorídrico, ácido fórmico, ácido sulfúrico suprapuro, ácido ortofosfórico 85%, ácido cítrico, acetona, álcool etílico, éter de petróleo, éter etílico, peróxido de hidrogênio (H_2O_2), D-glicose anidra, D-frutose, sacarose, foram obtidos pela Dinâmica Química Contemporânea® (São Paulo, Brasil). Reagentes grau HPLC como éter terc-metil-butílico (MTBE), acetonitrila, e metanol foram obtidos da Panreac® (Barcelona, Espanha).

O radical 2,2'-azino-bis-(ácido 3-etilbenzotiazolina-6-sulfonico) (ABTS), Trolox (±)-6-Hydroxy-2,5,7,8-tetrametillcromane-2-carboxilico acid), AAPH (2,2' Azobis (2 amidinopropane) dihydrochlorine), fluoresceína sódica, padrões de ácidos fenólicos como: gálico, clorogênico, catequina, epicatequina, galato de epigalocatequina, luteolina, miricetina, quercetina, resveratrol, ácido 3,5-dinitrosalicílico (DNS), pectina de maçã (ID 76282), ácido poligalacturônico e galacturônico foram obtidos da Sigma-Aldrich® (St Louis, Estados Unidos). Padrões de carotenoides como (all-*E*)-α-caroteno e zeaxantina foram obtidos da Fluka Analytical® (Munique, Alemanha) e de luteína da Indofine Chemical Company® (Hillsborough Township, Nova Jersey, EUA). Padrão de ácido ascórbico foi obtido pela Neon Comercial® (São Paulo, Brasil).

A água foi purificada pelo sistema Milli-Q® (modelo Integral 10). As amostras e solventes foram filtrados por membranas Millipore 0,45µm (Millex LCR 0,45 µm, 13 mm) antes de serem injetadas no cromatógrafo.

4.2 Metodologia

4.2.1 Análises físico-químicas

4.2.1.1 Rendimento

Os rendimentos dos sucos foram calculados de acordo com a Equação 1:

Eq. (1) $$\%\, Suco = \frac{massa\ do\ suco}{massa\ inicial\ da\ fruta} \times\ 100$$

4.2.1.2 Atividade Enzimática

As atividades de pectinase total (PE) e poligalacturonase (PG) foram determinadas de acordo com Dal Magro et al. (2016) e a quantidade de grupos redutores foi estimada pelo método do ácido 3,5-dinitrosalicíclico (DNS) de acordo com Miller (1959).

4.2.1.3 Potencial hidrogeniônico (pH) e acidez

Para o suco e a farinha, o pH foi determinado por método eletrométrico potenciométrico com pH-metro (Quimis®, modelo Q400AS, São Paulo, Brasil). A acidez para as amostras dos sucos e da farinha foram determinados por titulação com NaOH (0,1 N) (INSTITUTO ADOLFO LUTZ, 2008). Todas as medidas foram realizadas em triplicata.

4.2.1.4 Sólidos solúveis totais

As leituras do grau Brix foram feitas por refratometria, utilizando o refratômetro digital (ATAGO®, *pocket* 1-877, EUA), corrigido para 20° C. O aparelho foi calibrado a temperatura ambiente com água destilada (Índice de refração = 1,3330 e 0º Brix a 20ºC) e a leituras das amostras em triplicata (INSTITUTO ADOLFO LUTZ, 2008).

4.2.1.5 Açúcares redutores em glicose

Os valores de açúcares redutores em glicose, expressos em porcentagem de glicose, por reação de Fehling, foram determinados conforme o Instituto Adolfo Lutz (2008).

4.2.1.6 Análise de cor

A cor dos sucos e da farinha foram determinados com colorímetro (CR-400, Minolta Co. Ltd., Osaka, Japan) operando com iluminante C, e parâmteros de cor do sistema CIELAB. Os parâmetros utilizados foram L * (luminosidade), a * (variando de verde a vermelho) e b * (variando de azul a amarelo). Um disco branco foi usado como padrão (L0*: 91.52; a0*: -0.94; b0*: -1.12). As diferenças totais de cores (ΔE) das amostras serão calculadas utilizando a equação 2:

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

Onde: $\Delta L^* = L^* - L_0^*$, $\Delta a^* = a^* - a_0^*$ e $\Delta b^* = b^* - b_0^*$, onde L_0^*, a_0^* e b_0^* são os valores da cor do padrão L*, a* e b*.

Os valores do ângulo de Hue e do Croma indicam a tonalidade da amostra (0 ou 360 = vermelho, 90 = amarelo, 180 = verde, e 270 = azul) e a intensidade da saturação da cor, respectivamente. Eles foram calculados usando as Equações (3) e (4):

Eq. (3) $Hue = tan^{-1}(b^*/a^*)$

Eq. (4) $Chroma = [(a^*)^2 + (b^*)^2]^{1/2}$

Onde: a* e b* são parâmetros do sistema CIELAB.

4.2.1.7 Caracterização Reológica

A análise do comportamento reológico dos sucos foi feita em Reômetro (HAAKE Mars III; ThermoScientific, Alemanha), seguindo metodologia de De Castilhos et al. (2017). Foi utilizada geometria de cilindros concêntricos de 26 mm de diâmetro e temperatura de 20 °C. As curvas de viscosidade foram obtidas variando-se a taxa de deformação de 100 a 400 s^{-1}. A viscosidade aparente foi calculada a uma taxa de cisalhamento de 100 s^{-1}. Os modelos testados para os ajustes das curvas foram Newton e Ostwald-de Waele.

4.2.1.8 Medição do tamanho de partículas

A distribuição do tamanho das partículas foi determinada por difração a laser usando um Malvern Mastersizer (Mastersizer 3000, Malvern Instruments Limited, Reino Unido). Este analisador de tamanho de partículas pode fornecer tamanho de partículas distribuição de 10 nm a 3500 μm. Os valores 1,73 e 1,33 foram usados para os índices de refração de partículas de nuvens e fase de dispersão, respectivamente, e 0,1 foi usado para o índice de absorção de partículas de nuvens.

O diâmetro médio ponderado pelo volume foi expresso como diâmetro D [4,3], que é o diâmetro médio ponderado do volume, assumindo partículas esféricas com o mesmo volume que as partículas reais. Para calcular a distribuição do tamanho de partícula Equação 5 foi utilizado:

Eq. (5) $Span = \frac{d(0,9) - d(0,1)}{d(0,5)}$

Onde d (0,9), d (0,1) e d (0,5) são os diâmetros em 90, 10 e 50 % da curva de distribuição cumulativa de tamanho, respectivamente.

4.2.1.9 Composição centesimal

Todas as análises de composição centesimal da farinha foram realizadas de acordo com os procedimentos da AOAC (AOAC, 2012). A umidade foi determinada por gravimetria em estufa de ar forçado a 105 °C (DeLeo, Porto Alegre, Brasil), e as cinzas quantificadas por incineração em mufla a 550 °C (Linn High Therm, Elektro, Eschenfelden, Alemanha). O teor de proteína foi determinado pelo método Kjeldahl, usando um fator de conversão de nitrogênio para proteínas da farinha de trigo de 5,70. O concentrado lipídico foi mensurado por Soxhlet.

O conteúdo de carboidratos foi calculado pela diferença entre a porcentagem total de umidade, cinzas, proteínas, lipídios e fibra total. O valor de energia total foi calculado usando os fatores de conversão de nutrientes: 4 $kcal.g^{-1}$ de proteína, 4 $kcal.g^{-1}$ de conteúdo de carboidratos e 9 $kcal.g^{-1}$ de conteúdo de lipídios. Energia (kcal) = 4 × (g proteína + g carboidrato) + 9 × (g lipídeo) (peso fresco).

A fibra alimentar total e a fibra alimentar insolúvel foram determinadas pelo método enzimático gravimétrico, de acordo com o AOAC. A fibra alimentar total foi determinada com base na matéria seca. Para a digestão enzimática, as amostras foram aquecidas a 100 °C com α-amilase e incubadas a 60 °C com as enzimas protease e amiloglucosidase. A fibra alimentar solúvel foi calculada como a soma das frações total e insolúvel. Todas as medições foram realizadas em triplicata.

4.2.1.10 Propriedades funcionais

A solubilidade foi determinada de acordo com Cano-Chauca et al.(2005) . Foram adicionados 50 ml de água destilada a uma quantidade de 0,5 g de amostra em um tubo de centrífuga e a mistura foi homogeneizada em Vórtex (Multifunctional vortex K40-1020, Kasvi) por 1 minuto e posteriormente centrifugada (Hettich® MIKRO, 200R, Germany) a 3000 g por 5 minutos. Uma alíquota de 25 ml foi removida do sobrenadante e transferida para uma cápsula de porcelana previamente tarada e então armazenada em estufa a 105 °C por 24 horas. A solubilidade foi calcula pela diferença de peso.

As capacidades de retenção de óleo e água foram feitas segundo metodologia de Fernández-López *et al.*(2009). Um volume de 30 ml de água/óleo de girassol foi adicionado a 1 grama de amostra. A suspensão foi homogeneizada em Vórtex por 1 minuto e deixada em repouso em temperatura ambiente durante 24 horas. Foi então centrifugada a 3000 *g* por 20 minutos, o sobrenadante removido e o resíduo pesado.

A atividade de água (a_w) foi medida em aparelho medidor de atividade de água portátil Pre AquaLab Water Activity Analyser (Meter Group Inc, WA, USA).

4.2.2 Açúcares individuais e totais

Açúcares foram extraídos do suco da goiaba serrana, de acordo com o método descrito por Petkovsek; Stampar; Veberic, (2007). Para a extração de açúcares, 1 mL da amostra foram homogeneizados com Ultra-Turrax® (IKA, T25 digital) em 10 mL de etanol 80 %. Após a extração, as amostras foram centrifugadas a 20.000 *g* (Hitach CR21 GIII- HIMAC), por 15 minutos a 10 ºC. O sobrenadante foi filtrado por membranas Millipore 0,45µm (Millex LCR 0,45 µm, 13 mm). As amostras foram analisadas por cromatografia líquida de alta eficiência (CLAE) Waters Alliance 2695® (Milford, EUA) conectado a um detector de índice de refração (2414). Foi utilizada uma coluna Aminex® HPX-87H, 300 mm x 7,8 mm (Bio-Rad Laboratories Inc, Hercules, Califórnia, Estados Unidos). As condições cromatográficas foram as descritas por Mikulic-Petkovsek, et al. (2007) com adaptações: a fase móvel foi isocrática, com água MilliQ® e ácido sulfúrico 0,005 M, com fluxo de 0,5 $mL.min^{-1}$, volume de injeção de 20 µL e o tempo de corrida total foi de 30 minutos. O teor de açúcares individuais foi quantificado a partir de curvas analíticas de sete pontos de glicose (0,5-3,5 $g.L^{-1}$), frutose e sacarose (0,1-2,0 $g.L^{-1}$). Todas as curvas analíticas foram lineares (R^2=0,99). O limite de detecção (LOD) e o limite de quantificação foram respectivamente, 0,22 e 0,67 $g.L^{-1}$ para glicose; 0,10 e 0,29 $g.L^{-1}$ para frutose e 0,32 e 0,97 $g.L^{-1}$ para sacarose.

4.2.3 Vitamina C

A determinação de vitamina C foi baseada na metodologia proposta por Rosa *et al.*, (2007) com algumas modificações. Para os sucos foi utilizada amostra de 5 mL, e para a farinha 2 gramas, ambas homogeneizadas em um Ultra-Turrax® (IKA, T25 digital) com 20 mL de ácido sulfúrico 0,05 M a 96%, por 1 minuto, centrifugado (centrífuga

Hitach CR21 GIII- HIMAC) a 25.400 *g* durante 15 minutos e, em seguida, filtrada através de uma unidade de filtro hidrofílico de teflon.

As amostras foram analisadas por HPLC utilizando um cromatógrafo Waters Alliance 2695® (Milford, EUA) conectado a um detector de arranjo diodos (DAD 2996). Foi utilizada uma coluna polimérica C_{18} (250 mm x 4,6 mm i.d., 5 µm). A fase móvel foi de ácido sulfúrico 0,05 M, com fluxo de 1,0 $mL.min^{-1}$, volume de injeção de 10 µL e comprimento de onda de 254 nm. A vitamina C foi quantificada pela injeção do padrão de ácido ascórbico no dia da análise.

4.2.4 Compostos bioativos

4.2.4.1 Compostos fenólicos

A extração dos compostos fenólicos extraíveis seguiu a metodologia descrita por Rodrigues; Mariutti; Mercadante, (2013), com modificações. Para os sucos 5 mL da amostra foram centrifugados (Hitachi, Himac, CR21 GIII, Japão) a 30.000 *g* durante 15 minutos a 10 °C e o sobrenadante foi transferido para um frasco âmbar. Para a farinha, uma extração exaustiva por 2 minutos no Ultra-turrax (IKA®, T25 digital 1/min x 1000) adicionando-se 20 mL de uma mistura de metanol com água destilada (80:20%; v/v) à 0,4 g da amostra à temperatura ambiente foi realizada. Após o extrato foi centrifugado a 10.000 *g* durante 10 minutos a 20° C e o sobrenadante transferido para um balão volumétrico âmbar. Esse procedimento foi realizado em triplicata e repetido até a ausência de cor e os sobrenadantes combinados. Para a quantificação dos compostos fenólicos, os extratos foram rotaevaporados (Q334.2, Quimis®, Diadema, São Paulo, Brasil) e avolumados com a fase móvel A (água MilliQ® e ácido fórmico, na proporção de 99,5: 0,5%, v/v) para 25 mL em balão volumétrico.

A fração dos compostos fenólicos não extraíveis da farinha foi obtida a partir da hidrólise ácida do produto sólido (pellet), gerado na extração dos compostos fenólicos extraíveis. O método foi adaptado de Pérez-Ramírez et al. (2018). O pellet foi adicionado a 20 mL de metanol acidificado com HCl (15%, v/v) por 15 min em banho-maria a 90°C. O extrato foi mantido por 10 min em temperatura ambiente e depois centrifugado a 3000g por 5 min (4°C). O sobrenadante foi transferido para um frasco de fundo plano e o procedimento repetido. Após a segunda hidrólise, o pellet foi extraído com metanol 6 vezes para a extração exaustiva. Os extratos foram combinados e o metanol foi removido em um evaporador rotativo; o extrato quase seco foi ressuspenso em 5 mL de água.

Após a extração, as amostras do suco e da farinha foram filtradas por membranas Millipore 0,45μm (Millex LCR 0,45 μm, 13 mm) e injetadas em um cromatógrafo.

A ausência de compostos fenólicos no sobrenadante dos extratos foi verificada previamente utilizando o reagente Folin-Ciocalteau, seguindo a metodologia proposta por Becatti *et al.* (2010), com modificações. Foram adicionados 1375 μL do reagente de Folin-Ciocalteu (Sigma-Aldrich Chemical®, St. Louis, EUA) preparado (375 mL de água para 37,5 mL do reagente) à 25 μL da amostra, que reagiram por 8 minutos. Em seguida, adicionados 600 μL de água destilada e 500 μL de carbonato de sódio a 20%. A solução foi homogeneizada e deixada em repouso durante 30 min ao abrigo da luz. A absorbância foi lida a 750 nm à temperatura ambiente no espectrofotômetro (Shimadzu UV-Vis 1800/08302).

Os extratos foram purificados conforme descrito por Rodriguez-Saona; Wrolstad, (2001) Rodriguez-Saona & Wrolstad (2001) com algumas modificações. Os cartuchos de extração em fase sólida (SPE) C_{18} (Strata, Phenomenex, Torrance, CA) foram previamente condicionados com metanol e água acidificada com HCl 0,01 %. O cartucho foi carregado com a amostra. Os compostos polares foram eluídos com dois volumes de solução aquosa com HCl 0,01 %. Os compostos fenólicos eluídos usando acetato de etila. Os extratos foram concentrados em rotaevaporador rotativo (36 °C), e as amostras foram reconstituídas em 1 mL da fase móvel A (água MilliQ® e ácido fórmico, na proporção de 99,9: 0,1%, v/v). Após as amostras foram imediatamente injetadas no aparelho HPLC-DAD-MS/MS.

A identificação dos compostos fenólicos foi realizada em cromatógrafo HPLC (Shimadzu®, Kyoto, Japão), equipado com duas bombas (Shimadzu LC-20AD), um desgaseificador (Shimadzu DGU-20A) e forno de coluna (Shimadzu CTO-20A), conectado em série a um detector de arranjo de diodos (Shimadzu SPD-20A) e conectado a um espectrofotometro de massas (MS) com analisador quadrupolo por tempo de voo (Q-Tof) e fonte de ionização por eletropray (ESI) (Bruker Daltonics, modelo micrOTOF-QIII, Bremen, Alemanha). A coluna utilizada foi C_{18} LiChrospher® 100 RP, 150 x 3,2 mm, 5μm (Merck®, Darmstadt, Alemanha).

A fase móvel A constituída por uma mistura de água Milli-Q® e ácido fórmico (99,9:0,1 %, v/v) e a fase móvel B por uma mistura de acetonitrila e ácido fórmico, nas mesmas proporções (99,9:0,1 %, v/v), em um gradiente linear de 99:1 (v/v) fase móvel A/B para 50:50 (v/v) A/B por 50 minutos, e então 50:50 (v/v) A/B para 1:99 (v/v) A/B por 5 minutos. Essa razão foi mantida por mais 5 minutos. O tempo total de corrida foi

de 67 minutos e a taxa de fluxo da corrida foi de 0,7 mL.min^{-1} a 29 °C e o volume de injeção de 5 μL. Os espectros UV-Vis foram obtidos entre 200 e 600 nm, e os cromatogramas processados a 280, 320, 360 nm e 520 nm. Os espectros de massas foram adquiridos com uma varredura de m/z 100 a 1000. Os parâmetros de MS foram definidos da seguinte forma: fonte de ESI em modos positivo e negativo; tensão capilar, 3000 V; temperatura do dry gas (N_2), 310 °C; taxa de fluxo, 8 L.min^{-1}; gás de nebulização, 30 psi. MS^2 foi configurado no modo automático.

Os compostos fenólicos foram identificados com base na ordem de eluição e no tempo de retenção na coluna; características do espectro UV-Vis e de MS em comparação com padrões analisados nas mesmas condições e; dados disponíveis na literatura. Além disso, os compostos foram quantificados por cromatógrafo Waters Alliance 2695® (Milford, EUA) conectado a um detector de arranjo diodos (DAD 2996), sob as mesmas condições cromatográficas descritas no parágrafo anterior e utilizando uma curva analítica de sete pontos nas faixas mostradas na Tabela 1.

4.2.4.2 Carotenoides

O extrato de carotenoides foi preparado de acordo com Rodriguez-Amaya (2001), com algumas modificações. Para os sucos foi adicionado à um tubo falcon 3 mL da amostra, e realizado o particionamento com éter de petróleo (1 mL) e éter etílico (1 mL). As amostras foram homogeneizadas em vórtex (Multifunctional vortex K40-1020, Kasvi) por 1 minuto e centrifugados (Hitachi, Himac, CR21 GIII, Japão) a 25.000 *g* por 15 minutos a 5 ºC. O sobrenadante foi transferido para um frasco âmbar e o processo foi repetido mais uma vez (até a ausência de cor). A seguir, os sobrenadantes foram combinados e o extrato foi seco em um fluxo de nitrogênio.

Para a farinha as etapas principais foram: extração dos pigmentos com 3g de amostra e 30 mL de acetona homogeneizados por 1 minuto em Ultra-turrax (IKA®, T25 digital 1/min x 1000); e saponificação com metanol 10% KOH durante uma noite à temperatura ambiente. Após a remoção do álcali, o extrato foi concentrado em evaporador rotativo (Q334.2, Quimis®, Diadema, São Paulo, Brasil) (T<25 ºC) e seco em um fluxo de nitrogênio.

Os extratos do suco e da farinha foram armazenados no freezer (-18 °C) para posterior quantificação por cromatografia líquida de alta eficiência (CLAE). Para a análise, ambos os extratos concentrados foram diluídos em éter terc-metil-butílico

(MTBE), colocado em ultrassom (Unique, modelo USC 1400A) por 5 minutos e filtrado em filtro (Millex LCR 0,45 μm, 13 mm) para posterior injeção no cromatógrafo.

As análises foram realizadas em um cromatógrafo Agilent® (série 1100, Santa Clara, CA, EUA), equipado com um sistema solvente quaternário de bombeamento (G1311A – DE14917573 Agilent 1100 Series, Waldbronn, Alemanha) e um detector UV-Visível (G1314B – DE71358944 Agilent 1200 Series, Waldbronn, Alemanha). A coluna usada foi de 250 mm x 4,6 mm ID, 3 μm, de fase reversa C30 polimérica (YMC, modelo CT99SO3-2546WT). A fase móvel constitui um gradiente de eluição inicial de água:metanol:MTBE de 5:90:5, em 12 min a concentração foi para 0:95:5; em 25 min, 0:89:11, em 40 minutos foi para 0:75:25, e, finalmente, 00:50:50 em 60 min, com uma taxa de fluxo de 1 mL.min-1 a 22 ºC e volume de injeção de 5 μL. Os espectros foram obtidos entre 250 e 600 nm e os cromatogramas transformados em um comprimento de onda fixo de 450 nm para carotenoides.

Para a farinha do resíduo da goiaba serrana, a confirmação dos carotenoides identificados foi realizada na análise por HPLC-DAD-MS/MS nas mesmas condições descritas por Rodrigues et al (2013) com algumas modificações. Foi utilizada a mesma coluna descrita anteriormente, entretanto, com vazão de 0,9 $mL.min^{-1}$, volume de injeção de 20 μL e forno temperatura a 29 ºC. Utilizou-se um gradiente linear a partir da mistura de metanol-MTBE 95:5 (v/v) como fase móvel, atingindo 70:30 ao longo de 30 min, seguido de 50:50 ao longo de 20 min e mantendo essa proporção por 10 min. Aos 62 minutos, a condição inicial foi restabelecida e permaneceu por mais 5 minutos. Os espectros foram medidos entre 200 e 600 nm e os cromatogramas foram processados a 450 nm.

O eluato da coluna foi injetado diretamente em uma fonte de ionização química por pressão atmosférica (APCI), e os parâmetros do espectrômetro de massa seguiram as mesmas condições descritas anteriormente por de Rosso e Mercadante (2007). Os parâmetros do espectrômetro de massa foram definidos da seguinte forma: modo positivo, corona atual 4.000 nA, temperatura da fonte a 450 ºC, gás N2 (White Martins SA, Porto Alegre, Brasil), grau cromatográfico) com temperatura de 350 ºC e vazão de 4 L.min-1, nebulizador a 60 psi. Os experimentos MS/MS foram realizados no modo automático, com energia de fragmentação de 1,4 V. Os espectros de massa foram adquiridos com faixa de varredura de m/z de 100 a 2.200.

Para esta análise, foi utilizado um cromatógrafo (Shimadzu, Kyoto, Japão) equipado com duas bombas (Shimadzu LC-20AD), um desgaseificador on-line

(Shimadzu DGU- 20A3R), forno de coluna (Shimadzu CTO-20A), detector de matriz de diodos (Shimadzu SPDM20A) conectado em série a um espectrômetro de massa com uma fonte de ionização química por pressão atmosférica (APCI) e um analisador de captura de íons (modelo micrOTOF-QIII, Esquire 4000, Bruker Daltonics, Bremen, Alemanha).

A identificação e quantificação foi efetuada por comparação dos tempos de retenção dos picos da amostra e seus respectivos padrões, sob as mesmas condições cromatográficas. Os carotenoides foram identificados com base na ordem de eluição, características espectrais UV-Vis [comprimento de onda de absorção máxima (λmax), estrutura fina espectral (% III/II) e intensidade cis máxima (% AB/AII)] e características do espectro de espectrometria de massa. Além disso, esses parâmetros foram comparados aos dados disponíveis na literatura. Os cromatogramas foram processados a 450 nm. Para a quantificação, foi construída uma curva padrão para carotenoides nas faixas mostradas na Tabela 1.

Tabela 1. Faixas de concentração, coeficiente de correlação (R^2), limites de detecção (LOD) e limites de quantificação (LOQ) dos padrões de carotenoides e fenólicos.

Composto Bioativo	Faixa de concentração ($\mu g.mL^{-1}$)	Coeficiente de correlação (R^2)	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
Luteína	1-65	0,9991	$6,90 \times 10^{-3}$	$1,15 \times 10^{-2}$
Zeaxantina	1-40	0,9997	$9,56 \times 10^{-2}$	$1,59 \times 10^{-2}$
Criptoxantina	3-100	0,9911	$2,11 \times 10^{-2}$	$3,51 \times 10^{-2}$
α-caroteno	2-25	0,9934	$1,97 \times 10^{-2}$	$3,28 \times 10^{-2}$
β-caroteno	5-50	0,9998	$6,53 \times 10^{-2}$	$10,89 \times 10^{-2}$
Licopeno	10-100	0,9977	$7,0 \times 10^{-3}$	$33,0 \times 10^{-3}$
Ácido gálico	1-120	0,9939	0,30	0,90
Ácido caféico	1-120	0,9976	0,44	1,33
Rutina	1-120	0,9935	0,37	1,12

4.2.5 Determinação da capacidade antioxidante

4.2.5.1 Ensaio ABTS

A determinação da capacidade antioxidante total do suco e da farinha foi realizada pela captura do radical livre ABTS segundo a metodologia utilizada por Rufino *et al.* (2007). Para o extrato utilizou-se 1,5 mL de amostra de suco e 1 grama da farinha, adicionados a 20 mL de metanol 50%, homogeneizado em turrax e deixado em repouso por 60 minutos em ambiente escuro. Após o extrato foi centrifugado a 25.400 *g* por 15 minutos e o sobrenadante armazenado em balão âmbar de 50 mL. Esse processo foi

repetido com acetona 70% substituindo o metanol. O balão foi avolumado para 50 mL. A partir do extrato obtido foram preparados tubos de ensaio, com no mínimo, três diluições diferentes, em triplicata. Em ambiente escuro, foi transferido uma alíquota de 100 µL de cada diluição do extrato para tubos de ensaio com 1,0 mL do radical $ABTS^{\cdot+}$, os tubos foram homogeneizados e a leitura foi realizada a 734 nm em espectrofotômetro (Shimadzu UV-1800) após 6 minutos da mistura.

4.2.5.2 Ensaio ORAC

A atividade antioxidante dos sucos e da farinha foram determinados pelo ensaio ORAC (HUANG; BOXIN; PRIOR, 2005), para isto, foram realizados testes de concentrações previamente para ambas as amostras. Os sucos foram diluídos em uma concentração de 800 ppm com tampão fosfato de potássio. Em seguida, as amostras foram homogeneizadas em vórtex (Multifunctional vortex K40-1020, Kasvi) por 1 minuto, seguido pelo Ultrassom (Unique, modelo USC 1400A) por 3 minutos. Para a farinha, foi utilizado o extrato dos compostos fenólicos extraíveis e não extraíveis

O método ORAC verifica a capacidade sequestradora de um antioxidante frente a um radical peroxila induzido pelo AAPH a 37 ºC. Em cada poço de microplaca foram adicionados 25 uL dos extratos previamente diluídos em tampão fosfato de potássio 75 mM e 150 uL da solução de trabalho de fluoresceína (81nM). A placa foi incubada por 10 min a 37 °C, sendo os últimos 3 minutos sob agitação constante. Após, foram adicionados 25 uL da solução de AAPH (152 mM). Para o monitoramento do decaimento da fluorescência foi utilizado um leitor de fluorescência (Enspire 2300, Multimode Plate Reader, Perkin Elmer, USA) a 37 °C por 90 minutos ou até atingir menos de 0,5 % do valor inicial. Foram usados comprimentos de onda de excitação e emissão de 485 nm e 528 nm, respectivamente.

Após, para os cálculos, foi calculada a área sob a curva (AUC) e o valor do branco (sem antioxidante) foi subtraído para se obter a AUC líquida. A AUC é dada pela seguinte equação (6):

Eq. (6) $$AUC = 1 + {}^{f1}/_{f0} + {}^{f2}/_{f0} + {}^{f3}/_{f0} + \cdots {}^{fn}/_{f0}$$

Onde:

fn = fluorecência relativa em um ciclo de leitura (1 min)

$f0$ = fluorescência no tempo zero

Foi confeccionada uma curva padrão com trolox (0 – 96 uM) e os resultados expressos como μMol de equivalentes de Trolox por 100 mL de amostra.

Para a determinação da capacidade antioxidante e dos compostos bioativos, as análises foram realizadas sob temperatura controlada (22 ± 3 °C) e ambiente escuro.

4.3 Análise estatística

Todas as análises foram realizadas em triplicata e os resultados expressos em média ± desvio padrão. A análise estatística foi realizada utilizando ANOVA, com teste de comparação das médias pelo teste de Tukey ao nível de 5 % de significância, assim, em todos os casos foram considerados diferenças significativas quando $p \leq 0,05$. Todas as análises estatísticas foram efetuadas utilizando o software Statistica 12.0 (Statsoft Inc., Tulsa, USA).

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CAPÍTULO 5

5 MANUSCRITOS

Manuscrito 1

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Effect of enzymatic treatments and microfiltration on the physicochemical quality parameters of feijoa (Acca sellowiana) juice

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ABSTRACT: Feijoa fruit (*Acca Sellowiana*) has a short postharvest period, thus, processing is usually an alternative. The objective of this work was to evaluate the efficiency of three different commercial enzyme preparations in the extraction of feijoa juice in the yield and quality parameters. The juices with Pectinex®, Lallzyme® and Rohapect® were evaluated under three different concentrations (1, 5 and 10 $U.mL^{-1}$) before and after the pulping process. The samples were valued for total soluble solids, pH, reducing sugars, titratable acidity and colour. The juice that showed the most promise was evaluated for the microfiltration stage and rheological behaviour and particle size distribution were measured. The three treatments with Pectinex had an increase in juice yield in 43, 55 and 60%, while the samples with enzymes Lallzyme and Rohapect had no increase in yield compared to the control. All the tested enzymes had a positive impact on the colour of the juice, improving the colour parameters. The microfiltered juice had a behaviour index value of 1,091, indicating Newtonian behaviour. The fresh juice showed values below one (0.2178), indicating a non-Newtonian and typically pseudoplastic behaviour. Among three enzymes used, Pectinex® showed the better results for physical-chemical parameters for extraction of feijoa juice.

Keywords: Cellulase; Enzymes; Microfiltration; Pectinase; Rheology; Yield.

1. INTRODUCTION

The fruit juice market represents a major impact on the economy of many countries and on human nutrition. In recent years, consumers have become more aware of the many health benefits, which leads to an increase in the consumption of healthy, high-quality products. Whole fruit juices have aroused interest, due to their nutritional properties, through the presence of vitamins, minerals and compounds with antioxidant activity, which promote nutritional benefits for health (EPHREM *et al.*, 2018b; GOULAS; MANGANARIS, 2012; TAPRE; JAIN, 2014). In addition, there is a fast-growing market for varied fruit juices, such as exotic fruit juice and locally produced juices (AMARAL *et al.*, 2019; PERSIC *et al.*, 2017).

The species *Acca sellowiana* (Berg) Burret, popularly known as feijoa, belongs to the Myrtaceae Family. Native to South America (Southern Brazil and Northeastern Uruguay), in Brazil, it is still little known, and commercial production is incipient. The fruit is popular in international markets, such as New Zealand, Australia and Italy, due to its organoleptic and nutraceutical characteristics, aromas and exotic flavors (AMARAL *et al.*, 2019; POODI *et al.*, 2018). The peel of the fruit, in addition to being soft, remains in a green tone regardless of its degree of ripeness, the white mesocarp, with juicy pulp around the seeds. Due to its bitter skin, it is not usually consumed with the pulp and its yield can vary from 15 to 50% (CASTELLANOS; HERRERA; HERRERA, 2016; MORETTO; NODARI; NODARI, 2014; TUNCEL; YILMAZ, 2015).

The fruit has a short postharvest period, from three to four weeks, under refrigeration, due to the high respiration rates and ethylene production, in addition to the high activity of the enzymes polyphenoloxidase, peroxidase and phenylalanine amonialiasis. Therefore, as the ripening progresses, physical-chemical changes occur, such as the darkening of the pulp, and losses in nutritional and sensory quality, which results in serious limitations to meet the demands of the fresh fruit market (AMARANTE *et al.*, 2017a; CASTELLANOS; POLANÍA; HERRERA, 2016; PARRA C; FISCHER, 2013). For this reason, fruit processing is often an alternative for agribusiness. The development of products from feijoa is promising, since sensory tests carried out with the fruit, showed that participants consider the taste and aroma of feijoa as good or great and interesting for gastronomic uses, such as juice production (AMARAL *et al.*, 2019; BARNI *et al.*, 2004).

In the industrial production of juices, some technological aspects are fundamental to obtain a quality product. Food processing should lead to minimal nutrient losses and the final product should have a value that covers the cost of processing with a profit margin (Karabagias *et al.*, 2020). The juice processing occurs immediately after harvest, and bleaching, enzymatic extraction, clarification, filtration, and pasteurization being typical steps in juice production and presenting different parameters according to the raw material (RAWSON *et al.*, 2011; SILVA; SULAIMAN, 2018). The extraction process is one of the most important steps in the production of juice, since it directly affects the quality of the product, and thus, some treatments can be employed in this step. Enzymatic treatments, containing pectinases, cellulases and hemicellulases can be applied in the process of obtaining the juice. The use of these enzymes in the production of juices has been extensively researched in the food industry and their use varies with the raw material. There are a large number of commercial pectinases and cellulases capable of being applied in the juice industry, and therefore it is important to evaluate the capacity of each preparation for correct use in the beverage industry (Tapre and Jain, 2014; Kumar, 2015; Dal Magro *et al.*, 2016).

Due to the importance of enzymatic treatment in industrial juice production, the objective of this work was to evaluate and compare the efficiency of different commercial enzyme preparations in the extraction of feijoa juice. After the enzymatic treatment, the juices were evaluated for their yield and quality parameters (total soluble solids, pH, reducing sugars, titratable acidity and colour). In addition, the juices that showed the most promise were evaluated for the microfiltration stage. Rheological behaviour and particle size distribution were also studied.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Chemical

Lallzyme Beta was from Lallemand Wine (France), Pectinex Ultra Clear were kindly donated by Novozymes (Spain) and Rohapect 10L was from Amazon group (Brazil). Pectin from apple (ID 76282), polygalacturonic acid, galacturonic acid and 3,5-dinitrosalicyclic acid (DNS) were from Sigma-Aldrich (St. Louis, MO). Analytical grade reagents as sodium acetate, sodium phosphate monobasic and bibasic, potassium phosphate monobasic and bibasic, sodium hydroxide, sodium carbonate, sodium sulphate, sodium citrate and citric acid

were obtained from Comercial Neon© (Sao Paulo, Brazil). Hydrochloric acid (HCl), 85% orthophosphoric acid, formic acid, acetone, ethanol, methyl alcohol and were obtained by Dinâmica Química Contemporânea® (Sao Paulo, Brazil). Water was purified by Milli-Q® system Integral 10 (Millipore, Milford, MA, EUA). The samples and solvents were filtered through Millipore membranes (Millex LCR 0.45 µm, 13 mm).

2.1.2 Sample

The feijoa fruit (*Acca sellowiana* (O. Berg) Burret) was harvested in the city of Sao Joaquim, SC (GPS coordinates: 28º16'34.47 "S and 49º56'9.57" W), in the south of Brazil. Approximately 80 kg the fruit was collected at ideal maturation stage, when the fruits can be easily removed from the plant, according to Amarante et al. (2017a), from April 2018. The fruits were transported under refrigeration at the Federal University of Rio Grande do Sul (UFRGS) and kept in a cold chamber (-18 °C) until juices processing. The exsiccates were harvested and deposited at the Institute of the Biosciences Herbarium /UFRGS - Institute of Natural Sciences number ICN 187148.

2.1.3 Enzymes

In the processing of feijoa juice, the addition of three commercial enzymes was verified. The Pectinex Ultra Clear and Rohapect 10 L are enzymes with greater pectinase and polygalacturonase activity, while, Lallzyme Beta also has activity cellulase (DAL MAGRO et al., 2016). The enzymes were tested in 3 concentrations (1, 5 and 10 $U.mL^{-1}$) before and after pulping, according to the literature. In the first addition of enzyme, before pulping (BP), the objective was to increase the juice extraction yield. In the second addition, after pulping (AP), the main purpose was to clarify the juice.

2.2 Methods

2.2.1 Preparation of feijoa juice

The fruits were selected visually and the damaged fruits were removed. Subsequently, the fruits were washed with water and sanitized by immersion in sodium hypochlorite solution (200 $mg.L^{-1}$ for 15 min) and a new washing with water was done to eliminate residual sodium hypochlorite. After washing, the fruits were bleached in a water bath (Dubnoff NT 232 – Novatecnica®, Piracicaba, SP, Brazil) at 80 °C for 2.5 minutes, and cooled to 10 °C. Afterwards, the enzymatic solutions, properly diluted, was

added to the fruits and incubated at 50 °C for 40 min, under agitation in a water bath, and then cooled to 20 °C. The fruits were pulped and homogenized in a vertical pulping machine (DES-20, Braesi®, Caxias do Sul, RS, Brazil).

Table 1. Enzymes applied for the extraction of feijoa juice: Pectinex Ultra Clear, Lallzyme Beta and Rohapect 10L and three concentrations (1, 5 and 10 $U.mL^{-1}$) used before pulping (BP) and before filtration (BF).

Treatments	Enzymes	Before the pulp (BP)	Before filtration (BF)
Control		C0	C0
1		C1	C1
2		C1	C5
3		C1	C10
4		C5	C1
5	Lallzyme beta	C5	C5
6		C5	C10
7		C10	C1
8		C10	C5
9		C10	C10
Control		C0	C0
10		C1	C1
11		C1	C5
12		C1	C10
13		C5	C1
14	Pectinex Ultra Clear	C5	C5
15		C5	C10
16		C10	C1
17		C10	C5
18		C10	C10
Control		C0	C0
19		C1	C1
20		C1	C5
21		C1	C10
22		C5	C1
23	Rohapect	C5	C5
24		C5	C10
25		C10	C1
26		C10	C5
27		C10	C10

C= concentration; C0= without enzyme; C1 =1 $U.mL^{-1}$; C5 =5 $U.mL^{-1}$, C10 =10 $U.mL^{-1}$

Once again, the enzymatic solutions were added to the extracted juice and incubated (at 50 °C for 40 min), under agitation, in a water bath, and followed by cooling to 20 °C. The enzymes (Pectinex Ultra Clear, Lallzyme Beta or Rohapect 10L) and their

respective concentrations (1, 5 and 10 U.mL^{-1}) used before pulping (BP) and after pulping (AP) are described in Table 1. The juice was filtered through organza fabric, was transferred to100 mL glass bottles (Pilfer 24, Flint, Envidro, Porto Alegre, RS, Brazil), pasteurized (1 min at 90 °C), and cooled to 20 °C. The juices were stored under a nitrogen atmosphere and then closed with a screw cap. For all treatments, feijoa juice yield (JY) was calculated according to the Equation (Eq). 1:

$$JY(\%) = \frac{mass\ of\ juice}{initial\ mass\ of\ feijoa} \times 100 \quad (1)$$

The most promising juices from each enzyme treatment were subjected to the microfiltration (MF) process, which occurred, shortly after the filtration step in the organza fabric. A polyamide membrane (PAM Selective Membranes, Rio de Janeiro, RJ, Brazil) with a pore diameter of 0.4 μm and a filtration area of 0.7 m² was used for MF. Fixed conditions of 20 ± 2 °C and 300 kPa were used during the experiment. After the MF step, the juice was pasteurized (1 min at 90 °C). The flowchart of the juices preparation is shown in Figure 1.

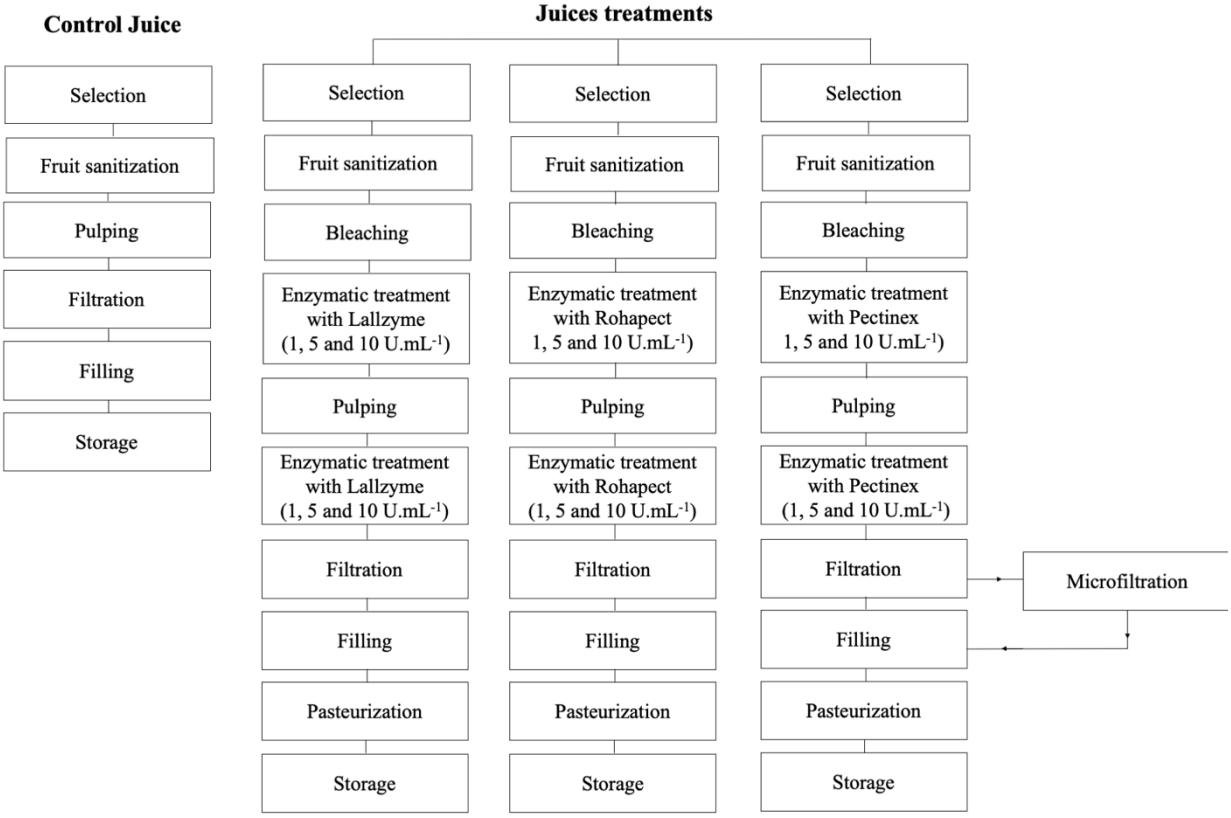

Figure 1. Flowchart for processing feijoa (*Acca Sellowaiana*) juice

For all juices experiments, a control juice (fresh juice) was implemented without enzyme. In the fresh juice, after washing, the fruits were pulped and homogenized in a vertical pulping machine. Afterward, the juice was filtered through organza fabric. The juice was transferred to 100 mL glass bottles stored under a nitrogen atmosphere and subsequently, they were closed.

2.2.2 Enzymatic Activities

Total pectinase (PE) activity were determined according to Dal Magro et al. (2016) and the amount of reducing groups was estimated by the 3,5-dinitrosalicyclic acid (DNS) method according to Miller (1959). One unit of PE and PG activity was defined as the amount of enzyme required to release 1 μmol of reducing groups, expressed as glucose, per minute under the reaction conditions.

2.2.3 Total Acidity, pH, Total Soluble Solids (°Brix) and Reducing Sugars

The total titrable acidity (TTA) was determined by the titration using sodium hydroxide (NaOH 0.1 N solution), and the results are expressed as % acidity according to the Instituto Adolfo Lutz (2008). The pH was performed by a potentiometric electrometric method with a pH-meter (Quimis®, model Q400AS, Sao Paulo, Brazil). The total soluble solids (TSS) was determined by using a portable digital refractometer (Atago® U.S.A, Pocket, Model Pal-3, Bellevue, WA, USA) and the TSS/TTA ratio was calculated. The values of glucose-reducing sugars, expressed as a percentage of glucose, by Fehling's reaction were determined (INSTITUTO ADOLFO LUTZ, 2008). All analyses were performed in triplicates.

2.2.4 Color parameters

Th color was performed by a Minolta Colorimeter (Model CR-400, Konica Minolta Sensing, Osaka, Japan) working with illuminant C, and the color parameters according to the CIELAB system. The parameters used were L^* (lightness; 0 = dark and 100 = white), a^* (component red-green; <a* = green and >a* = red) and b^* (component yellow-blue; <b* = blue and >b* = yellow). A white ceramic tile was used for calibration before carrying out any measurements. The hue angle (h^*) chroma (C^*) and ΔE were calculated following the Eqs. 2, 3 and 4 respectively:

$$h^* = \text{atan}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.2.5 Particle size distribution

Particle size distribution was measured with a Malvern Mastersizer (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK) to which a dry dispersion module Sirocco 2000 was attached, using the laser diffraction technique. The volume-weighted mean diameter was expressed as diameter D[4,3], which is the weighted average volume diameter, assuming spherical particles with the same volume as the actual particles. To calculate the particle size distribution Eq. (4) was used:

$$Span = (d(0.9) - d(0.1)/d(0.5) \quad (5)$$

Where d0.9, d0.1, and d0.5 are the diameters at 90, 10, and 50% of the cumulative size distribution curve, respectively.

2.2.6 Rheological Characterization

The analysis of the rheological behavior of the juices was carried out by a rheometer (HAAKE Mars III; ThermoScientific, Germany), following the methodology of Castilhos, Betiol, de Carvalho, & Telis-Romero, (2017). The geometry of concentric cylinders of 26 mm in diameter and temperature of 20 ° C was used. The viscosity curves were obtained by varying the strain rate from 100 to 400 s^{-1}. The apparent viscosity was calculated at a shear rate of 100 s^{-1}. The tested models for adjusting the curves were Newton (Eq. (5)) and Ostwald-de Waele (Eq. (6)).

$$\tau = \mu\gamma \quad (6)$$

$$\tau = K(\gamma)^n \quad (7)$$

The different values of η indicate the fluid behavior, i.e., η =1 for Newtonian, η < 1 for pseudoplastic fluid and η > 1 for dilatant fluid.

2.2.7 Statistical analysis

All experiments were performed in triplicates, and the results are expressed as the mean ± standard deviation. The results were analyzed by one-way analysis of variance (ANOVA) to evaluate the differences between samples and the Tukey test for means

comparison at 5% significance using Statistica 12.0 software (STATSOFT Inc.). The software OriginPro® 8.0 (Northampton, MA, USA) was used.

3. RESULTS AND DISCUSSION

Initially, the three enzyme preparations were evaluated regarding enzymatic activities (pectinase activity). Among the commercial enzyme preparations, Pectinex® Ultra Clear (Novozymes) has the highest total pectinase activity (6413.93 $U.mL^{-1}$), followed by Lallzyme Beta® (5949.35 $U.mL^{-1}$) and Rohapect 10L® (3554.15 $U.mL^{-1}$), Dal Magro et al. (2016) and Rojas-Garbanzo et al. (2019) had already reported, a similar result.

As for yield parameter, it was seen that the samples of the juices treated with the enzymes Lallzyme Beta and Rohapect 10L in the three concentrations (1, 5 e 10 $U.mL^{-1}$) before the pulping process, did not have a significant difference compared with the control sample ($p \geq 0.05$ data not shown), without increasing this parameter. However, for juices treated with Pectinex there was an increase in yield.

The highest yield (60.19%) was obtained by the treatment with Pectinex Ultra Clear in the highest concentration used (10 $U.mL^{-1}$), where there are statistical differences at 5% of significance. When comparing this sample with the control, 26.6 % increase in the amount of extracted juice was observed. Kaur, Sarkar, Sharma, & Singh, (2009) working with pectinase enzymes, obtained an increase in yield of 25 to 42% for guava juice. The results of the feijoa juice yield with the Pectinex enzyme are shown in Figure 2.

The results found can be explained by the good activity of the enzyme of the pectinolytic complex (polygalacturonase, pectin methyl esterase and pectinlyase) of the preparation with Pectinex (Dal Magro *et al.*, 2016). Furthermore, the lower yields presented by Lallzyme and Rohapect could also be explained by the low pectinylases content, that are responsible for catalysing the β elimination of the principal chain of the pectic polysaccharide, acting on pectin with a high degree of methylation (Kashyap *et al.*, 2001; Tapre and Jain, 2014; Dal Magro *et al.*, 2016).

The control juice had a yield of 33.5%. This value is in accordance with the literature, which finds variations of 15 to 50% for the yield of feijoa pulp (Tuncel and Yılmaz, 2015; Castellanos *et al.*, 2016a).

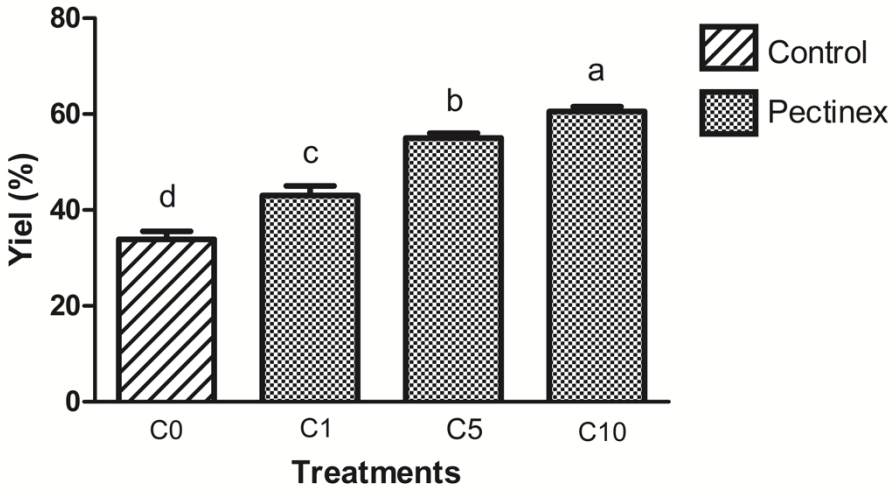

Figure 2. Yield of feijoa juice extraction using the commercial enzyme Pectinex® Ultra Clear in three different concentrations (1, 5 and 10 U.mL^{-1}). Means followed by different letters differ by Tukey's test at 5% probability.

Samples feijoa juice treated with Lallzyme were evaluated by pH, total titratable acidity (TTA), total soluble solids (TSS), reducing sugar and colour parameters, the yield after the filtration stage was also evaluated at concentrations (1, 5 and 10 U.mL^{-1}) and the results for nine treatments are presented in Table 2. It can be observed from Table 2 that, the yield after filtration was highest in treatment T6, in which 10 U.mL^{-1} of the enzyme was added and, consequently, this treatment was the one with the highest total yield, since treatments T4, T5 and T6 were the ones that had the highest yield of juice after pulping for the Lallzyme enzyme. It is seen that the treatments in which the lowest enzyme concentration was added, before the filtration process, were the ones with the lowest yield (T1, T4, T7). The treatments T2 and T3, as well as T8 and T9, showed similar yield after filtration, with no difference between the pairs, respectively, that is, the addition of double enzyme in T3 and T9 did not increase the juice yield after filtration, therefore, the concentration of 5 U.mL^{-1} added in T2 and T8 would be plenty.

Table 2. Analysis of pH, total acidity, total soluble solids, TSS/ TTA, reducing sugars and color parameters of feijoa juices prepared with Lallzyme Beta enzymatic preparations under different concentrations

Treatments	Yield AF[a] (%)	pH	TTA	TSS	TSS/TTA	Reducing sugar	*L**	*a**	*b**	ΔΣ	*C**	Hue (°)
Control	-	2.85 ± 0.04^{b}	1.84 ± 0.15^{e}	13.90 ± 0.00^{a}	7.60 ± 0.63^{a}	8.37 ± 0.78^{a}	32.18 ± 2.32^{e}	1.15 ± 0.01^{f}	7.21 ± 0.60^{f}	-	7.28 ± 0.57^{f}	80.71 ± 1.00^{a}
T1	66.67^{d}	2.60 ± 0.01^{c}	2.28 ± 0.06^{d}	9.07 ± 0.06^{e}	3.97 ± 0.12^{b}	5.31 ± 0.37^{bcd}	51.20 ± 1.56^{a}	4.47 ± 0.20^{e}	19.47 ± 0.21^{bc}	22.84 ± 1.35^{a}	19.91 ± 0.10^{bc}	77.32 ± 0.35^{b}
T2	70.83^{c}	2.59 ± 0.01^{c}	2.31 ± 0.07^{d}	9.03 ± 0.23^{e}	3.94 ± 0.24^{bc}	5.83 ± 0.53^{bc}	50.99 ± 1.00^{a}	4.47 ± 0.31^{e}	18.66 ± 0.25^{cd}	22.75 ± 0.67^{a}	19.29 ± 0.14^{cde}	77.21 ± 0.96^{b}
T3	71.67^{c}	2.59 ± 0.01^{c}	2.33 ± 0.11^{d}	8.97 ± 0.06^{e}	2.84 ± 0.21^{bc}	6.03 ± 0.38^{b}	50.50 ± 2.43^{ab}	4.52 ± 0.21^{e}	18.60 ± 0.40^{cd}	22.58 ± 1.05^{ab}	18.92 ± 0.31^{cde}	76.52 ± 0.26^{b}
T4	68.00^{cd}	2.57 ± 0.00^{c}	2.68 ± 0.08^{c}	8.87 ± 0.23^{e}	3.29 ± 0.21^{c}	4.40 ± 0.16^{d}	46.74 ± 0.20^{d}	5.32 ± 0.43^{d}	17.91 ± 0.49^{de}	18.31 ± 0.17^{cd}	18.46 ± 0.06^{de}	72.27 ± 1.35^{c}
T5	71.20^{c}	2.57 ± 0.01^{c}	2.64 ± 0.22^{c}	9.07 ± 0.06^{e}	3.44 ± 0.32^{c}	5.78 ± 0.19^{bc}	45.38 ± 0.15^{d}	6.08 ± 0.17^{bc}	17.30 ± 0.49^{e}	17.25 ± 0.06d	18.11 ± 0.02^{e}	70.48 ± 0.50^{d}
T6	79.20^{a}	2.55 ± 0.06^{c}	2.30 ± 0.01^{d}	9.00 ± 0.00^{e}	3.92 ± 0.02^{bc}	5.69 ± 0.15^{bc}	47.77 ± 0.23^{bcd}	5.57 ± 0.08^{cd}	18.57 ± 0.30^{cd}	19.63 ± 0.25c	19.40 ± 0.23^{cd}	73.16 ± 0.04^{c}
T7	69.10^{c}	2.94 ± 0.01^{a}	4.47 ± 0.19^{b}	9.60 ± 0.00^{b}	2.15 ± 0.09^{d}	5.46 ± 0.33^{bcd}	47.07 ± 0.08^{cd}	6.77 ± 0.01^{a}	19.79 ± 0.05^{b}	20.28 ± 0.09^{bc}	20.91 ± 0.04^{b}	71.12 ± 0.06^{d}
T8	75.32^{b}	2.89 ± 0.00^{ab}	4.42 ± 0.04^{b}	9.60 ± 0.00^{b}	2.17 ± 0.02^{d}	4.53 ± 0.02^{cd}	50.00 ± 0.06^{abc}	6.16 ± 0.01^{b}	21.65 ± 0.02^{a}	23.47 ± 0.06^{a}	22.50 ± 0.02^{a}	74.11 ± 0.02^{c}
T9	75.50^{b}	2.90 ± 0.01^{ab}	4.91 ± 0.07^{a}	9.47 ± 0.06^{b}	1.93 ± 0.02^{d}	4.79 ± 0.34^{cd}	45.75 ± 0.04^{d}	6.82 ± 0.01^{a}	18.83 ± 0.01^{bcd}	18.74 ± 0.03^{cd}	20.02 ± 0.00^{bc}	70.09 ± 0.04^{d}

[a]AF: yield after filtration. Values are the mean of 3 replications (± standard deviation). Different lowercase superscript letters in the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). TTA: Total Titratable Acidity is expressed as percent of acid citric. TSS: Total Soluble Solids is expressed in °Brix. Reducing Sugars expressed in %. *L** brightness, *a** redness/greenness, *b** yellowness/blueness, ΔΣ ab color difference, *C** chroma, hue angle.

For the pH parameter, the result T1 to T6 was similar, and from T7 to T9, the pH had a slight increase, being like to the control juice. For TTA there was a tendency to increase and for TSS to decrease. The titratable acidity of all enzyme-treated juices was significantly ($p \leq 0.05$) higher than that of the control juice, most likely rom the good balance in the pectinases activities in this preparation, considering the pectinase, polygalacturonase, and pectinlyase activities. The higher concentration of enzyme added before the fruit pulping process seems to have influenced the acidity, since, the value almost doubled in T7, T8 and T9, in relation to the other treatments. This can be evidenced by T9, in which the highest concentration of enzyme was added (10 $U.mL^{-1}$ before and after pulping) and had the highest value acidity (4.91%) with a significant difference for all other treatments. For TSS, the control juice had the highest concentration, followed by T7, T8, and T9. The other treatments (T1 to T6) had a lower concentration and had no difference between them. Regarding the TSS/TTA ratio, it was observed that all treatments had a lower ratio than the control juice. The amount was lower around 50% for treatments T1, T2, T4, T5 and T6, reaching around 70% for T3, T7, T8 and T9. For reducing sugars, the treatments had an amount around 25 to 50% less than the control juice.

Concerning the colour, it is seen that the parameters L^* (brightness), a^* (redness), b^* (yellowness) and C^* (chroma), increased compared to the control juice. The juice became clearer, yellow, and with a more intense colour, improving the colour of the juice. As for the Hue angle, there is a slight decrease in relation to the control juice. The treatments had a Hue angle around 70-77, while the control juice had 80, however, all are in the first quadrant of the CIELab colour chart, indicating variation between shades of yellow. The $\Delta\Sigma$ was measured to determine the total colour difference of the juices in comparison with the control, where can be highlighted the treatments T1, T2, T3, and, T8, which presented the highest values, compared to the control. Fruit juices are naturally cloudy, especially due to the presence of polysaccharides (pectin, cellulose, hemicelluloses and starch), proteins, tannins and metal ions. Therefore, clarification is an important step, which aims to remove pectic substances, through enzymatic depectinization treatment (Vaillant *et al.*, 2001).

Regarding the preparation with the Lallzyme enzyme, it was observed that, for feijoa juice, the colour parameters have improved, however, there was no increase in the juice yield and some quality parameters, such as TSS/TTA and reducing sugars, on the contrary, decreased.

For characterization of feijoa juice treated with Pectinex the results for nine treatments are presented in Table 3. As for the yield after filtration, it is seen that the treatments T10 to T15 had no significant difference between them. Although, the T12 treatment showed the highest yield after filtration, it did not have the highest yield in the pulping. Overall, the T17 was the treatment that had the highest total yield. Because from T16 to T18, they presented the highest yield in the pulping step (Fig. 2), and T17 also showed the highest yield after filtration. For pH, most treatments had a slight decrease compared to the control, however T12, T17 and T18 had no significant difference. For TTA, none of the samples showed the highest (1.97 and 1.95%, respectively).

For the parameters TSS, TSS/TTA and reducing sugars, the treatments with the enzyme Pectinex had a different behaviour from the treatments with the enzyme Lallzyme. Whereas with Lallzyme, all treatments showed a significant reduction compared to the control for these three parameters, treatments with Pectinex maintained more the sweetness of the juice. For TSS, with the exception of T13, T14 and T15, all the others showed no difference in relation to the control. As for TSS/TTA, only T10 and T11 were similar to fresh juice, however, it is seen that all samples with Pectinex had a higher ratio than samples with Lallzyme. This ratio is important, since, sugars and organic acids contribute appreciably to fruit juice flavour.

For reducing sugars, it is observed that the T17 had a higher content of reducing sugars than the control juice and T15, T16 and T18 had slightly higher content. These four samples, stood out in this attribute. It is seen that for reducing sugars the concentration of the enzyme influenced the content of this parameter, the highest concentrations utilized, both in the stage before pulping and filtration, had a higher content of this parameter. This is due to the fact that pectin molecules release reducing sugar during enzymatic hydrolysis. An increase in the enzyme concentration accelerates this process due to intensified degradation. In the fruit juice industry, pectinases are used to improve sensory quality, with a decrease in titratable acidity and an increase in sugars, which is seen as a desirable effect, since the greater this relationship, the greater the perception of sweet taste by the consumer (Ninga *et al.*, 2018).

Table 3. Analysis of pH, total acidity, total soluble solids, TSS/ TTA, reducing sugars and color parameters of feijoa juices prepared with Pectinex Ultra Clear enzymatic preparations under different concentrations

Treatments	Yeld AF (%)	pH	TTA	TSS	TSS/TTA	Reducing Sugars	*L**	*a**	*b**	ΔΣ	*C**	Hue (°)
Control		2.80 ± 0.05^{a}	1.66 ± 0.35^{ab}	12.47 ± 1.57^{a}	7.91 ± 0.65^{a}	8.74 ± 0.46^{bc}	33.36 ± 0.0^{1i}	-0.38 ± 0.01^{j}	6.80 ± 0.01i	-	6.81 ± 0.01^{i}	86.84 ± 0.04^{e}
T10	90.1^{abc}	2.62 ± 0.01^{b}	1.64 ± 0.06^{ab}	11.73 ± 0.06^{ab}	7.16 ± 0.27^{abc}	5.24 ± 0.17^{e}	60.52 ± 0.08^{a}	1.88 ± 0.01^{b}	11.16 ± 0.01^{f}	28.62 ± 0.08^{a}	11.32 ± 0.01^{f}	80.44 ± 0.08^{h}
T11	92.0^{ab}	2.62 ± 0.13^{b}	1.56 ± 0.05^{b}	11.43 ± 0.06^{ab}	7.32 ± 0.21^{ab}	6.19 ± 0.19^{de}	57.85 ± 0.02^{f}	1.39 ± 0.01^{a}	9.08 ± 0.01^{h}	25.74 ± 0.02^{e}	9.19 ± 0.02^{h}	81.33 ± 0.03^{g}
T12	94.6^{a}	2.78 ± 0.02^{ab}	1.82 ± 0.02^{ab}	12.13 ± 0.12^{ab}	6.64 ± 0.16^{bcd}	7.07 ± 0.25^{cde}	45.30 ± 0.01^{h}	2.75 ± 0.00^{a}	10.26 ± 0.01^{g}	13.56 ± 0.01^{g}	10.62 ± 0.01^{g}	74.99 ± 0.01^{i}
T13	89.6^{abc}	2.75 ± 0.02^{ab}	1.97 ± 0.07^{a}	11.00 ± 0.00^{b}	5.60 ± 0.19^{e}	6.19 ± 0.24^{de}	55.45 ± 0.00^{g}	0.48 ± 0.00^{g}	11.46 ± 0.00^{e}	23.67 ± 0.00^{f}	11.47 ± 0.00^{e}	87.60 ± 0.00^{c}
T14	91.2^{ab}	2.63 ± 0.02^{b}	1.88 ± 0.02^{ab}	11.00 ± 0.00^{b}	5.84 ± 0.05^{de}	7.35 ± 0.33^{cd}	59.92 ± 0.01^{c}	0.92 ± 0.01^{d}	13.96 ± 0.00^{a}	28.55 ± 0.01^{a}	13.99 ± 0.00^{a}	86.25 ± 0.03^{f}
T15	92.8^{ab}	2.65 ± 0.05^{b}	1.86 ± 0.03^{ab}	11.07 ± 0.06^{b}	5.94 ± 0.12^{de}	10.69 ± 0.76^{ab}	60.19 ± 0.39^{b}	0.59 ± 0.02^{f}	13.59 ± 0.09^{b}	28,73 ± 0.36^{a}	13.60 ± 0.09^{b}	87.53 ± 0.11^{c}
T16	81.8^{d}	2.65 ± 0.08^{b}	1.86 ± 0.02^{ab}	11.80 ± 0.00^{ab}	6.35 ± 0.08^{cde}	10.54 ± 0.26^{ab}	59.27 ± 0.00^{d}	0.71 ± 0.00^{e}	13.93 ± 0.01^{a}	27.92 ± 0.00^{b}	13.95 ± 0.01^{a}	87.08 ± 0.00^{d}
T17	88.0^{bc}	2.68 ± 0.01^{ab}	1.95 ± 0.05^{a}	11.63 ± 0.00^{ab}	5.97 ± 0.14^{de}	11.34 ± 0.90^{a}	58.56 ± 0.02^{e}	0.18 ± 0.01^{i}	12.08 ± 0.01^{d}	26.85 ± 0.02^{c}	12.08 ± 0.01^{d}	89.17 ± 0.03^{a}
T18	80.9^{d}	2.68 ± 0.01^{ab}	1.86 ± 0.05^{ab}	11.70 ± 0.00^{ab}	6.29 ± 0.17^{de}	10.61 ± 0.79^{ab}	58.01 ± 0.01^{f}	0.44 ± 0.01^{h}	12.27 ± 0.01^{c}	26.33 ± 0.01^{d}	12.27 ± 0.01^{c}	87.95 ± 0.06^{b}

AF: after filtration. Values are the mean of 3 replications (± standard deviation). Different lowercase superscript lettersin the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). TTA: Total Titratable Acidity is expressed as percent of acid citric. TSS: Total Soluble Solids is expressed in °Brix. Reducing Sugars expressed in %. *L** brightness, *a** redness/greenness, *b** yellowness/blueness, ΔΣ ab color difference, *C** chroma, hue angle

Regarding the colour of the juices, it can be seen from Table 3 that all treatments increased the *L**, *b** and *C** components by 70 to 100% and for all treatments and control, the Hue angle indicates the yellow colour and T17 presented highest value for this parameter. The juice became lighter, with more intense yellow. This colour difference in relation to the control can be observed by ΔΣ, in which all samples, with the exception of T12, presented values from 23 to 28.

The Table 4 shows the results of the treatments in which the preparation with the enzyme Rohapect was used. It is observed that the different concentrations used (1 to 5 $U.mL^{-1}$) did not interfere in the yield after filtration, since in each trio of samples the three treatments were statistically equal (Table 4), and only the concentrations of enzymes used before pulping the fruit, is that in fact interfered the yield (Fig. 2). The T21 sample had the highest pH value (3.01) and the samples from T25 to T27 had the lowest (2.24), these samples differed from the control juice.

As with Lallzyme, for the enzyme Rohapect, there was a tendency to slightly increase TTA and decrease TSS. The titratable acidity of all enzyme-treated juices was significantly higher than that of the control juice ($p \leq 0.05$). For TTA and TSS, it can be seen that samples T22 to T24 had similar behaviour, being statistically different from the other samples. The control juice had the highest TSS content, reducing sugars and TSS/TTA, this ratio was almost 2x higher than that found in the treatments. It is observed that the T19 in which it had the least addition of enzyme, in the previous pulping step (1 $U.mL^{-1}$), and in the previous step filtration (1 $U.mL^{-1}$), presented the lowest value of reducing sugars, around 50% less compared to the control.

As for the colour parameters, it is seen that for the *L** component, only T25, T26 and T27 had an improvement compared to the control sample. In addition to the brightness, these three treatments showed higher values of parameters *b**, ΔΣ and *C** among all samples and the control juice. For components *a**, *b**, ΔΣ and *C** all samples treated enzymatically were improved as compared to the control, however, T25, T26 and T27 showed, overall, the best results for colour. These three treatments had the highest concentration of enzyme added initially (10 $U.mL^{-1}$). For the Hue angle, these three samples showed no difference for control, but they had for T19, T20 and T21. The colour of the juice, as well as for the other enzymes, is yellow, indicated by the angle Hue and component *b**.

Table 4. Analysis of pH, total acidity, total soluble solids, TSS/ TTA, reducing sugars and color parameters of feijoa juices prepared with Rohapect 10L enzymatic preparations under different concentrations

Treatments	Yeld AF (%)	pH	TTA	TSS	TSS/TTA	Reducing Sugars	*L**	*a**	*b**	ΔΣ	*C**	Hue (°)
Control	-	2.91 ± 0.01^{bc}	2.21 ± 0.11^{d}	13.30 ± 0.17^{a}	6.02 ± 0.31^{a}	8.99 ± 0.40^{a}	39.67 ± 0.02^{c}	1.60 ± 0.01^{h}	7.47 ± 0.16^{g}	-	7.64 ± 0.19^{g}	79.67 ± 3.19^{ab}
T19	84.34^{ab}	2.95 ± 0.04^{b}	2.50 ± 0.00^{ab}	9.77 ± 0.06^{b}	3.91 ± 0.02^{b}	3.99 ± 0.12^{e}	40.09 ± 0.01^{c}	5.39 ± 0.01^{c}	18.76 ± 0.01^{c}	14.62 ± 0.02^{b}	19.52 ± 0.02^{c}	73.97 ± 0.03^{c}
T20	85.54^{ab}	2.92 ± 0.01^{bc}	2.51 ± 0.01^{ab}	9.80 ± 0.00^{b}	3.91 ± 0.01^{b}	6.23 ± 0.12^{bc}	40.66 ± 0.04^{c}	5.19 ± 0.01^{b}	18.68 ± 0.03^{c}	14.82 ± 0.04^{b}	19.39 ± 0.03^{c}	74.49 ± 0.04^{c}
T21	89.16^{a}	3.01 ± 0.02^{a}	2.51±0.01^{ab}	9.83 ± 0.06^{b}	3.92 ± 0.03^{b}	5.66 ± 0.42^{cd}	40.58 ± 0.06^{c}	5.15 ± 0.01^{b}	18.77 ± 0.03^{c}	14.84 ± 0.06^{b}	19.46 ± 0.03^{c}	74.67 ± 0.00^{c}
T22	82.61^{b}	2.94 ± 0.01^{b}	2.35± 0.04^{c}	9.30 ± 0.17^{c}	3.95 ± 0.13^{b}	6.80 ± 0.19^{b}	40.22 ± 0.01^{c}	3.10 ± 0.01^{f}	15.28 ± 0.01^{d}	11.55 ± 0.00^{c}	15.59 ± 0.01^{d}	78.53 ± 0.06^{b}
T23	83.48^{b}	2.87 ± 0.03^{c}	2.39 ± 0.01^{c}	9.20 ± 0.00^{c}	3.85 ± 0.02^{b}	5.58 ± 0.18^{cd}	40.02 ± 0.04^{c}	2.80 ± 0.01^{f}	14.91 ± 0.02^{e}	11.11 ± 0.01^{c}	15.16 ± 0.02^{e}	79.38 ± 0.01^{ab}
T24	82.61^{b}	2.90 ± 0.00^{bc}	2.41 ± 0.01^{bc}	9.23 ± 0.15^{c}	3.84 ± 0.06^{b}	5.16 ± 0.16^{d}	36.59 ± 0.01^{d}	3.35 ± 0.07^{e}	13.01 ± 0.01^{f}	7.61 ± 0.02^{d}	13.43 ± 0.03^{f}	75.56 ± 0.28^{c}
T25	84.20^{ab}	2.24 ± 0.01^{d}	2.58 ± 0.03^{a}	9.70 ± 0.00^{b}	3.76 ± 0.04^{b}	6.31 ± 0.40^{bc}	55.15 ± 0.03^{b}	3.62 ± 0.02^{d}	23.07 ± 0.06^{a}	30.51 ± 3.52^{a}	23.35 ± 0.01^{a}	81.08 ± 0.02^{ab}
T26	86.50^{ab}	2.24 ± 0.01^{d}	2.60 ± 0.02^{a}	9.97 ± 0.12^{b}	3.83 ± 0.06^{b}	6.08 ± 0.27^{bc}	57.23 ± 0.01^{ab}	3.32 ± 0.00^{e}	22.23 ± 0.01^{b}	31.14 ± 2.62^{a}	22.48 ± 0.00^{b}	81.50 ± 0.02^{a}
T27	85.40^{ab}	2.25 ± 0.00^{d}	2.58 ± 0.01^{a}	9.87 ± 0.06^{b}	3.82 ± 0.02^{b}	6.88 ± 0.28^{b}	57.37 ± 0.00^{a}	3.75 ± 0.00^{c}	23.47 ± 0.00^{a}	31.55 ± 2.05^{a}	23.77 ± 0.01^{a}	80.92 ± 0.02^{ab}

AF: after filtration. Values are the mean of 3 replications (± standard deviation). Different lowercase superscript letters in the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). TTA: Total Titratable Acidity is expressed as percent of acid citric. TSS: Total Soluble Solids is expressed in °Brix. Reducing Sugars expressed in %. *L** brightness, *a** redness/greenness, *b** yellowness/blueness, ΔΣ ab color difference, *C** chroma, hue angle.

As with Lallzyme, treatments with Rohapect did not show an improvement in the contents of TSS, TSS / TTA and reducing sugars. However, it can be seen that for all the enzymes utilized, the colour of the juice improved considerably, and overall, the concentration applied before the pulping step, was more significant in colour than the concentration applied before filtration.

Among the three enzymes used in this work, Pectinex stood out for feijoa juice. It presented the highest juice yield, and the higher the concentration of enzyme applied, the greater the quantity of juice in the stage to pulping. As for the costs of enzymatic treatments for extracting and clarifying juices, it was seen that the expenses with enzymes compensate in comparison with other alternative processes, and would have a significant production yield. For the tropical fruit juice industries, it represents a real alternative to diversify production and increase the market segment (Tapre and Jain, 2014). Among the nine treatments with Pectinex, T17 was noteworthy, it had the highest overall yield. In addition, this preparation presented a good balance for the quality parameters and, therefore, was chosen as a standard for the next experiments.

Membrane-based technologies, being easily scalable, non-thermal, and physical in nature without using any external additives can be an attractive alternative in enhancing the shelf-life of feijoa juice (Panigrahi *et al.*, 2018). This process is gaining more attention and focus in food industry due to its advantages (environmental friendliness, cost saving, and product improvement) as compared with other conventional methods. Thus, microfiltration has been successfully applied to preserve and clarify many fruit juices. However, one of the critical factors that limits the general performance of this technique is the significant loss of membrane permeability, due to the phenomenon of membrane fouling. To prolong the activity of the microfiltration membrane, the juice is treated with enzymes immediately before filtration (Echavarría *et al.*, 2011; Urošević *et al.*, 2017).

Microfiltered feijoa fruit juice was characterized by pH, TTA, TSS, reducing sugars, colour, viscosity and particle as can be seen in Table 5. The pH was not affected by microfiltration, nevertheless, TTA, TSS, TSS/TTA and reducing sugars was influenced. The microfiltered juice had a slight increase in acidity (2.52 %), the TSS content decreased by around 18% and, consequently, the TSS/TTA ratio reduced 37% when compared to the same pasteurized juice (T17). Reducing sugars also decreased in microfiltered juice, approximately 40%. The T17 sample had the highest increase in this parameter (11.34 %) compared to the control (8.74 %), however the microfiltration process, reduced to a lower value (6.47 %) than the control juice. Hence, as can be seen,

the microfiltered juice, presented a lower content of TSS and reducing sugars, probably owing to the microfiltration process that removes larger-sized, high molecular weight gel-forming materials from the feed itself (Panigrahi *et al.*, 2018).

Table 5. Analysis of pH, total acidity, total soluble solids, TSS/ TTA, reducing sugars and color parameters of feijoa juices prepared with Pectinex Ultra Clear enzyme (T17) with addition of the microfiltration process

Treatment	pH	TTA	TSS	TSS/TTA	Reducing Sugars	*L**	*a**	*b**	ΔΣ	*C**	Hue (°)
T17 microfiltered	2.63 ± 0.01	2.52 ± 0.02	9.50 ± 0.00	3.76 ± 0.01	6.47 ± 0.84	70.40 ± 0.01	0.88 ± 0.01	25.67 ± 0.01	39.33 ± 0.02	25.68 ± 0.01	87.75 ± 0.03

Values are the mean of 3 replications (± standard deviation). TTA: Total Titratable Acidity is expressed as percent of acid citric. TSS: Total Soluble Solids is expressed in °Brix. Reducing Sugars expressed in %. *L** brightness, *a** redness/greenness, *b** yellowness/blueness, ΔΣ ab color difference, *C** chroma, hue angle.

Meanwhile, the colour parameters improved significantly in microfiltered juice. All parameters increased with the exception of the Hue angle. The high values *L** component (70.40) and *b** (25.67), in T17 microfiltered, compared to the other treatments indicate a bright and yellowish characteristic of this sample. The *L** component of 58 (T17) enhanced to 70. The *b** and the *C** parameters have doubled in value. Chroma represents the degree of saturation or intensity of colour, which is a better measure for assessing overall colour of a products, the higher the *C** value, the superior the intensity of the juice. The total colour difference compared to the control is large, as shown by ΔΣ. The yellow-coloured juice, as indicated by Hue's angle, became more translucent and shinier. The colour differences of the three feijoa fruit juices can be seen in Figure 3.

The fresh juice, the treatment T17 and the T17 microfiltered were evaluated for particle size and viscosity, and the results of these parameters are shown in Table 6. It is observed that the Ostwald-de Waele model tested to describe the rheological behaviour of feijoa juice was satisfactorily adapted for the three samples, presenting low values of χ^2, and high values for the coefficient of determination (R^2), higher than 0.92 for the control sample and 0.99 for the other two samples. It appears that for the control juice the behaviour index (n) showed values below one (0.2178), indicating a non-Newtonian and typically pseudoplastic behaviour for the raw juice sample. A consequence of the behaviour of pseudoplastic liquids is low mixing efficiency when such a fluid is homogenized with a mechanical stirrer (Vandresen, 2007). For the T17 and T17 microfiltered samples, the value of **n** was equal to 1 (1.066 and 1.091, respectively), indicating a Newtonian behaviour. In addition, it can be seen that for these two treatments, the consistency index (κ) decreased significantly compared to the control, nevertheless,

in microfiltered juice, the retention of suspended solids larger than the membrane pore resulted in a less consistent drink when compared to T17. Enzymatic treatment and microfiltration process reduced the viscosity significantly compared to control sample. The knowledge of the rheological parameters, as well as the rheological behaviour of food products allows to assist from the design of the equipment, implying cost reduction in the food industry, up to the final quality of the product. In many food industry operations, measuring the viscosity of a fluid is important for quality control of raw materials and for assessing the effect of variations, both on processing conditions on products during manufacture and on the final product. Knowing of viscosity can contribute to process optimization, cost reduction in ingredients and improvement of product consistency.

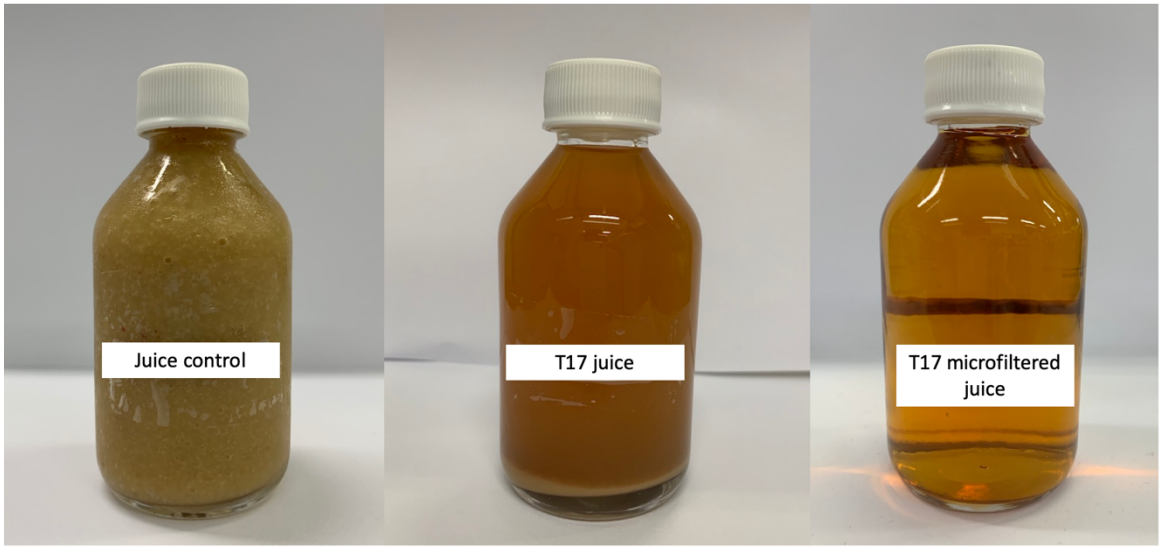

Figure 3. Three feijoa juices - juice control: raw; T17 juice: treated with Pectinex enzyme (10 $U.mL^{-1}$ and 5 $U.mL^{-1}$) and pasteurized; and T17 microfiltered juice: with addition of the microfiltration process.

Enzymatic treatment by pectinase has been widely used as a pre-treatment in the juice processing industry, before clarification by microfiltration. Researches have shown that enzymatic pre-treatment helps in maintaining a higher permeate flux level in microfiltration in case of clarification of citrus juices by reducing the juice viscosity and pectic materials (De Bruijn *et al.*, 2003). Rai et al. (2007) investigated the impact of some pre-treatment methods to determine the efficiency of MF for clarifying the juice. Among the pre-treatment methods, they concluded that the treatment with pectinase was the most

successful and they observed that there was an increase in the permeate flow in the microfiltration stage when the enzyme was added at two different moments in the juice production stage (Bhattacharjee *et al.*, 2017; Urošević *et al.*, 2017).

Table 6. Rheological and particle size parameters of the control (raw juice), T17 (enzymatic and pasteurized treatment) and T17 microfiltered

Parameters	Control	T17	T17 microfiltered
Rheological parameters			
n	0.2178 ± 0.02^{b}	1.066 ± 0.006^{a}	1.091 ± 0.004^{a}
κ (Pa.s^n)	8.8660 ± 0.87^{a}	0.00102 ± 0.001^{b}	0.0009417 ± 0.000^{c}
χ^2	0.17138,8660	4.3^{-10}	3.578^{-9}
R^2	0.9224	0.9922	0.9938
η_{app}	0.241728 ± 0.002^{a}	0.001382 ± 0.000^{c}	0.001431 ± 0.000^{b}
Particle Distribution Parameters			
Span	1.51 ± 0.03^{b}	4.55 ± 0.44^{a}	1.86 ± 0.05^{b}
Vol Weighted Mean [4,3] (μm)	818.92 ± 12.05^{a}	96.28 ± 8.24^{b}	85.95 ± 5.23^{b}

Values are the mean of 3 replications (± standard deviation). Different lowercase superscript letters in the same line indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). Rheological parameters from Ostwald-de Waele equation, n: behavior index; κ: consistency index; η_{app}: apparent viscosity calculated at 100 s^{-1}.

Regarding the particle size, it can be observed from this Figure 4, that a multimodal distribution profile is present in the samples juices and the three samples had differences in the overall appearance. The control juice was characterized mainly by large particles (greater than 100 μm). Regarding the T17 juice, its size distribution was described by two distinct modes (around 10 μm and 100 μm). The microfiltered T17 juice was illustrated mainly by smaller particles (with size less than 1 μm), although note that there is also a small volume with large particles.

The average value D[4.3] for each juice is given in Table 6. The highest D[4.3] was obtained for control juice, which is characterized by a mode near to 818 μm in large particles domain and the presence of particles larger than 1000 μm. The D[4.3] was significantly smaller in juices with enzymatic treatment and microfiltration (T17 and T17 microfiltered). These two samples had no difference in relation to the average diameter. The T17 juice had a higher span value (4.55) showing that it is characterized by a high distribution polydispersity. The control juice and microfiltered juice presented a value span of 1.51 and 1.86, respectively. This difference in the size distribution of the juice T17 could be attributed to the fact that this sample had been subjected to some pre-treatments with the objective to break and/or eliminate the largest particles. All these

observations show that the three studied juices are different concerning their particle size distribution.

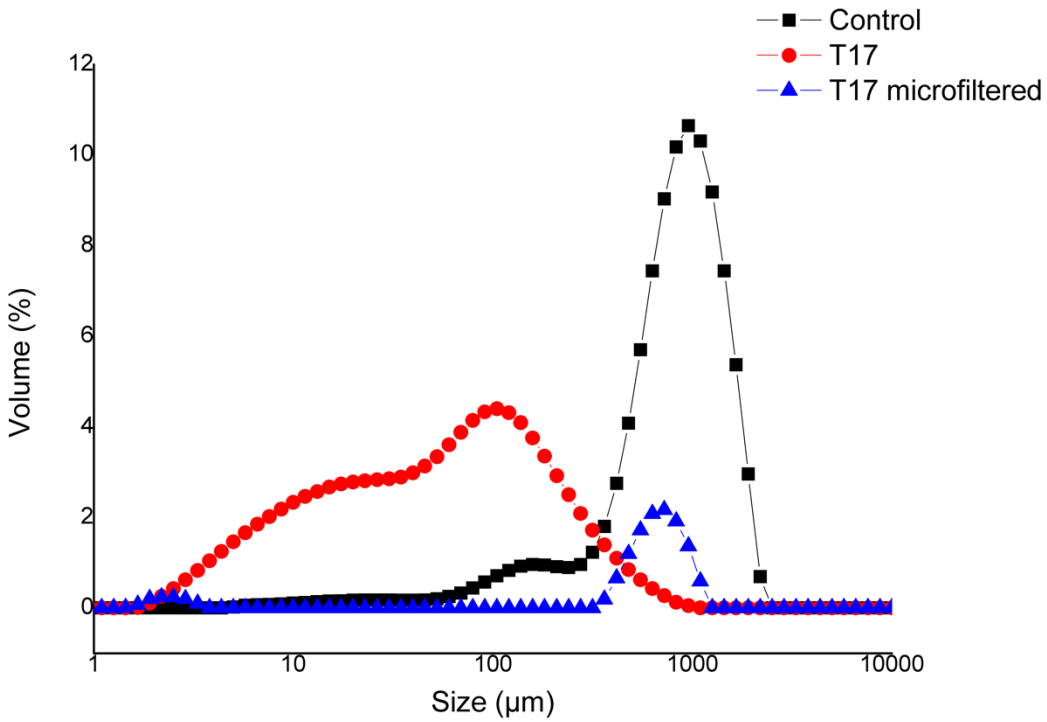

Figure 4. Particles size distribution of the three samples feijoa juices: raw juice), T17 (enzymatic and pasteurized treatment) and T17 microfiltered.

The feijoa juice showed to be promising, due to its physicochemical characteristics presented in this study. Bio-functional drinks made from fruit juice have been achievement space in the market and are showing excellent physicochemical and sensory characteristics (Karabagias *et al.*, 2020).

4. CONCLUSION

The present work studied the effect of three commercial enzymes on the yield and physical-chemical properties of feijoa juice. In overall, all the tested enzymes had a positive impact on the colour of the juice.

The Pectinex® Ultra Clear enzyme showed the better results for physical-chemical parameters, being the most effective for extracting feijoa juice. The added concentration

of 10 $U.mL^{-1}$ in the step prior to pulping increasing yield and enhancing the concentration of sugars. The T17 had the highest overall yield and it was effective in improving the clarity of juice and in decreasing viscosity. The microfiltration altered the TSS and TTA content in the juice, but improved the clarity of the juice.

Modelling the rheological behaviour of raw feijoa juice showed that it is a non-Newtonian fluid, and that it has pseudoplastic characteristics, whereas feijoa juices treated with enzyme and microfiltration showed Newtonian fluid behaviour. The three juices studied are different in terms of the size distribution of their particles, the raw juice had the largest size, while the microfiltered juice the smallest.

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Influence of processing conditions on the composition of feijoa (*Acca sellowiana*) juices during storage

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ABSTRACT: The feijoa has an excellent potential to be processed to juice. However, the effect of processing and storage conditions on feijoa juice composition is not known. Thus, the aim of this study was to evaluate the effect of the commercial pectinolytic enzyme, pasteurization, and microfiltration on some bioactive compounds and physicochemical quality of feijoa (*Acca sellowiana*) juice during 90 days of storage. The phenolic compounds were analyzed by LC-DAD-MS. Carotenoids, ascorbic acid and individual sugars, were analyzed by HPLC. Physicochemical parameters were studied. Three treatments were evaluated: fresh juice (F), enzyme-treated and pasteurized juice (P), enzyme-treated, microfiltered and pasteurized juice (MP). The enzymatic treatment had a positive impact on juice yield, with an increase of up to 10%. The ascorbic acid content was higher in the microfiltered juice (6.4 mg.100 mL^{-1}), while the fresh juice and pasteurized juice had similar concentrations (4.0 mg.100 mL^{-1}). The P and MP juices had a significantly higher amount of total phenolic compounds (21,061.30 and 22,198.71 μg.100 mL^{-1}, respectively) than fresh juice (1591.18 μg.100 mL^{-1}). The use of enzyme improved mainly the yield, color parameters, and phenolic compounds.

Keywords: Food processing; enzyme; microfiltration; pasteurized juice; phenolic compounds.

1. INTRODUCTION

Feijoa (*Acca sellowiana* (O. Berg) Burret) is a fruit native to South America, members of the Myrtaceae family. Due to its easy adaptability in subtropical regions, it is currently grown in many countries, such as Australia, New Zealand, and Colombia, and is considered an exotic fruit in Europe. In recent years, there has been an increase in worldwide interest in fruit, due to its unique sweet-acidic flavor, in addition to being highly aromatic and nutritious (Pasquariello et al., 2015; Peng et al., 2020).

The fruit is a source of vitamins and minerals, mainly ascorbic acid, pantothenic acid, sodium, potassium, and magnesium. It contains phenolic compounds, as the main bioactive components, but has fewer carotenoids (Phan et al., 2019; H. Schmidt et al., 2020). Among the carotenoids, lutein and β-carotene have been identified in greater quantities. The major phenolic acids identified were pedunculagin isomer and ellagic acid derivatives. The fruit has a high antioxidant capacity, which is mainly due to polyphenols (H. Schmidt et al., 2019). Phenolic compounds, carotenoids and vitamin C exhibit many bioactivities in the organism, such as anti-inflammatory, anti-obesity, and anti-cancer properties and due to their antioxidant activity, they protect cells against oxidative damage (Aguilera et al., 2016; Kabir et al., 2022; Pisoschi et al., 2022; Santos-Buelga et al., 2019).

In addition to fresh consumption, fruits can be processed and used in the production of food and beverages. The processing of feijoa is often necessary, as studies report a short period of *in natura* conservation after harvest, due to high ethylene production and high activity of deteriorating enzymes, followed by the appearance of brown pigments in the peel and the browning of the pulp, which compromises its quality (Amarante et al., 2017). A processing option for fruits is the production of industrialized juices.

One of the main problems in the production of fruit juice is turbidity. Therefore, clarification is an important step, which aims to remove pectic substances, through the enzymatic treatment of depectinization (Dal Magro et al., 2016; Siddiq et al., 2018). Associated with the use of enzymes, microfiltration is used in many industries, mainly as a technology for clarifying juices, and thus, it can be used as an adjuvant to obtain a clear juice (Dahdouh et al., 2018). Conventional pasteurization, combined with other preservation methods, such as bleaching and microfiltration, allows the destruction of microorganisms and the inactivation of enzymes, and thus guarantees a stable shelf life, without the use of additives, even at room temperature storage (Wibowo et al., 2015).

A wide variety of fruit juices are available on the market, however, to date, there is no research available on the stability of the physicochemical properties during the storage of pasteurized and clarified by enzymatic treatment and/or microfiltration feijoa juices. Therefore, this study aimed to evaluate the stability of physicochemical properties and bioactive compounds in feijoa juice at room temperature storage (20 °C) for 3 months and to estimate the effect of processing pasteurized and clarified juice compared to fresh juice.

2. MATERIALS AND METHODS

2.1 Chemical

High-performance liquid chromatography (HPLC) grade reagents such as acetonitrile and methanol were acquired from Panreac AppliChem© (Barcelona, Spain), (all-E)-β-carotene, (all-E)-β cryptoxanthin, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, ellagic acid, catechin, epicatechin, epigallocatechin gallate, luteolin, quercetin, quercetin 3 glucoside, rutin, kaempferol and myricetin, were purchased from Sigma-Aldrich® (St Louis, USA). Zeaxanthin were donated by Fluka Analytical® (Buchs, Switzerland), (all-E)-Lutein were obtained from Indofine Chemical Company® (New Jersey, USA). Water was purified by Milli-Q® system Integral 10 (Millipore, Milford, MA, EUA). The samples and solvents were filtered through Millipore membranes (Millex LCR 0.45 μm, 13 mm).

2.2 Material

The feijoa fruit was harvested in the south of Brazil, in the city of São Joaquim, SC (GPS coordinates: 28º16'34.47 "S and 49º56'9.57" W). The fruit (approximately 60 kg) was harvested at optimal maturation stage, when can be easily separated from the plant, according to Amarante et al. (2017) in April 2019. The fruits were transported under refrigeration to the Bioactive Compounds Laboratory at the Federal University of Rio Grande do Sul (UFRGS) and kept in a cold chamber (-18 °C) until juices were processed.

2.3 Preparation and storage of feijoa juice

The fruits were selected visually, washed in running water and sanitized by immersion in chlorinated solution (200 mg.L-1 for 15 min). A new wash with running water was done to remove residual chlorine. The flowchart of the juice preparations is shown in Figure 1. The yields of feijoa juices (JY) were calculated according to Equation 1:

$$JY(\%) = \frac{mass\ of\ juice}{initial\ mass\ of\ feijoa} \times 100 \quad (1)$$

2.3.1 Fresh juice

After washing, the fruits were pulped and homogenized in a vertical pulping machine (DES-20, Braesi®, Caxias do Sul, RS, Brazil). Afterward, the juice was filtered through organza fabric. The juice was transferred to 100 mL glass bottles (Pilfer 24, Flint, Envidro, Porto Alegre, RS, Brazil), stored under a nitrogen atmosphere and subsequently, they were closed with a screw cap.

2.3.2 Enzyme-treated and pasteurized juice

After cleaning the fruits, they were bleached in a water bath (Dubnoff NT 232 – Novatecnica®, Piracicaba, Brazil) at 80 °C for 2.5 minutes, and cooled to 10 °C. Subsequently, the enzyme Pectinex Ultra Clear® (Novozymes, Spain) was applied (10 U.mL-1), at 50 °C for 40 min, under agitation, and cooled to 20 °C. The fruits were pulped and homogenized in a vertical pulping machine. Then, the enzyme Pectinex Ultra Clear has added again (5 U.mL-1), at 50 °C for 40 min, under agitation, and followed by cooling to 20 °C. In the first enzyme addition, before pulping, the objective was to increase the juice extraction yield. In the second addition, after pulping, the main purpose was to clarify the juice. Successively, the juice was filtered through organza fabric, was bottled (100 mL glass bottles), pasteurized (1 min at 90 °C), followed by cooling to 20 °C. The juices were stored under a nitrogen atmosphere and then closed.

2.3.3 Enzyme-treated, microfiltered and pasteurized juice

In this juice, the same procedure as in the previous item was performed, however, with the addition of the microfiltration (MF) process, as can be seen in the flowchart (Figure 1). Enzyme clarification was used as a pre-treatment for removal of the suspended solids and juice clarification. After the filtration step in organza fabric, the juice underwent microfiltration.

For MF was used, a polyamide membrane (PAM Selective Membranes, Rio de Janeiro, RJ, Brazil) with a pore diameter of 0.4 μm and a filtration area of 0.7 m². Fixed conditions of 20 ± 2 °C and 300 kPa were applied during the experiment. After the MF, the juice was pasteurized (1 min at 90 °C) and all steps after pasteurization were carried out in the same way as described previously in *section 2.3.2.*

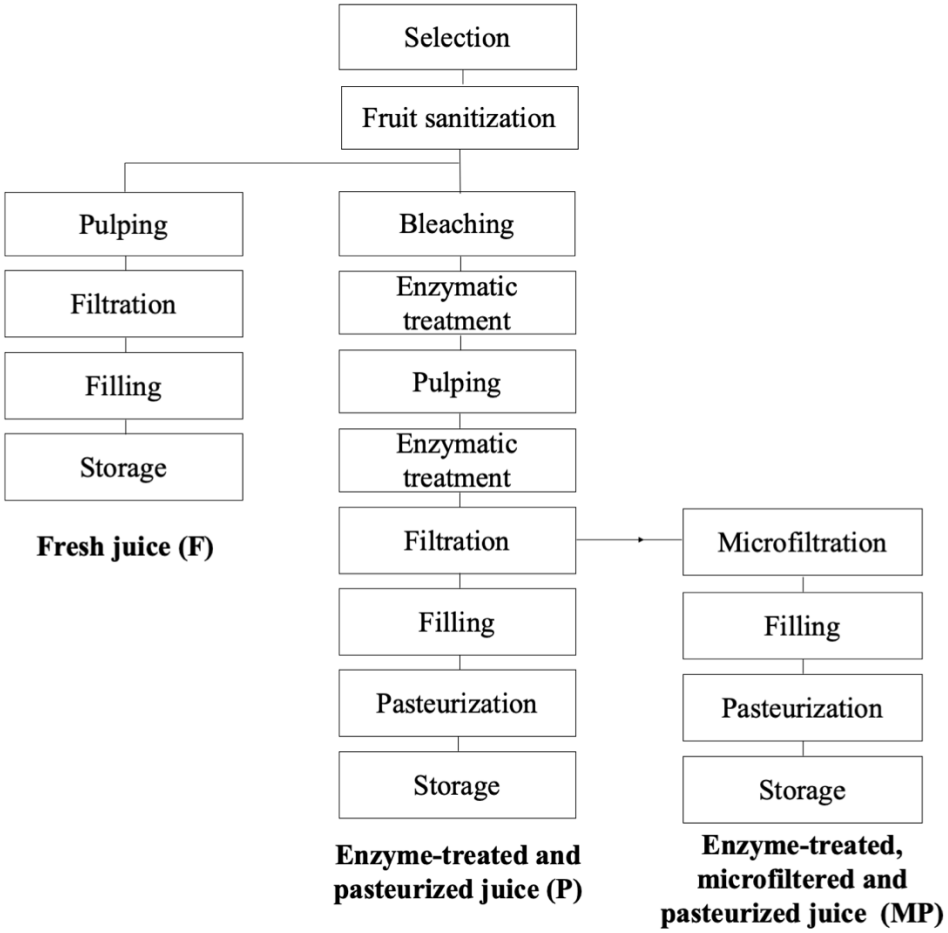

Figure 1. Flowchart of three feijoa (*Acca sellowiana*) juice processing.

2.3.4 Storage conditions

For the fresh juice (F), the bottles were stored under refrigeration at 4 ºC for one day and analyzed in triplicate. The juice clarified by enzymatic treatment and pasteurized (P) and the juice clarified by enzymatic treatment and microfiltration and pasteurized (MP) were stored at 20 ºC for 90 days. All samples were stored in a biochemical oxygen demand (BOD) camera (NL-41-01 A, NewLab, Piracicaba, SP, Brazil) under a 12-h photoperiod (light/dark cycle), to mimic the type of storage used in the local market. The relative humidity and chamber temperature were monitored (50.7 ± 3.5% and 4.7 ± 0.5 °C, respectively) for the fresh juice (65.3 ± 4.6% and 20.7 ± 0.3°C, respectively) and for the pasteurized juice and microfiltered and pasteurized juice. These two treatments were analyzed in triplicate at 1, 7, 14, 21, 30, 60 and 90 days of storage time. An aliquot of all

treatments was collected for physicochemical analysis. The remaining juice was stored in 15 and 50 mL falcon tubes and frozen (-18 ºC), and to perform the analysis, the samples were defrosted before use at 4 ºC during one night.

The fresh juice was taken as a reference sample in order to study the effect of the different treatments on the analyzed parameters.

2.4 Physicochemical analyses

The pH was determined by a potentiometric electrometric method with a pH-meter (Quimis®, model Q400AS, São Paulo, Brazil). The total titratable acidity (TTA) was determined by the titration method using sodium hydroxide solution (NaOH 0.1 N), and the results are expressed as % citric acid following the Analytical Standards (Instituto Adolfo Lutz 2008). The total soluble solids (TSS) were determined by direct reading in a portable digital refractometer (Atago® U.S.A, Pocket, Model Pal-3, Bellevue, WA, USA). All analyses were performed in triplicates and the TSS/TTA ratio was calculated for each replicate. The percentage of dry matter (DM) was determined by gravimetry. Five mL of well-homogenized juice was added in an aluminum capsule containing 10 g of purified sand and dried in a forced air oven at 105 °C for 24 h.

The color was measured by a Minolta Colorimeter (Model CR-400, Konica Minolta Sensing, Osaka, Japan) operating with illuminant C, and color parameters of the CIELAB system. The parameters used were L^* (lightness; 0 = dark and 100 = white), a^* (component red-green; <a* = green and >a* = red) and b^* (yellow-blue component; <b* = blue and >b* = yellow). A white ceramic tile was used for calibration before carrying out any measurements. The hue angle (h^*), chroma (C^*), and color difference (ΔE^*_{ab}) were calculated following the Equations. 2, 3, and 4, respectively:

$$h^* = \text{atan}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.5 Individual and total sugars

Sugars were extracted according to the method described by Petkovsek et al. (2007). For the extraction, 1 mL of the sample was homogenized with Ultra-Turrax® (T25 digital,

IKA, Staufen, Germany) in 10 mL of 80% ethanol. After extraction, the samples were centrifuged at 20,000 g (Himac CR21 GIII, Hitachi Koki Co., Tokyo Japan), for 15 min at 10 ºC. The supernatant was filtered through Millipore membranes (Millex LCR 0.45 µm, 13 mm). The samples were analyzed by HPLC.

For the analysis of individual sugars, the samples were analyzed by Waters Alliance 2695® (Milford, MA, USA) HPLC connected to a refractive index detector (2414). An Aminex® HPX-87H column, 300 mm x 7.8 mm (Bio-Rad Laboratories Inc, Hercules, California, United States) was used. The chromatographic conditions were those described by Petkovsek et al. (2007) with adaptations: the mobile phase was isocratic, with Milli-Q® water and 0.005 M sulfuric acid, with a flow of 0.5 mL.min-1, injection volume of 20 µL and the total running time was 30 minutes. The concentration ranges, correlation coefficient (R^2), detection limits (LOD) and limits of quantification (LOQ) of the sugar patterns are found in Table 1.

2.6 Ascorbic acid

The ascorbic acid (vitamin C) content was quantified on the methodology proposed by Rosa et al. (2007) with modifications. Each 5 mL sample was homogenized in an Ultra-Turrax® with 20 mL of 96% sulfuric acid (0.05 M) for 1 min. Afterward, samples were centrifuged at 25,400g for 15 min and then filtered through Millipore membranes for HPLC injection. Vitamin C was analyzed in Waters Alliance 2695®, chromatograph equipped with a quaternary solvent pump system connected to a diode-array detector (DAD 2996) using a C18 polymer column (250 mm x 4.6 mm, 5 µm). The mobile phase was 0.05 M sulfuric acid, with a flow rate of 1.0 mL.min-1, an injection volume of 10 µL and a wavelength of 254 nm. Vitamin C was quantified by the injection of the ascorbic acid standard on the day of analysis.

2.7 Carotenoids

The exhaustive extraction of carotenoids was prepared according to Rodriguez-Amaya (2001) with some modifications. For extraction, 3 ml of the sample was added to a falcon tube and the partitioning with petroleum ether and ethyl ether mixture [1:1 (v/v)] was performed. The samples were homogenized in an Ultra-Turrax® for 1 min and centrifuged at 25,000g for 15 min at 5 ºC. The supernatant was transferred to an amber flask and the process was repeated (until no color). Then, the supernatants were combined and the extract was dried in a nitrogen flow and stored in amber tubes in the freezer (-18

°C) for further quantification by HPLC. For HPLC analysis, the concentrated extract was diluted in methyl tert-butyl ether (MTBE), placed in ultrasound (Unique, model USC 1400A) for 5 min and filtered through Millipore 0.45 μm membranes.

The analyzes for carotenoids were performed on an Agilent chromatography equipment, 1100 series (Santa Clara, CA, USA), previously described by Rockett et al. (2020). The carotenoids were separated on a C_{30} reversed phase polymeric column, YMC (250 x 4.6 mm, 3μm) according to Rodriguez-Amaya (2001). The mobile phase was water, methanol and MTBE, starting at 5:90:5 (v/v/v), reaching 0:95:5 (v/v/v) in 12 min, 0:89:11 (v/v/v) in 25 min, 0:75:25 (v/v/v) in 40 min and finally 0:50:50 (v/v/v) after a total of 60 min, with a flow rate of 1 $mL.min^{-1}$ and an injection volume of 5 μL at 22 ºC. The spectrum was processed at a fixed wavelength of 450 nm. The concentration ranges, R^2, LOD and LOQ of of the carotenoid patterns are found in Table 1.

2.8 Phenolic compounds

The phenolic compounds were extraction by following the methodology described by Rodrigues et al. (2013) with modifications. The extraction of the phenolic compounds was carried out using 5 mL of the sample, centrifuged at 30,000g, 10 °C for 15 min, and the supernatant was transferred to an amber flask. Afterward, the samples were filtered through 0.45 μm Millipore membranes and injected in a HPLC. To qualitatively analyze the extracted phenolic compounds, the extracts were previously purified by solid-phase extraction (SPE). The extracts were purified according to Schmidt et al. (2020) with C_{18} solid-phase extraction cartridges (Phenomenex®, Strata, Torrance, USA).

The phenolic compounds were identified using a Shimadzu HPLC (Kyoto, Japan), equipped with two pumps (Shimadzu LC-20AD), a degasser (Shimadzu DGU-20A), column oven (Shimadzu CTO-20A), DAD (Shimadzu SPD-M20A) and MS with quadrupole analyzer–time-of-flight (Q-TOF) and electrospray ionization (ESI) sources (model microTOF-QIII, Bruker Daltonics, Bremen, Germany). Chromatographic separation of the compounds was achieved using a C_{18} LiChrospher® column, (150 x 3,2 mm, 5μm) (Merck®, Darmstadt, Germany). The mobile phase A was composed of a mixture of Milli-Q® water and formic acid (99.9:0.1%, v/v). Mobile phase B was a mixture of acetonitrile and formic acid (99.9:0.1%, v/v). A linear gradient was used, starting at 99:1 (v/v) mobile phase A/B, reaching 50:50 (v/v) A/B in 50 min, and then 50:50 (v/v) A/B to 1:99 (v/v) A/B for 5 min. This ratio was maintained for another 5 min.

The conditions used were as follows: flow rate, 0.7 mL.min^{-1}; temperature, 29 °C; injection volume, 5 µL. The spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280, 320 and 360 respectively. The mass spectra were acquired over a mass range of *m/z* 100–1000. The MS parameters were described as follows: ESI source in negative ion modes; capillary voltage, 3000 V; dry gas (N_2) temperature, 310 °C; flow rate, 8 L.min^{-1}; nebulizer gas, 30 psi. MS^2 was set to automatic mode. The phenolic compounds were identified based on the order of elution and retention time on the column, characteristics of the UV–Vis and MS spectra compared with standards analysed (caffeic acid, chlorogenic acid, coumaric acid, ellagic acid, ferulic acid, gallic acid, catechin, epigallocatechin gallate, kaempferol, luteolin, myricetin, quercetin, quercetin-3-glucoside and rutin) under the same conditions, and data available in the literature. The phenolic compounds were quantified by HPLC−DAD, using nine-point analytical curves of gallic acid, caffeic acid, and rutin (1−120 µg.mL^{-1}). The concentration ranges, R^2, LOD and LOQ of the phenolic compounds patterns are found in Table 1.

Table 1. Concentration ranges, correlation coefficient (R^2), detection limits (LOD) and limits of quantification (LOQ) of the carotenoid, phenolic and sugar patterns.

Bioactive Compound	**Concentration range (µg.mL^{-1})**	**(R^2)**	**LOD (µg.mL^{-1})**	**LOQ (µg.mL^{-1})**
	Carotenoids			
Lutein	0.7-65	0.9991	6.90 x 10^{-3}	1.15 x 10^{-2}
Zeaxanthin	1-40	0.9997	9.56 x 10^{-2}	1.59 x 10^{-2}
Cryptoxanthin	3-100	0.9911	2.11 x 10^{-2}	3.51 x 10^{-2}
α-carotene	1-25	0.9934	1.97 x 10^{-2}	3.28 x 10^{-2}
β-carotene	4-48	0.9998	6.53 x 10^{-2}	10.89 x 10^{-2}
Lycopene	10-100	0.9977	7.0 x 10^{-3}	33.0 x 10^{-3}
	Phenolic Compounds			
Caffeic acid	1-120	0.9976	0.44	1.33
Chlorogenic acid	0.75 – 24	0.9960	0.77	2.34
Coumaric acid	0.05 – 12	0.9935	0.28	0.86
Ellagic acid	0.05 – 12	0.9925	0.55	1.67
Ferulic acid	0.05 – 12	0.9940	0.85	2.56
Gallic acid	1-120	0.9939	0.30	0.90
Catechin	0.70 – 70	0.9905	1.50	4.53
Epigallocatechin gallate	0.10 – 70	0.9964	0.09	0.26
Kaempferol	0.05 – 12	0.9925	0.03	0.11
Luteolin	0.10 – 50	0.9931	0.34	1.04
Myricetin	0.28 – 50	0.9938	1.23	3.71
Quercetin	0.40 – 50	0.9942	0.71	2.15

Quercetin 3-glucoside	0.11 – 50	0.9997	0.07	0.20
Rutin	1-120	0.9935	0.37	1.12
Sugar Compounds	Concentration range ($g.mL^{-1}$)	(R^2)	LOD ($g.mL^{-1}$)	LOQ ($g.mL^{-1}$)
Glucose	0.5-3.5	0.9963	0.22	0.67
Fructose	0.1-2.0	0.9965	0.10	0.29
Sucrose	0.1-2.0	0.9907	0.32	0.97

2.9 Statistical analysis

All samples were analyzed in triplicates. The statistical analysis was performed by one-way analysis of variance (ANOVA) to evaluate the differences between samples and the Tukey test to find significant differences at significance level of 5 %. using Statistica 12.0 (STATSOFT Inc.) and the OriginPro® 8.0 (Northampton, MA, USA) softwares.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters of juices

Natural juices have become economically important, and their consumption is significantly increasing in the last years, due to the health benefits, pleasant taste, and practicality, making it an alternative to other drinks (Amarante et al., 2017). This study is the first to evaluate, for 90 days of storage, the physical-chemical stability and bioactive compounds of enzyme-treated feijoa juices and by the membrane microfiltration process.

The juice yields ranged from 54 to 64%, with statistical differences between the three treatments. The highest yield (64%) was obtained by enzyme-treated and pasteurized juice (P). When comparing with the fresh juice (54%), which did not use enzyme treatment, 10% increase in the amount of extracted juice was observed.

In the study by Schmidt et al. (2021), extraction of feijoa juice with other enzymes and concentrations was analyzed. It is observed that the enzyme complex (polygalacturonase, pectin methyl esterase and pectinlyase) from Pectinex improves the extraction and yield of juice, in addition to highlighting the improvement of physicochemical parameters when compared to the other enzymes studied. Commercial pectinolytic enzymes are used as processing aids for the degradation of pectin, causing solubilization of polysaccharides, improving the extraction and yield of juice (Kumar, 2015; Sandri et al., 2013; Tapre & Jain, 2014). Kaur et al. (2009) achieved a 25% increase in yield for guava juice (*Psidium guajava* L.) with the addition of pectinases and in

enzyme-treated grape juices there was an increase in yield of approximately 8.7% (Dal Magro et al., 2016). The enzyme-treated, microfiltered and pasteurized juice (MP) had a yield of 60%.

The two treatments in which enzymes were added increased juice yield. However, the lower yield value MP juice may be justified by the addition of the microfiltration step, in which it removes the largest particle size classes from the liquid. Despite the several known advantages, the application of microfiltration is still limited in the fruit juice industries, due to the known phenomenon of membrane encrustation, and for this reason, previous processing such as enzymatic treatment, filtration and centrifugation are strategies to improve the performance of microfiltration (Dahdouh et al., 2015; De Oliveira et al., 2012; Yu & Lencki, 2004).

For characterization of feijoa juice, pH, TTA, TSS, dry matter (DM), individual, total sugars and color parameters were evaluated, and the results for three treatments are presented in Table 2. It can be observed from Table 1 that, there is a significant decrease in the total solid content after microfiltration (around 50%). The dry matter of the MP juice treatment was 7.6%, while for the other two treatments it was around 13%, showing that the suspended solids were removed by the microfiltration membrane. The two treated juices showed pH variation (up and down) during the 90-day storage. It is observed that the pH values did not change during juice processing, since off all juices sampled were below 3.0, which is similar to the found for whole fruit (Amaral et al., 2019). The TTA of the enzyme-treated juices (P and MP) was higher ($p \leq 0.05$) than the control juice (F), probably due to the high activity of the pectin methyl esterase (PME) of the enzymatic preparation. The PME catalyzes the de-esterification of the methoxyl group of pectin, forming pectic acid (Dal Magro et al., 2016). This finding is corroborated by the work of Dal Magro et al. (2016) who evaluated 8 enzymatic preparations, in which the two enzymes with the highest PME activity (including Pectinex Ultra Clear) had a significant increase in the TTA parameter. Comparing the P and MP treatments in the same time of storage (Table 1), the results indicate that there are significant differences for TTA and TSS, the P treatment showed higher values of these parameters. For MP treatment, a reduction in % acidity was observed in D7 and remained constant until the end of the storage period. On the first day of storage, the three treatments showed about 10% TSS, which agrees with the results found for whole fruits, which varies from 8.56% to 11.4% (Amaral et al., 2019). However, with the follow-up of storage, there was a reduction in the content of soluble solids for both juices, and this was more pronounced in the MP

treatment, which on the 7th day decreased by 2.0 °Brix. The MP juice, presented a lower content of TTA, TSS and dry matter, during storage, possibly due to microfiltration process that eliminates larger-sized, high molecular weight gel forming materials from the feed itself (Panigrahi et al., 2018).

Organic acids are a useful index of authenticity in the fruit product. These acids, sugar and their ratios are among the main compounds found in fruit juices and play important roles in the character and quality of the flavor and organoleptic properties (Kelebek et al., 2009; Petkovsek et al., 2007).

The main portions of carbohydrates in feijoa juices on the first day of follow-up was the three simple sugars: sucrose, glucose, and fructose (Table 2). Sucrose is present in the largest amounts for feijoa juices. In fresh juice, sucrose represented 57% of the sugar content, whereas in P and MP it was 51%, followed by fructose (28% for F juice and 33% for P and MP juices) and glucose (14% for F juice and 15% for P and MP juices). The composition of these sugars agrees with the literature, for other fruit juices studied, such as orange juice (Kelebek et al., 2009), mango juice (Wibowo et al., 2015) and guava (Rojas-Garbanzo et al., 2019). The sugar profile and ratios of specific sugars have been suggested as an indicator for determining the authenticity of juice samples (Kelebek et al., 2009). The content of each sugar changed significantly during storage, with reduced sucrose content and increased glucose and fructose. In P and MP juice, the sucrose content decreased approximately 60-70% in 90 days of storage. The glucose and fructose content increased respectively, about 4 and 3-fold for P treatment and 3 and 2-fold for MP treatment, between the first and the last day of storage. For total sugars, until the end of the follow-up, the content of the MP juice increased (from 21.76 to 30.44 $g.L^{-1}$) and doubled for the P juice (24.97 to 48.91 $g.L^{-1}$). This increase in the content of total sugars for two treatments can be attributed to the hydrolysis of sucrose in the presence of acids, leading to the formation of glucose and fructose. This observation agreed with Wibowo et al. (2015), who studied citric juice and saw that during storage, the concentrations of fructose and glucose also increased, while the concentration of sucrose decreased. The decrease in the sucrose content may not coincide with the increase in the fructose and glucose content. The hydrolysis of carbohydrates, such as fibers, maybe an explanation for this since the juice pulp consists of pectins, cellulose, and hemicellulose (Wibowo et al., 2015). In our study, P juice had a greater increase in total sugars than MP juice, suggesting that as P juice did not undergo microfiltration, it still contained a higher cellulose content, and consequently more glucose remaining.

Table 2. Evaluation of pH, TTA, TSS, sucrose, glucose, fructose, total sugars and color parameters of feijoa fresh juice (F), clarified by enzymatic treatment and pasteurized juice (P) and clarified by enzymatic treatment and microfiltration, pasteurized juice (MP) during 90 days of storage

Parameter	Treatment	Storage time (days)						
		D1	D7	D14	D21	D30	D60	D90
Dry matter (%)	F	13.51 ± 0.08^{A}	-	-	-	-	-	-
	P	13.19 ± 0.01^{aB}	8.35 ± 0.12^{cA}	8.45 ± 0.16^{bcA}	8.64 ± 0.09^{bA}	8.66 ± 0.05^{bA}	8.44 ± 0.05^{bcA}	8.27 ± 0.06^{cA}
	MP	7.64 ± 0.03^{aC}	6.37 ± 0.27^{bcB}	6.37 ± 0.09^{bcB}	6.44 ± 0.07^{bB}	6.07 ± 0.02^{cB}	6.16 ± 0.04^{bcB}	6.30 ± 0.11^{bcB}
pH	F	2.74 ± 0.02^{A}	-	-	-	-	-	-
	P	2.54 ± 0.02^{dB}	2.61 ± 0.00^{cdA}	2.64 ± 0.01^{bcA}	2.57 ± 0.07^{cdB}	2.56 ± 0.06^{cdA}	2.76 ± 0.01^{aA}	2.73 ± 0.01^{abB}
	MP	2.71 ± 0.01^{cA}	2.53 ± 0.01^{dB}	2.59 ± 0.00^{fB}	2.94 ± 0.02^{aA}	2.47 ± 0.02^{eA}	2.58 ± 0.01^{fB}	2.76 ± 0.00^{bA}
Total Titratable Acidity (TTA)	F	1.35 ± 0.02^{C}	-	-	-	-	-	-
	P	1.67 ± 0.03^{bcA}	1.65 ± 0.02^{bcA}	1.74 ± 0.02^{aA}	1.75 ± 0.04^{aA}	1.66 ± 0.03^{bcA}	1.62 ± 0.01^{cA}	1.72 ± 0.03^{abA}
	MP	1.60 ± 0.01^{aB}	1.32 ± 0.01^{bB}	1.31 ± 0.04^{bB}	1.26 ± 0.01^{bcB}	1.23 ± 0.01^{cB}	1.24 ± 0.02^{cB}	1.27 ± 0.02^{bcB}
Total Soluble Solids (TSS)	F	10.53 ± 0.06^{A}	-	-	-	-	-	-
	P	10.13 ± 0.06^{abB}	10.17 ± 0.06^{aA}	10.10 ± 0.10^{abA}	9.60 ± 0.10^{dA}	9.90 ± 0.00^{cA}	9.96 ± 0.06^{cA}	9.57 ± 0.06^{dA}
	MP	10.03 ± 0.00^{aB}	8.07 ± 0.06^{bcB}	7.97 ± 0.06^{cdB}	7.90 ± 0.00^{dB}	8.10 ± 0.00^{bB}	7.17 ± 0.06^{fB}	7.60 ± 0.00^{eB}
TSS/TTA	F	7.82 ± 0.19^{A}	-	-	-	-	-	-
	P	6.02 ± 0.09^{abC}	6.16 ± 0.06^{aA}	5.79 ± 0.01^{bcB}	5.50 ± 0.19^{dB}	5.97 ± 0.10^{abB}	6.13 ± 0.06^{aA}	5.57 ± 0.06^{cdB}
	MP	6.33 ± 0.05^{abB}	6.10 ± 0.06^{bcA}	6.08 ± 0.14^{bcA}	6.26 ± 0.06^{bcA}	6.57 ± 0.19^{aA}	5.76 ± 0.08^{dB}	6.00 ± 0.08^{cdA}
Sucrose ($g.L^{-1}$)	F	15.02 ± 0.79^{A}	-	-	-	-	-	-
	P	12.77 ± 0.26^{bB}	14.42 ± 1.16^{aB}	13.18 ± 0.87^{abB}	7.97 ± 0.26^{cB}	3.12 ± 0.05^{eB}	4.18 ± 0.13^{dB}	4.48 ± 0.05^{dB}
	MP	11.25 ± 0.85^{dB}	17.52 ± 0.80^{abA}	15.08 ± 0.16^{cA}	15.87 ± 0.22^{bcA}	18.82 ± 0.20^{aA}	8.88 ± 0.74^{eA}	3.39 ± 0.20^{fA}
Glucose ($g.L^{-1}$)	F	3.76 ± 0.12^{AB}	-	-	-	-	-	-
	P	3.94 ± 0.05^{eA}	8.79 ± 0.10^{dA}	15.47 ± 0.22^{cA}	18.52 ± 0.81^{aA}	$16,71 \pm 0.65^{bcA}$	16.06 ± 0.57^{bcA}	16.76 ± 0.84^{bA}
	MP	3.39 ± 0.21^{cB}	5.43 ± 0.33^{bB}	5.54 ± 0.04^{bB}	6.38 ± 0.12^{bB}	9.86 ± 0.23^{aB}	10.46 ± 0.75^{aB}	9.75 ± 0.41^{aB}
Fructose ($g.L^{-1}$)	F	7.32 ± 0.23^{B}	-	-	-	-	-	-
	P	8.25 ± 0.62^{fA}	16.22 ± 0.24^{eA}	25.61 ± 0.92^{dA}	28.97 ± 0.41^{abA}	29.94 ± 1.12^{aA}	26.95 ± 0.57^{cdA}	27.67 ± 0.77^{bcA}
	MP	7.11 ± 0.46^{cB}	11.07 ± 0.56^{bB}	11.11 ± 0.08^{bB}	12.55 ± 0.15^{bB}	18.20 ± 0.23^{aB}	18.62 ± 1.13^{aB}	17.30 ± 0.45^{aB}
Total sugar ($g.L^{-1}$)	F	26.10 ± 1.12^{A}	-	-	-	-	-	-
	P	24.97 ± 0.63^{dA}	40.15 ± 0.38^{cA}	54.25 ± 1.82^{aA}	55.46 ± 0.85^{aA}	49.77 ± 1.73^{bA}	47.19 ± 0.86^{bA}	48.91 ± 1.73^{bA}
	MP	21.76 ± 1.52^{eB}	34.02 ± 1.69^{cB}	31.74 ± 0.26^{cdB}	34.81 ± 0.42^{cB}	46.87 ± 0.49^{aB}	37.96 ± 1.23^{bA}	30.44 ± 0.63^{dB}
*L**	F	42.28 ± 0.40^{C}	-	-	-	-	-	-
	P	45.65 ± 0.12^{cB}	51.30 ± 0.76^{aB}	51.08 ± 0.53^{aB}	50.85 ± 0.21^{aB}	47.79 ± 0.03^{bB}	46.56 ± 0.01^{bcB}	45.33 ± 0.95^{cB}

	MP	62.53 ± 0.18^{cA}	68.71 ± 0.06^{aA}	68.23 ± 0.06^{aA}	64.44 ± 0.14^{bA}	64.21 ± 0.08^{bA}	63.98 ± 0.29^{bA}	61.74 ± 0.82^{cA}
*a**	F	2.19 ± 0.22^{A}	-	-	-	-	-	-
	P	2.05 ± 0.05^{bA}	0.22 ± 0.03^{eA}	0.30 ± 0.06^{eA}	0.24 ± 0.01^{eA}	0.91 ± 0.06^{dA}	1.61 ± 0.03^{cA}	2.29 ± 0.19^{aA}
	MP	-1.85 ± 0.02^{bB}	-2.90 ± 0.02^{eB}	-3.47 ± 0.02^{fB}	-2.92 ± 0.01^{eB}	-2.46 ± 0.01^{dB}	-2.01 ± 0.02^{cB}	-1.10 ± 0.05^{aB}
*b**	F	15.50 ± 0.10^{C}	-	-	-	-	-	-
	P	17.18 ± 0.09^{dB}	19.35 ± 0.15^{cB}	19.67 ± 0.13^{bB}	19.98 ± 0.02^{aB}	19.33 ± 0.16^{cB}	19.36 ± 0.01^{cB}	19.39 ± 0.09^{bcB}
	MP	25.49 ± 0.13^{aA}	21.36 ± 0.15^{dA}	22.56 ± 0.00^{bA}	21.83 ± 0.29^{cA}	22.11 ± 0.19^{bcA}	22.40 ± 0.12^{bA}	21.89 ± 0.17^{cA}
ΔΣ	F	-	-	-	-	-	-	-
	P	-	6.57 ± 0.47^{aB}	6.23 ± 0.58^{aA}	6.18 ± 0.22^{aA}	3.25 ± 0.21^{bB}	2.41 ± 0.13^{bB}	2.37 ± 0.82^{bB}
	MP	-	7.51 ± 0.12^{aA}	6.62 ± 0.15^{bA}	4.26 ± 0.25^{cB}	3.82 ± 0.10^{deA}	3.42 ± 0.06^{eA}	3.85 ± 0.12^{dA}
*C**	F	15.65 ± 0.13^{C}	-	-	-	-	-	-
	P	17.30 ± 0.09^{dB}	19.35 ± 0.15^{cB}	19.67 ± 0.13^{bB}	19.98 ± 0.02^{aB}	19.35 ± 0.16^{cB}	19.43 ± 0.01^{bcB}	19.53 ± 0.08^{bcB}
	MP	25.55 ± 0.13^{aA}	21.55 ± 0.15^{eA}	22.83 ± 0.00^{bA}	22.02 ± 0.28^{cdA}	22.25 ± 0.19^{cdA}	22.49 ± 0.12^{bcA}	21.92 ± 0.17^{deA}
Hue (°)	F	81.95 ± 0.77^{C}	-	-	-	-	-	-
	P	83.21 ± 0.12^{dB}	89.35 ± 0.09^{aB}	89.14 ± 0.17^{aB}	89.32 ± 0.02^{aB}	87.31 ± 0.19^{bB}	85.24 ± 0.08^{cB}	83.27 ± 0.57^{dB}
	MP	94.15 ± 0.02^{eA}	97.74 ± 0.01^{bA}	98.75 ± 0.05^{aA}	97.61 ± 0.11^{bA}	96.36 ± 0.04^{cA}	95.14 ± 0.03^{dA}	92.87 ± 0.10^{fA}

Different uppercase superscript letters in the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). Different lowercase superscript letters in the same line indicate statistically significant difference for the same treatment over the storage period by Tukey test ($p<0.05$). Values are the mean of 3 replications (± standard deviation). Total dry matter expressed in weight/weight. TTA expressed as percent of acid citric. TSS expressed in °Brix. L* brightness, a* redness/greenness, b* yellowness/blueness, ΔΣ ab color difference, C* chroma, hue angle.

As for the content of total sugars, the value found for feijoa juices (about 23 $g.L^{-1}$) was lower than other fruit juices, such as orange (about 120 $g.L^{-1}$) and guava (38 $g.L^{-1}$) (Kelebek et al., 2009; Rojas-Garbanzo et al., 2019). Comparing the two juices treated in the same storage time, it is observed that the P treatment had a higher concentration of glucose, fructose and total sugars. In microfiltration, depending on working conditions (enzymatic treatment and operating pressure), monosaccharides and pectin showed greater retention. This fact can be attributed to the concentration polarization, cake formation and pore blocking, which did not allow the sugars to pass to the permeate side (Machado et al., 2016; Urošević et al., 2017).

3.2. Juice color parameters

Color is one of the most important attributes affecting consumer perception of quality and it plays a key role in food preference and acceptability and may even influence sweetness perception (Barrett et al., 2010). The MP juice had the highest values of L^* and b^*, that indicates greater brightness and yellow color, respectively (Table 2). For the component a^*, the treatments F and P are characterized by positive values (slightly redness), while in the treatment MP by negative values (slightly greenness). Over the storage period, the b^* value of MP treatment decreased (21), while for P treatment it increased (19). The $\Delta\Sigma$ was measured to determine the total color difference of the samples over the shelf life compared to the first day in which the juice would be fresher. For both juices the value of $\Delta\Sigma$ decreased significantly during the storage period, namely as it extends, the total color difference for the first day is lower. The MP juice showed a Hue angle of about 90, whereas the P and F juices showed 80, all in the first quadrant of the CIELab color chart, indicating a variation between yellow tones, and with different intensities due to the differences in the Chroma factor. The results allow to identify that the MP treatment presented a pronounced yellow color, has greater brightness and superior color intensity than the other treatments (L^*, b^* Chroma and hue parameters). Enzymatic treatment and microfiltration significantly influenced the color of the juices. For the two enzyme-treated and pasteurized juices, there was a tendency to increase the color, luminosity and color intensity of the juice, when compared to fresh juice. However, the reduction of turbidity was achieved more intensely with microfiltration.

Fruit juices are generally cloudy due to the presence of vegetable residues, which are not soluble in water (fiber, cellulose, hemicellulose, starch and fat) and the particles of colloidal size (pectin, protein and soluble parts of the starch, certain polyphenols and

their oxidized ones). These dispersed substances must be partially or completely eliminated in the production of clear juice to avoid blurring and subsequent deposition and to improve the sensory characteristics (taste, odor and color). In practice, the most widely used method is a combination of physical, chemical and mechanical methods. The enzymatic hydrolysis of macromolecules in juices is very important, as it allows high permeate flux in membrane filtration processes. Also, microfiltration removes almost all the suspended particles that make the juice cloudy in this way, a completely clear juice is obtained (Dahdouh et al., 2018; Urošević et al., 2017).

3.3. Quantification of ascorbic acids and carotenoids

The effect of the three treatments was also analyzed to vitamin C and carotenoids profile in the feijoa juices and the results can be seen in Table 3. The vitamin C content was higher in the MP treatment (6.4 mg.100 mL^{-1}), while the F and P treatments had similar concentrations (4.0 mg.100 mL^{-1}). In Brazil, the dietary reference intake (DRI) for vitamin C for adults is 45 mg per day, therefore, the daily consumption of one american cup (190 mL) of feijoa juice makes up around 30% of the DRI. This shows that feijoa juice can contribute to vitamin C intake.

During the storage period, the two treated juices had their concentration increased by around 20% and 50%, with the highest concentration in D7 (7.96 mg.100 mL^{-1}) and in D30 (8.17 mg.100 mL^{-1}) for MP and P, respectively. However, it was seen that there was a reduction in the vitamin C content of 75.5% for MP juice and 64.5% for P juice, after 30 days of storage. Amarante et al., (2017) studying the quality of feijoas, found that the vitamin C concentration in the fruits also increased by 24% during storage, both in the skin and in the pulp. In most fruits and vegetables, substantial loss of vitamin C is common during storage due to enzymatic oxidation of ascorbic acid, mainly by the action of ascorbate oxidase (Cánovas et al., 2020). However, pectin degradation intermediates, such as D-galacturonate, already have been identified as a precursor to ascorbic acid in plants. That is, the degradation of the cell wall leads to the release of D-galacturonate, that alternative precursor, increases the ascorbic acid biosynthesis (Locato et al., 2013). This could justify, in the case of feijoa, the increase in vitamin C observed during storage. It is also important to comment that vitamin C is more stable in an acid, therefore, the maintenance of the vitamin C content in feijoa juice could have been favored by the low pH values of the fruits.

Table 3. Vitamin C and Carotenoids profile and in the three feijoa juices: fresh juice (F), clarified by enzymatic treatment and pasteurized juice (P) and clarified by enzymatic treatment and microfiltration, pasteurized juice (MP) during 90 days of storage

		Storage time (days)						
Parameter	**Treatment**	**D1**	**D7**	**D14**	**D21**	**D30**	**D60**	**D90**
Vitamin C (mg ascorbic acid.100 mL^{-1})	F	4.09 ± 0.31^{B}	-	-	-	-	-	-
	P	4.06 ± 0.11^{dB}	6.88 ± 0.28^{cB}	7.93 ± 0.19^{abA}	7.28 ± 0.38^{bcA}	8.17 ± 0.09^{aA}	2.90 ± 0.35^{eA}	3.08 ± 0.03^{eA}
	MP	6.42 ± 0.23^{bA}	7.96 ± 0.22^{aA}	7.91 ± 0.01^{aA}	6.24 ± 0.55^{bA}	7.03 ± 0.47^{abA}	1.72 ± 0.11^{cB}	1.85 ± 0.48^{cB}
Lutein (µg.100mL^{-1})	F	11.53 ± 1.39^{A}	-	-	-	-	-	-
	P	11.84 ± 0.90^{aA}	6.91 ± 0.26^{b}	7.26 ± 0.12^{b}	7.24 ± 0.06^{b}	7.17 ± 0.19^{b}	7.31 ± 0.11^{b}	7.60 ± 0.20^{b}
	MP	ND	ND	ND	ND	ND	ND	ND
Zeaxanthin (µg.100mL^{-1})	F	1.25 ± 0.08^{A}	-	-	-	-	-	-
	P	0.85 ± 0.06^{B}	ND	ND	ND	ND	ND	ND
	MP	ND	ND	ND	ND	ND	ND	ND
Cryptoxanthin (µg.100mL^{-1})	F	1.06 ± 0.01^{A}	-	-	-	-	-	-
	P	1.22 ± 0.10^{bA}	0.93 ± 0.12^{c}	1.27 ± 0.00^{b}	1.77 ± 0.12^{a}	1.60 ± 0.10^{a}	0.92 ± 0.07^{c}	0.96 ± 0.07^{c}
	MP	ND	ND	ND	ND	ND	ND	ND
β-carotene (µg.100mL^{-1})	F	33.17 ± 2.69^{AB}	-	-	-	-	-	-
	P	37.15 ± 3.36^{aA}	31.80 ± 2.93^{abA}	33.77 ± 2.16^{abA}	31.85 ± 1.89^{abA}	31.36 ± 1.34^{ab}	30.80 ± 1.24^{b}	29.74 ± 0.81^{b}
	MP	30.51 ± 0.24^{aB}	31.08 ± 0.03^{aA}	29.31 ± 0.56^{bB}	28.72 ± 0.19^{bB}	ND	ND	ND
Total Carotenoids (µg.100mL^{-1})	F	46.94 ± 4.01^{A}	-	-	-	-	-	-
	P	51.14 ± 4.16^{aA}	39.67 ± 3.24^{bA}	42.74 ± 2.28^{bA}	40.85 ± 1.83^{bA}	40.13 ± 1.31^{b}	39.04 ± 1.39^{b}	38.30 ± 0.99^{b}
	MP	30.51 ± 0.24^{aB}	31.08 ± 0.03^{aB}	29.31 ± 0.56^{bB}	28.72 ± 0.19^{bB}	ND	ND	ND

Different uppercase superscript letters in the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$). Different lowercase superscript letters in the same line indicate statistically significant difference for the same treatment over the storage period by Tukey test ($p<0.05$). Values are the mean of 3 replications (± standard deviation).

The carotenoid profile of feijoa juices (Table 3), shows that β-carotene was the principal carotenoid in the all treatments and in the MP juice, only this carotenoid was detected. Lutein was the second major carotenoid found in the F and P treatments. Except of zeaxanthin, for all other carotenoids, as well as for total carotenoids, the treatments F and P had no difference between them. However, the P and MP treatments had a difference between each other for β-carotene and total carotenoid content, once microfiltered juice presented a lower amount for these two parameters. The results of this study indicate that storage leads to a decrease in the proportion of carotenoids in the treated juices. Regarding the total carotenoid content, P treatment had a slight decrease after D1, but it remained constant until day 90 of storage, and for MP treatment, the concentration decreased after day 7, and from D30 onwards it cannot be quantified.

3.4. Identification and quantification of phenolic compounds

This study identified and characterized the phenolic compounds in feijoa juices, using HPLC–ESI–MS/MS analysis. The HPLC–DAD–MS/MS method allowed for the separation and identification of the phenolic compounds based on the interpretation of their fragmentation patterns obtained from the mass spectra, retention time, and by comparison of the mass spectra with the literature. In addition, 13 standard compounds were also used to aid in the identification of the compounds. The three treatments showed similar profile of phenolic compounds, although different regarding the concentration of the compounds. A total of 44 phenolic compounds were identified in the feijoa juices, as shown in Table 4.

The P and MP feijoa juices had a similar percentage of the phenolic classes and it was mainly characterized by the presence of hydroxybenzoic acids. This class represented about 64% and 70% of total phenolic compounds in P and MP treatment, respectively, and, flavan-3-ols, hydroxycinnamic acids, flavonol and flavones constituted about 14, 6-12, 9-10 and 1%, respectively, with slight variations over the 90 days of storage, for both treatments. Fresh juice had a different profile, the main classes found were flavonols and hydroxycinnamic acids with 29 and 27%, respectively, 22% for hydroxybenzoic acids, 10% for flavan-3-ols and 9% for flavones. Considering that the identification of some of these phenolic compounds has already been described in detail in feijoa fruit and fresh juice (Aoyama et al., 2018; Peng et al., 2020; H. Schmidt et al., 2020), only the molecules that were not previously identified in this specie, are discussed in this work.

Peak **6**, **11, 15 and 20** had a $[M–H]^-$ at *m/z* 633 and main fragment ion at *m/z* 301 (MS^2), with a characteristic loss of galloyl glucose, thereby identifying it as corilagin (HHDP-galloyl-hexoside isomer I, II, III and IV, respectively) (Bakr et al., 2017). Compound at peak **12** was identified as valoneic acid dilactone (*m/z* 469) and fragments ion at *m/z* 425 and 299 (Bakr et al., 2017). In the MS^2 spectrum, peak **14** showed a base peak at *m/z* 951 and an intense secondary peak at *m/z* 301, consistent with the fragmentation pattern of trisgalloyl HHDP glucose (Singh et al., 2016). Compound **23** was identified as casuarinin (galloyl-bis-HHDP-glucose), with ion at *m/z* 935 and MS/MS fragment ions at *m/z* 633 (loss of HHDP) and 301 (loss of galloyl-HHDP-glucose) (Zhu et al., 2015). Galloyl-bis-HHDP-glucose is a basic unit of many ellagitannins. Compound **24** had a negative ion at *m/z* 497, which gave fragment ions at *m/z* 335, 179 and 135 (MS^2), and was identified as di-caffeoyl shikimic acid (Khallouki et al., 2018). These compounds had not previously been identified in feijoa.

The three principal compounds found in the treated juices belong to hydroxybenzoic acids, namely corilagin, pedunculagin isomers and trisgalloyl HHDP glucose. In addition to these compounds, (Epi)catechin derivative, methyl cirsimaritin coumaric acid, cirsilineol methyl caffeoyl, casuarinin, catechin and epicatechin and procyanidin B, were other abundant phenolic compounds identified in these two treatments. Peng et al. (2020) analyzed fresh juices from different feijoa cultivars and identified only seven phenolic compounds and the major compounds were procyanidin B1 and catechin. In the present study, pedunculagin isomer V was a major compound identified in enzyme treated feijoa juices with 5477.57 e 5094.80 μg.100 mL^{-1} for the P and MP treatment, respectively (Table 5). This compound was present in high concentrations in feijoa fruit in a previous study (H. Schmidt et al., 2020). Pedunculagin isomer is derived from ellagic acid and well-known as ellagitannins and this compound show anti-mutagenic and anti-oxidative activities (Berdowska et al., 2018; Rojas-Garbanzo et al., 2019).

On the other hand, in the fresh juice the principal compound was cirsilineol methyl caffeoyl (151.50 μg.100 mL^{-1}), followed by methyl cirsimaritin coumaric acid (131.48 μg.100 mL^{-1}), which belongs to hydroxycinnamic acids. The other main compounds found in this treatment are the flavonols: quercetin pentoside, isorhamnetin pentoside, quercetin glucoside. Hydroxycinnamic acid derivatives and flavonols are widespread in plants and belong to the group of polyphenols, are of interest due to their antioxidant activity.

Table 4. Chromatographic and Spectroscopic Characteristics of phenolic compounds in the three feijoa juices: fresh juice (F), clarified by enzymatic treatment and pasteurized juice (P) and clarified by enzymatic treatment and microfiltration, pasteurized juice (MP) obtained by LC-DAD-ESI-MS/MS

Peak[a]	Compound	T_R (min)[b]	λ_{max} (nm)[c]	$[M-H]^-$ (*m/z*)	MS^2 (-) (*m/z*)[d]
1	(Epi)catechin derivative	13.0	280		**289**; 191
2	Pedunculagin isomer I	13.4	320	783.0545	**301**; 275; 191
3	Caffeoyl Hexoside derivative	13.4	320	539.1194	341; 179
4	Pedunculagin Isomer II	13.9	320	783.0708	**301**; 275
5	Pedunculagin isomer III	14.1	320	783.0710	**301**; 275; 191
6	Corilagin (HHDP-galloyl-hexoside isomer I)	14.7	320	633.0759	481; 421; **301**; 275
7	Phyllanthusiin E methyl ester	15.2	320	305.0689	225; 191; 96
8	Not identified	15.5	320	705.2131 352.1054	308; 218; 160; 146
9	Castalagin	15.8	280	933.0656	**301**; 275
10	Pedunculagin isomer IV	16.0	289	783.0695	**301**; 275
11	Corilagin (HHDP-galloyl-hexoside isomer II)	16.1	280	633.0734	481; **301**; 275
12	Valoneic acid dilactone	16.2	280	469.0044	425; 299
13	Pedunculagin isomer V	16.3	280	783.0555	423; 363; **301**; 275
14	Trisgalloyl HHDP glucose isomer I	16.4	280	951.0748	783; 481; **301**; 275
15	Corilagin (HHDP-galloyl-hexoside isomer III)	16.8	320	633.0782	**301**; 275
16	Procyanidin B dimer	17.0	280	577.1342	451; 425; 407; 339; **289**; 245; 125
17	Caffeic acid derivative	17.4	320	491.1418	323; 179; 121
18	Caffeoyl Hexoside	18.4	278	341.0892	179; 135
19	Derivative ellagic acid	18.5	279	588.0664;	**301**; 249; 169
20	Corilagin (HHDP-galloyl-hexoside isomer IV)	18.8	280	633.0731	**301**; 275; 249; 169; 125
21	Catechin	18.9	275	289.0741	221; 203; **123**; 109
22	(Epi)catechin Dimer or Procyanidin B dimer	19.0	276	579.1497	**289**; 245; 221; 203; 191.0227; 179; 123
23	Casuarinin	19.5	280	935.0836	633; **301**; 275; 249
24	Di-caffeoyl shikimic acid	20.1	280	497.1343	**335**; 179; 135
25	Procyanidin B dimer	20.3	280	577.1382	43; 421; 407; 339; **289**; 245; 125
26	Phyllanthusiin E	21.0	360	291.0165	247; 191
27	Ellagic acid derivative	21.2	280	799.0647	745; 435; **301**; 275
28	Epicatechin	21.7	280	289.0728	191
29	Not identified	22.2	280	537.2014	**327**; 195; 165; 119; 113

30	Quercetin 3 glucoside	22.6	360	463.0526	301
31	Cirsilineol	22.7	360	539.2166	**491**; 343; 329; 195; 165
32	(Epi)catechin Dimer or Procyanidin B dimer	23.4	280	602.2140 575.1929	**289**; 195
33	(Epi)Catechin derivative	23.4	280	729.1468	577; 451; 407; **289**; 125
34	Quercetin pentoside	25.5	360	433.0431	**301**
35	Isorhamnetin hexoside	26.3	363	477.0695	**315**; 299
36	Quercetin 3 glucoside	27.2	357	463.0846	301
37	Ellagic acid	27.4	365	301.0011	245; 257; 229; 201; 185
38	Not identified	27.6	367	793.0883	**301**; 283
39	Methyl Cirsimaritin coumaric acid	27.6	368	491.0827	476; **328**; 313
40	Quercetin pentoside	28.2	360	433.0781	**301**; 271
41	Quercetin pentoside	28.7	360	433.0771	**301**; 261; 191
42	Quercetin derivative	28.8	360	415.1982	**301**; 179
43	Not identified	29.1	280	417.2122 407.1862	**301**; 191
44	Quercetin pentoside	29.6	360	433.0804	**301**; 271; 191; 178
45	Cirsilineol derivative	30.0	360	423.0054	343; **328**; 300, 279; 191
46	Caffeoyl acid derivative	31.7	320	555.3113	519; **179**
47	Cirsilineol Methyl Caffeoyl	33.5	367	551.1036	**343**; 328; 313
48	Cirsilineol	34.1	360	521.0937	491; **343**

[a] Retention time on the C18 LiChrospher® (5 μm) column

[b] Maximum absorbance. Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid

[c] In the MS/MS, the most abundant ion shown in boldface.

As shown in Table 5, variations were observed in the total phenolic content of fresh feijoa juice and treated feijoa juices. The fresh juice presented a much smaller amount of total phenolic compounds (1591.18 μg.100 mL^{-1}), while the treated juices presented 21,061.30 and 22,198.71 μg.100 mL^{-1}, for P and MP, respectively. These two treatments were not significantly different from each other, but both were for fresh juice. Growing evidence from epidemiological studies, *in vivo* and *in vitro* studies, as well as clinical trials and meta-analyses suggest that high intake of polyphenols, can reduce the risk of chronic diseases, improving human health (Costa et al., 2017).

In fruit juice processing, pectinases hydrolyze a wide range of polysaccharides structural from the plant cell walls. This degradation helps to release bioactive compounds, thus enhancing the recovery of polyphenols, which otherwise would be lost. The higher levels of the phenolic compounds from enzyme treated juices relative to the fresh juice indicate a breakdown of the plant cell walls with subsequent recovery of the phenolic compounds. This finding is corroborated by several studies (Dal Magro et al., 2016; Siddiq et al., 2018) who found concentrations of total phenolic was significantly higher in fruit juices prepared with enzymatic preparations that had in its composition pectinases.

To the best of our knowledge, this is the first study in which the changes in phytochemical properties of MF-clarified feijoa juice during storage are reported. When comparing the two treated juices, it is observed that the process of microfiltration not allowed a higher retention of bioactive compounds, as observed in other studies (Panigrahi et al., 2018). However, over the storage period, the content of phenolic compounds decreased more severely in the MP treatment. During 3 months of storage at 20 °C, the total phenols content of MP juice decreased around 60%. On the other hand, it is observed that in the P treatment the amount of total phenolic compounds increased up to 21th day, and afterwards there was a reduction around 30%. Nevertheless, for this treatment, the TPC values in D1 and D90 were statistically equal.

In the juice industry, one of the principal challenges is to produce juices with characteristics similar to whole fruits, assuring consumers a quality product. Understanding the stability of product characteristics during storage can support producers to identify appropriate storage conditions (Saeeduddin et al., 2015). The development of products from native species, such as feijoa, contributes to the conservation of biodiversity, in addition to providing income for local populations.

Table 5. Content (μg.100 mL^{-1}) of phenolic compounds in the three feijoa juices: fresh juice (F), clarified by enzymatic treatment and pasteurized juice (P) and clarified by enzymatic treatment and microfiltration, pasteurized juice (MP) during 90 days of storage

	Compound	Treatment	Storage time (days)						
			D1	D7	D14	D21	D30	D60	D90
1	(Epi)catechin derivative	F	32.53 ± 0.70^{B}	-	-	-	-	-	-
		P	836.32 ± 42.38^{eA}	980.65 ± 12.88^{deA}	1139.72 ± 110.90^{dA}	2534.63 ± 116.80^{aA}	2105.14 ± 193.08^{bA}	1462.62 ± 77.52^{cA}	1705.51 ± 51.87^{cA}
		MP	860.25 ± 21.83^{aA}	566.99 ± 22.56^{bB}	548.58 ± 54.30^{bcB}	516.37 ± 27.43^{bcdB}	520.62 ± 47.86^{bcdB}	459.43 ± 33.53^{cdB}	444.26 ± 17.44^{eB}
2	Pedunculagin isomer I	F	33.77 ± 0.69^{C}	-	-	-	-	-	-
		P	411.94 ± 27.74^{bB}	502.94 ± 15.88^{aA}	424.68 ± 7.76^{bA}	n.d	n.d	n.d	n.d
		MP	579.18 ± 16.61^{aA}	344.80 ± 14.35^{bB}	338.77 ± 33.53^{bB}	306.09 ± 18.33^{b}	n.d	n.d	n.d
3	Caffeoyl Hexoside derivative	F	25.90 ± 0.54^{C}	-	-	-	-	-	-
		P	68.26 ± 2.88^{bB}	82.05 ± 0.91^{aA}	68.32 ± 0.42^{bA}	70.74 ± 1.61^{bA}	35.10 ± 2.86^{dB}	37.96 ± 1.62^{dA}	44.05 ± 1.27^{cA}
		MP	94.29 ± 6.91^{aA}	60.62 ± 3.90^{bB}	63.43 ± 5.65^{bA}	57.19 ± 3.52^{bB}	57.40 ± 0.40^{bA}	22.30 ± 0.76^{cB}	32.24 ± 1.52^{cB}
4	Pedunculagin isomer II	F	27.51 ± 0.02^{C}	-	-	-	-	-	-
		P	3428.64 ± 110.66^{cdB}	3953.00 ± 60.00^{bA}	3829.92 ± 123.08^{bcA}	5000.85 ± 179.32^{aA}	4120.99 ± 324.34^{bA}	4067.07 ± 234.56^{bA}	3147.22 ± 89.91^{dA}
5	Pedunculagin isomer III	MP	2653.14 ± 72.06^{aA}	1779.32 ± 64.19^{cB}	1795.81 ± 172.03^{cB}	1707.20 ± 99.51^{cdB}	1909.02 ± 103.50^{bcB}	2082.31 ± 75.56^{bB}	1463.11 ± 25.65^{dB}
6	Corilagin	F	20.74 ± 0.51^{C}	-	-	-	-	-	-
		P	2493.78 ± 115.93^{dB}	3126.33 ± 71.85^{abcA}	3369.01 ± 243.01^{abA}	3432.00 ± 160.48^{aA}	$2906.44 \pm 266.23^{bcdA}$	2479.33 ± 173.50^{dA}	2653.48 ± 91.81^{cdA}
		MP	2802.05 ± 81.86^{aA}	1842.82 ± 60.37^{bB}	1861.77 ± 191.01^{bB}	1767.11 ± 101.12^{bB}	1945.54 ± 62.35^{bB}	1874.63 ± 67.57^{bB}	1164.61 ± 26.27^{cB}
7	Phyllanthusiin E methyl ester	F	26.21 ± 0.51	-	-	-	-	-	-
		P	n.d	n.d	n.d	n.d	n.d	n.d	n.d
		MP	n.d	n.d	n.d	n.d	n.d	n.d	n.d
8	Not identified	F	24.03 ± 1.55^{C}	-	-	-	-	-	-
		P	55.89 ± 2.85^{cdB}	69.78 ± 0.90^{aA}	60.56 ± 5.50^{bcA}	67.57 ± 3.17^{abA}	58.99 ± 4.44^{bcA}	48.47 ± 1.32^{dA}	55.37 ± 2.01^{cd}
		MP	70.27 ± 0.16^{aA}	45.59 ± 4.76^{bB}	49.65 ± 3.94^{bB}	46.82 ± 3.48^{bB}	49.41 ± 1.09^{bB}	34.33 ± 0.51^{cB}	n.d
9	Castalagin	F	23.16 ± 0.28^{C}	-	-	-	-	-	-
		P	524.55 ± 31.76^{dB}	672.58 ± 29.80^{bcA}	813.05 ± 40.13^{aA}	738.87 ± 61.96^{abA}	616.14 ± 55.73^{cdA}	402.66 ± 15.07^{eA}	516.73 ± 6.46^{dA}
		MP	607.88 ± 18.05^{aA}	378.51 ± 25.81^{bB}	379.09 ± 25.41^{bB}	361.35 ± 27.51^{bB}	386.12 ± 22.05^{bB}	386.34 ± 9.64^{bA}	202.42 ± 14.13^{cB}
10	Pedunculagin isomer IV	F	n.d	-	-	-	-	-	-
		P	n.d	n.d	n.d	n.d	n.d	n.d	n.d
11	Corilagin isomer II	MP	n.d	n.d	n.d	n.d	n.d	n.d	n.d

	Compound	Treatment	Storage time (days)						
			D1	D7	D14	D21	D30	D60	D90
12	Valoneic acid dilactone	F	n.d	-	-	-	-	-	-
		P	n.d	n.d	n.d	n.d	n.d	n.d	n.d
		MP	n.d	n.d	n.d	n.d	n.d	n.d	n.d
13	Pedunculagin isomer V	F	123.85 ± 5.79^{C}	-	-	-	-	-	-
		P	5477.57 ± 203.81^{cA}	6675.34 ± 170.21^{bA}	6707.33 ± 498.50^{bA}	7647.96 ± 314.52^{aA}	6356.31 ± 573.73^{bcA}	4401.72 ± 112.72^{dA}	5544.19 ± 170.27^{cA}
		MP	5094.80 ± 147.18^{aB}	3492.69 ± 120.25^{bB}	3529.25 ± 347.99^{bB}	3353.20 ± 200.74^{bB}	3683.87 ± 134.19^{bB}	3530.41 ± 131.33^{bB}	2178.09 ± 40.41^{cB}
14	Trisgalloyl HHDP glucose isomer I	F	n.d	-	-	-	-	-	-
		P	n.d	n.d	n.d	n.d	n.d	n.d	n.d
15	Corilagin isomer III	MP	n.d	n.d	n.d	n.d	n.d	n.d	n.d
16	Procyanidin B dimer	F	n.d	-	-	-	-	-	-
		P	n.d	n.d	n.d	n.d	n.d	n.d	n.d
		MP	n.d	n.d	n.d	n.d	n.d	n.d	n.d
17	Caffeic acid derivative	F	24.59 ± 0.95^{B}	-	-	-	-	-	-
		P	19.69 ± 1.36^{bC}	19.37 ± 0.31^{bB}	19.93 ± 0.05^{bB}	21.90 ± 0.46^{bB}	19.98 ± 0.57^{bB}	54.01 ± 2.69^{aA}	54.40 ± 1.10^{aA}
		MP	96.20 ± 0.33^{aA}	29.43 ± 0.75^{bcA}	32.13 ± 1.45^{bA}	30.66 ± 1.85^{bcA}	31.90 ± 2.72^{bcA}	27.20 ± 2.03^{cB}	22.36 ± 0.83^{dB}
18	Caffeoyl Hexoside Derivative ellagic acid	F	35.72 ± 1.08^{C}	-	-	-	-	-	-
		P	171.61 ± 11.78^{dB}	228.55 ± 1.31^{abcA}	252.40 ± 24.60^{aA}	234.04 ± 7.72^{abA}	195.95 ± 12.00^{cdA}	192.14 ± 6.17^{dA}	203.64 ± 0.61^{bcdA}
19		MP	211.16 ± 5.13^{aA}	147.83 ± 14.45^{bB}	155.00 ± 15.32^{bB}	148.45 ± 8.13^{bB}	$164.60 \pm 11.16b^{B}$	141.22 ± 8.02^{bB}	151.83 ± 5.61^{bB}
20	Corilagin isomer IV	F	37.42 ± 0.88^{B}	-	-	-	-	-	-
		P	522.28 ± 23.13^{abA}	575.41 ± 34.71^{aA}	437.46 ± 15.24^{cA}	475.80 ± 35.12^{bcA}	253.51 ± 25.45^{d}	n.d	n.d
21	Catechin	MP	$546.02 \pm 14{,}56^{aA}$	327.11 ± 15.65^{bB}	292.86 ± 15.32^{bcB}	262.34 ± 15.57^{dB}	n.d	n.d	n.d
22	(Epi)catechin Dimer or Procyanidin B dimer	F	26.58 ± 0.67^{C}	-	-	-	-	-	-
		P	315.41 ± 15.94^{cB}	396.54 ± 25.83^{aA}	335.45 ± 14.99^{bcA}	391.56 ± 36.89^{abA}	349.05 ± 12.62^{abcA}	253.83 ± 19.64^{d}	n.d
		MP	413.96 ± 8.13^{aA}	259.86 ± 11.53^{cB}	252.01 ± 26.14^{cB}	231.94 ± 11.79^{cB}	302.87 ± 16.06^{bB}	n.d	n.d
23	Casuarinin	F	n.d	-	-	-	-	-	-
		P	623.50 ± 31.90^{cA}	828.50 ± 55.45^{bA}	977.32 ± 101.48^{abA}	952.84 ± 92.50^{abA}	882.88 ± 13.59^{abA}	640.85 ± 66.24^{cA}	1041.93 ± 36.04^{aA}
		MP	485.25 ± 20.08^{aB}	306.33 ± 16.52^{cB}	321.19 ± 31.06^{bcB}	323.94 ± 26.70^{bcB}	366.27 ± 16.06^{bcB}	381.33 ± 15.50^{bB}	109.42 ± 1.65^{dB}
24		F	24.76 ± 1.81^{C}	-	-	-	-	-	-

	Compound	Treatment	Storage time (days)						
			D1	D7	D14	D21	D30	D60	D90
	Di-caffeoyl shikimic acid	P	45.01 ± 1.23^{dB}	76.52 ± 1.34^{abA}	65.11 ± 1.05^{cA}	80.21 ± 3.72^{aA}	68.83 ± 4.76^{bcA}	68.13 ± 2.48^{cA}	73.02 ± 3.29^{abcA}
		MP	100.47 ± 3.87^{aA}	79.75 ± 2.01^{bA}	77.18 ± 8.20^{bcA}	71.99 ± 3.00^{bcB}	80.06 ± 9.93^{bA}	36.01 ± 1.39^{dB}	62.99 ± 0.42^{B}
25	Procyanidin B dimer	F	25.01 ± 0.27^{B}	-	-	-	-	-	-
		P	584.98 ± 23.25^{dA}	728.70 ± 35.66^{cdA}	983.28 ± 95.42^{aA}	942.32 ± 93.71^{aA}	893.29 ± 30.88^{abA}	728.10 ± 22.58^{cdA}	786.68 ± 4.80^{bcA}
		MP	551.08 ± 26.59^{bA}	718.51 ± 33.96^{aA}	717.79 ± 73.17^{aB}	685.36 ± 40.78^{aB}	735.18 ± 35.87^{aB}	694.16 ± 31.56^{aA}	141.05 ± 16.14^{cB}
26	Phyllanthusiin E	F	24.63 ± 0.23^{C}	-	-	-	-	-	-
		P	250.27 ± 21.87^{eB}	353.03 ± 26.30^{dA}	435.32 ± 30.4^{8dA}	768.53 ± 20.49^{abA}	840.13 ± 12.75^{aA}	658.58 ± 62.92^{cA}	735.19 ± 29.54^{bc}
		MP	421.71 ± 36.77^{aA}	377.18 ± 27.78^{abA}	267.41 ± 21.78^{cB}	310.66 ± 33.37^{bcB}	354.29 ± 27.11^{abB}	264.26 ± 4.65^{cB}	n.d
27	Ellagic acid derivative	F	n.d	-	-	-	-	-	-
		P	440.96 ± 21.01^{bB}	580.40 ± 46.84^{aA}	484.38 ± 29.99^{bA}	122.55 ± 9.15^{cB}	109.73 ± 2.30^{cdB}	57.71 ± 2.65^{dB}	87.62 ± 3.50^{cd}
		MP	652.74 ± 26.05^{aA}	328.26 ± 30.28^{bB}	391.59 ± 30.78^{bB}	257.86 ± 3.50^{cA}	374.60 ± 31.46^{bA}	382.78 ± 16.73^{bA}	n.d
28	Epicatechin	F	28.08 ± 1.20^{C}	-	-	-	-	-	-
		P	537.59 ± 34.58^{dB}	749.62 ± 74.07^{bcA}	892.26 ± 85.39^{aA}	943.00 ± 17.50^{aA}	832.34 ± 23.12^{abA}	671.75 ± 14.36^{cA}	741.20 ± 23.63^{bcA}
		MP	846.59 ± 26.82^{aA}	519.25 ± 22.01^{cB}	528.45 ± 54.5^{7cB}	513.97 ± 35.78^{cB}	630.94 ± 32.82^{bB}	562.59 ± 40.02^{bcB}	106.26 ± 4.03^{dB}
29	Not identified	F	24.92 ± 0.56^{C}	-	-	-	-	-	-
		P	297.35 ± 13.95^{cB}	407.26 ± 37.73^{bA}	480.07 ± 24.37^{aA}	482.17 ± 2.77^{aA}	419.71 ± 12.77^{bA}	399.27 ± 11.75^{bA}	383.58 ± 10.14^{bA}
		MP	465.84 ± 18.74^{aA}	276.59 ± 15.80^{bcB}	263.45 ± 3.88^{cB}	266.49 ± 16.12^{bcB}	297.95 ± 0.48^{bB}	277.76 ± 6.50^{bcB}	57.95 ± 0.32^{dB}
30	Quercetin 3 glucoside	F	89.87 ± 2.63^{C}	-	-	-	-	-	-
31	Cirsilineol	P	230.89 ± 14.44^{cB}	269.11 ± 0.90^{bA}	255.57 ± 22.86^{bcA}	312.18 ± 9.07^{aA}	237.66 ± 17.91^{bcB}	133.95 ± 0.72^{dB}	141.76 ± 12.70^{dB}
		MP	324.32 ± 9.50^{aA}	248.22 ± 5.87^{bcdB}	271.87 ± 5.02^{bcA}	272.07 ± 23.79^{bcA}	276.09 ± 0.41^{bA}	237.00 ± 21.66^{cdA}	220.24 ± 2.64^{dA}
32	(Epi)catechin Dimer or Procyanidin B dimer	F	26.56 ± 0.61^{C}	-	-	-	-	-	-
		P	361.71 ± 18.76^{cB}	607.58 ± 53.29^{aA}	606.03 ± 61.05^{aA}	573.97 ± 4.15^{abA}	490.60 ± 11.90^{bA}	343.13 ± 8.42^{cA}	377.22 ± 11.39^{c}
33	(Epi)Catechin derivative	MP	552.67 ± 14.58^{aA}	312.72 ± 15.56^{bB}	304.97 ± 29.57^{bB}	276.90 ± 17.56^{bB}	319.43 ± 27.47^{bB}	274.25 ± 10.33^{bB}	n.d
34	Quercetin pentoside	F	103.38 ± 1.91^{C}	-	-	-	-	-	-
		P	216.58 ± 12.22^{aB}	186.87 ± 2.01^{bB}	133.53 ± 6.51^{cB}	170.05 ± 9.04^{bB}	141.92 ± 8.44^{cB}	nd	nd
		MP	298.43 ± 10.15^{aA}	256.10 ± 7.61^{bA}	264.68 ± 24.62^{abA}	256.02 ± 15.39^{bA}	252.34 ± 2.80^{bcA}	218.93 ± 7.06^{cd}	201.17 ± 3.75^{d}
35	Isorhamnetin hexoside	F	122.02 ± 3.09^{C}	-	-	-	-	-	-
		P	305.88 ± 13.31^{aB}	304.21 ± 0.50^{aA}	311.58 ± 14.89^{aA}	265.87 ± 9.42^{bA}	213.77 ± 12.14^{cB}	98.77 ± 2.74^{dB}	115.68 ± 3.84^{dB}

	Compound	Treatment	Storage time (days)						
			D1	D7	D14	D21	D30	D60	D90
		MP	330.24 ± 6.76^{aA}	251.98 ± 5.97^{bB}	257.41 ± 22.98^{bB}	246.72 ± 13.73^{bA}	263.46 ± 2.01^{bA}	244.80 ± 7.73^{bA}	263.61 ± 1.21^{bA}
36	Quercetin 3 glucoside	F	82.12 ± 4.11^{C}	-	-	-	-	-	-
		P	158.39 ± 5.24^{cB}	482.27 ± 18.84^{bB}	571.33 ± 29.67^{aB}	605.59 ± 38.82^{aB}	560.86 ± 0.28^{aB}	n.d	n.d
		MP	420.26 ± 30.21^{cA}	722.27 ± 33.53^{bA}	976.21 ± 98.25^{aA}	1090.80 ± 76.53^{aA}	1077.96 ± 15.84^{aA}	786.85 ± 48.10^{b}	n.d
37	Ellagic acid	F	67.09 ± 4.27^{C}	-	-	-	-	-	-
		P	561.44 ± 33.07^{deA}	977.64 ± 100.19^{aA}	839.12 ± 55.09^{abA}	837.73 ± 8.80^{bA}	734.57 ± 19.36^{bcB}	474.84 ± 46.24^{eB}	644.51 ± 23.90^{cdA}
		MP	456.88 ± 31.80^{eB}	562.04 ± 30.42^{deB}	741.39 ± 75.28^{abcA}	820.63 ± 58.94^{abA}	847.66 ± 17.62^{aA}	631.20 ± 37.18^{cdA}	657.78 ± 18.27^{bcdA}
	Not identified	F	131.48 ± 3.41^{C}	-	-	-	-	-	-
38	Methyl Cirsimaritin coumaric acid	P	702.79 ± 40.15^{cB}	1131.44 ± 2.49^{aA}	1000.96 ± 108.28^{abA}	1037.10 ± 21.49^{aA}	896.93 ± 46.26^{bA}	475.94 ± 19.23^{dB}	675.09 ± 25.24^{cA}
39		MP	907.93 ± 37.21^{aA}	765.37 ± 22.47^{bcB}	819.12 ± 74.23^{abA}	796.63 ± 40.14^{bcB}	812.94 ± 11.94^{abcB}	705.52 ± 19.72^{cA}	556.09 ± 38.82^{dB}
		F	n.d	-	-	-	-	-	-
40	Quercetin pentoside	P	138.07 ± 7.23^{aA}	62.94 ± 0.07^{bA}	134.50 ± 4.50^{aA}	62.36 ± 1.51^{bA}	61.62 ± 2.10^{bA}	n.d	n.d
		MP	64.92 ± 0.29^{aB}	62.82 ± 1.00^{aA}	64.70 ± 5.60^{aB}	63.24 ± 3.06^{aA}	61.54 ± 0.68^{aA}	63.19 ± 1.22^{a}	n.d
	Quercetin pentoside	F	n.d	-	-	-	-	-	-
41		P	114.75 ± 4.61^{cA}	143.70 ± 0.43^{aA}	118.27 ± 4.07^{cA}	132.93 ± 2.15^{bA}	118.73 ± 4.64^{cA}	84.49 ± 2.80^{eA}	95.12 ± 3.13^{dA}
42	Quercetin derivative	MP	115.11 ± 1.80^{aA}	106.64 ± 2.21^{aB}	116.16 ± 11.38^{aA}	113.01 ± 5.04^{aB}	107.36 ± 0.67^{aB}	83.22 ± 1.67^{bA}	105.61 ± 6.17^{aA}
		F	n.d	-	-	-	-	-	-
43	Not identified	P	120.14 ± 8.20^{bA}	172.81 ± 0.17^{aA}	126.95 ± 1.80^{bA}	130.42 ± 3.43^{bA}	122.98 ± 4.10^{bA}	93.06 ± 3.02^{cA}	102.45 ± 3.22^{cA}
		MP	114.18 ± 1.84^{aA}	114.22 ± 2.30^{aB}	118.54 ± 11.36^{aA}	116.30 ± 5.62^{aB}	112.42 ± 0.62^{aB}	78.97 ± 2.14^{bB}	79.20 ± 7.63^{bB}
		F	76.54 ± 3.48^{C}	-	-	-	-	-	-
44	Quercetin pentoside	P	127.74 ± 6.04^{aA}	58.38 ± 0.10^{b}	n.d	n.d	n.d	n.d	n.d
		MP	89.26 ± 2.38^{B}	n.d	n.d	n.d	n.d	n.d	n.d
		F	75.34 ± 3.73^{B}	-	-	-	-	-	-
45	Cirsilineol derivative	P	131.38 ± 7.71^{bA}	145.78 ± 0.03^{aA}	117.37 ± 1.26^{cA}	137.43 ± 4.24^{abA}	113.49 ± 4.92^{cdA}	98.31 ± 3.34^{eA}	105.30 ± 3.33^{deA}
		MP	124.63 ± 2.81^{aA}	99.40 ± 6.74^{bB}	100.92 ± 1.41^{bB}	104.28 ± 4.89^{bB}	101.36 ± 1.17^{bB}	93.55 ± 2.55^{bA}	74.02 ± 4.77^{cB}
		F	n.d	-	-	-	-	-	-
46	Caffeoyl acid derivative	P	21.71 ± 1.22^{aA}	18.69 ± 0.01^{b}	n.d	n.d	n.d	n.d	n.d
		MP	22.85 ± 0.50^{A}	n.d	n.d	n.d	n.d	n.d	n.d
47		F	151.50 ± 5.54^{C}	-	-	-	-	-	-

	Compound	Treatment	Storage time (days)						
			D1	D7	D14	D21	D30	D60	D90
	Cirsilineol Methyl Caffeoyl	P	664.91 ± 28.41^{bcB}	785.16 ± 3.32^{aA}	770.06 ± 74.06^{aA}	731.73 ± 20.16^{abA}	624.19 ± 39.62^{cA}	327.13 ± 10.46^{dB}	379.50 ± 11.67^{dB}
		MP	715.31 ± 19.01^{aA}	527.49 ± 12.62^{bB}	534.62 ± 51.66^{bB}	510.42 ± 30.43^{bB}	559.00 ± 6.63^{bB}	533.80 ± 17.74^{bA}	528.41 ± 0.25^{bA}
48	Cirsilineol	F	75.88 ± 3.71^{B}	-	-	-	-	-	-
		P	99.32 ± 3.98^{abA}	105.95 ± 0.23^{aA}	98.76 ± 9.67^{abcA}	105.96 ± 3.09^{aA}	98.07 ± 3.76^{abcA}	86.09 ± 2.33^{cA}	89.82 ± 2.53^{bcA}
		MP	98.84 ± 2.74^{aA}	86.94 ± 1.82^{abB}	89.75 ± 8.61^{abA}	86.87 ± 5.05^{abB}	87.59 ± 0.64^{abB}	86.74 ± 2.61^{abA}	82.63 ± 7.48^{bA}
	Total Phenolic Compounds	F	1591.18 ± 13.43^{B}	-	-	-	-	-	-
		P	21,061.30 ± 876.04^{cA}	26,459.12 ± 532.05^{bA}	26,859.50 ± 1223.64^{bA}	30,029.75 ± 1192.92^{aA}	25,479.91 ± 1622.11^{bA}	18,838.93 ± 588.50^{cA}	20,520.28 ± 565.01^{cA}
		MP	22,198.72 ± 681.98^{aA}	15,997.53 ± 581.18^{bB}	16,525.74 ± 1526.04^{bB}	15,973.88 ± 903.62^{bB}	16,940.97 ± 221.93^{bB}	15,195.30 ± 547.33^{bB}	8905.83 ± 106.84^{cB}
	Hydroxybenzoic acids	F	354.49 ± 9.78^{B}	-	-	-	-	-	-
		P	14,734.92 ± 587.22^{cdA}	18,245.18 ± 469.03^{abA}	18,317.49 ± 843.80^{abA}	19,996.05 ± 856.97^{aA}	16,820.71 ± 1243.11^{bcA}	13,182.76 ± 443.98^{dA}	14,390.87 ± 450.46^{dA}
		MP	14,299.67 ± 463.67^{aA}	9739.05 ± 360.30^{bB}	9919.11 ± 948.76^{bB}	9470.39 ± 517.85^{bB}	10,045.29 ± 169.19^{bB}	9533.26 ± 320.36^{bB}	5775.43 ± 81.45^{cB}
	Flavan-3-ols	F	168.65 ± 0.83^{C}	-	-	-	-	-	-
		P	2636.00 ± 125.93^{dB}	3463.09 ± 56.76^{cA}	3956.74 ± 358.16^{cA}	5385.47 ± 246.94^{aA}	4670.42 ± 228.73^{bA}	3459.43 ± 90.47^{cA}	3610.61 ± 34.12^{cA}
		MP	3224.55 ± 96.62^{aA}	2377.33 ± 105.11^{bB}	2351.80 ± 237.39^{bB}	2224.53 ± 132.63^{bcB}	2509.05 ± 30.04^{bB}	1990.43 ± 109.39^{cB}	691.57 ± 27.10^{dB}
	Flavonols	F	473.93 ± 11.68^{C}	-	-	-	-	-	-
		P	1412.45 ± 68.82^{bB}	1680.29 ± 21.82^{aA}	1651.74 ± 52.67^{aB}	1679.39 ± 71.90^{aB}	1457.54 ± 41.46^{bB}	409.27 ± 8.54^{cB}	455.01 ± 21.85^{cB}
		MP	1756.72 ± 61.46^{bA}	1762.25 ± 56.80^{bA}	2069.56 ± 171.30^{aA}	2159.17 ± 138.14^{aA}	2151.16 ± 21.09^{aA}	1712.97 ± 82.48^{bA}	869.83 ± 10.33^{cA}
	Hydroxycinnamic acids	F	442.89 ± 7.99^{C}	-	-	-	-	-	-
		P	2047.23 ± 84.36^{bcB}	2818.83 ± 34.54^{aA}	2717.41 ± 195.00^{aA}	2725.44 ± 55.80^{aA}	2319.69 ± 110.21^{bA}	1603.06 ± 50.45^{dB}	1868.66 ± 54.09^{cdA}
		MP	2694.32 ± 62.38^{aA}	1932.56 ± 62.06^{bcB}	1994.58 ± 159.96^{bcB}	1928.65 ± 106.10^{bcB}	2046.53 ± 16.54^{bB}	1778.35 ± 43.79^{cA}	1412.36 ± 44.77^{dB}
	Flavones	F	151.22 ± 7.43^{B}	-	-	-	-	-	-
		P	230.70 ± 11.63^{abA}	251.73 ± 0.26^{aA}	216.12 ± 9.71^{bcA}	243.39 ± 7.32^{aA}	211.57 ± 8.66^{bcA}	184.40 ± 5.65^{cdA}	195.13 ± 5.86^{cA}
		MP	223.46 ± 5.55^{aA}	186.34 ± 8.15^{bB}	190.67 ± 8.67^{bB}	191.15 ± 9.94^{bB}	188.94 ± 1.73^{bB}	180.29 ± 5.02^{bA}	156.65 ± 11.88^{cB}

Values are the mean of 3 replications (± standard deviation).
Content of phenolic compounds expressed in μg.100 mL^{-1}; n.d. = below LOQ.
Different uppercase superscript letters in the same column indicate statistically significant difference between treatments for the same compound by Tukey test ($p<0.05$).
Different lowercase superscript letters in the same line indicate statistically significant difference for the same treatment over the storage period by Tukey test ($p<0.05$
[a] Quantified as catechin, [b] Quantified as quercetin, [c] Quantified as quercetin 3 glucoside, [d] Quantified as chlorogenic acid, [e] Quantified as ellagic acid, [f] Quantified as gallic acid.

4. CONCLUSION

In feijoa juices, a total of 44 phenolic compounds were identified and pedunculagin isomer was a major compound identified in enzyme-treated juices. This study is the first to report the identification of corilagin, casuarinin, valoneic acid dilactone, trisgalloyl HHDP glucose, caffeoyl shikimic acid in feijoa products. The enzyme-treated juices had a widely higher amount of total phenolic compounds than fresh juice. Juice stored for 90 days led to a decrease in carotenoids and vitamin C (64-75%). Nevertheless, the microfiltration stage in feijoa juice proved to be a suitable alternative to prolong the shelf life of this beverage. Furthermore, the microfiltration stage had a great improvement in color, with a pronounced brightness and superior color intensity than the other treatments. Feijoa juice proved to be a promising option for the beverage market composed of bioactive substances.

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Manuscrito 3

Physicochemical characterization and bioactive compounds of feijoa (*Acca sellowiana*) waste flour

Manuscrito em preparação para submissão ao periódico *Food Research International*

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ABSTRACT: In processing feijoa, the peel and the pomace are often discarded, giving rise to a significant amount of fruit residue. This work aims to characterize the flour from the processing residue of feijoa fruit (*Acca sellowiana*) and to evaluate the profile of bioactive compounds and antioxidant capacity. The drying of the samples was carried out in an air circulation oven to obtain flour. For the proximate composition, moisture, ash, protein, lipids,and fiber content were evaluated, as well as the water activity, pH, WHC, OHC, and carotenoid profile by HPLC. Antioxidant capacity was determined by ABTS radical and ORAC assay. The feijoa residue flour had a low content of lipids and carbohydrates and a high content of crude fiber (54.88 ± 0.40%). The moisture content (%) and water activity were 8.03 ± 0.14 e 0.3650 ± 0.04, respectively, demonstrating good conservation for the product. The bioactive compounds of feijoa waste flour were mainly characterized by zeaxanthin (184.76 μg.100g^{-1}), β-cryptoxanthin (136.25 μg.100g^{-1}) and β-carotene (112 μg.100g^{-1}), followed by lutein (84.40 μg.100g^{-1}) and α-carotene (68.53 μg.100g^{-1}), that were identified by HPLC, contributing to a greater valorization of the feijoa waste flour. The total carotenoid content was 586.33 μg.100g^{-1}. The antioxidant capacity determined by ORAC assay exhibited a strong potency (1324.30 μM Trolox.g^{-1}), even as the ascorbic acid content showed a value of 27 mg.100g^{-1}. The results showed that the feijoa residue flour could be used as a food supplement or as an ingredient in food because it showed high levels of fiber (54%). Due to its nutritional characteristics, the feijoa residue proved to be a good alternative as an ingredient for functional foodscs.

Keywords: Feijoa waste; Fruit peel; Dietary fibers; Antioxidant activity; Carotenoids.

1. INTRODUCTION

Native fruits have become the focus of ample research. These are a fruit not commonly found in global markets, but they t have great potential due to their nutritional and sensory characteristics, in addition to conserving biodiversity and promoting local agriculture. However, due to the high nutritional value of many native species, consumption has increased both in the national and international markets (Sun-Waterhouse et al., 2013; Teixeira et al., 2019).

Feijoa (*Acca sellowiana*) is a native fruit from South America, belonging to the Myrtaceae family. Due to its easy adaptability in subtropical regions, it is currently cultivated in many countries and considered an exotic fruit in Europe. It is used on a larger scale in countries such as Australia, New Zealand,and Colombia; however, in Brazil, the species is still little known, despite the country being the center of its origin (Pasquariello et al., 2015). There has been an increase in worldwide interest in feijoa due to its sweet-acidic flavor, aroma, and nutritional composition. The fruit has a high content of phenolics and antioxidant capacity, which contribute to the protection f rom the harmful effects of oxidative stress on human health (Peng et al., 2020; Weston, 2010). In addition to these bioactive compounds, it has an excellent content of dietary fiber (30-50%), minerals such as potassium, calcium, magnesium, phosphorus, vitamin C and pantothenic acid (Omer & Sadiye, 2011; Schmidt et al., 2019).

In addition to fresh consumption, fruits can be processed and used in the production of food and beverages. Due to the relatively high perishability of the fruit, feijoa has a short post-harvest period, which results in serious limitations to meet market demands. So there is a need to process the fruit into various food products, which allows people to consume products based on mountain guava out of season through foods rich in the fruit'bioactive (Peng et al., 2020; Schmidt et al., 2022).

Approximately half of the feijoas grown commercially in New Zealand undergo further processing into juices, alcoholic beverages and preserves, with the other half going to the fresh fruit market (Sun-Waterhouse et al., 2013). The juice production process results in large amounts of waste material. The production of large volumes of waste is a problem caused by the fruit and vegetable processing industry, managing of this waste is becoming increasingly important as it generates economic and environmental impacts worldwide (O'Shea et al., 2015).

During juice processing by the industry, for example, only 40 to 50% of the weight of the whole fruit is used, and by-products such as rinds and seeds that represent 50% of the total weight are discarded (Nascimento et al., 2013). In the processing of feijoa, the peel and the pomace, which represent about 50% of the weight of the fruit, are sour and bitter, being often discarded during the industrial processing of the fruit, which gives rise to a significant amount of waste (Santos et al., 2019; Schmidt et al., 2021). This discarded material is generally referred to as a by-product or waste, and can consist of the kernel, rind, seeds, and pit of the fruit to be processed (O'Shea et al., 2015). Although these matrices are residues from fruit processing, it has been observed that they can have a high amount of nutrients and bioactive compounds, even superior to the main product, and therefore, beneficial to human health (Wijngaard et al., 2009). However, many of these important compounds are still unexplored.

The use of waste from fruit processing is economically viable, creating value for the product and solving disposal problems. In this way, flours from waste are a great strategy for the full use of vegetables that would otherwise be discarded. It can become a long-lasting ingredient for application in a wide variety of food products and incorporated including gluten-free foods. In addition to providing benefits to the consumer's health, due to their nutritional composition, flours based on fruit by-products have the potential to improve the structure and flavor of foods (O'Shea et al., 2015), and have a lower economic impact. and environmental, and therefore can be used as a new naturally healthy ingredient (Siddiq et al., 2018; Silva et al., 2014). Another advantage of using flour, especially in the case of native fruits, is that availability is guaranteed in all seasons.

Based on the above, this study aimed to develop a flour from feijoa (*Acca sellowiana*) residue from juice processing, and evaluate its physicochemical and techno-functional properties. In addition, identify the bioactive compounds and determine the antioxidant capacity in the residue flour.

2. MATERIALS AND METHODS

2.1 Chemical

Analytical grade reagents as sodium phosphate monobasic and bibasic, potassium phosphate monobasic and bibasic were obtained from Comercial Neon© (São Paulo, Brazil). Hydrochloric acid (HCl), 85% orthophosphoric acid, acetone, ethanol, methyl alcohol, and

hydrogen peroxide (H_2O_2) were obtained by Dinâmica Química Contemporânea® (São Paulo, Brazil). High-performance liquid chromatography (HPLC) grade reagents such as acetonitrile, metil tert-butyl ether (MTBE), and methanol were acquired from Panreac AppliChem© (Barcelona, Spain). Trolox® (±)-6-Hydroxy 2,5,7,8-tetramethylchromane-2 carboxylic acid), $ABTS^{\bullet+}$ radical [2,2'-azino-bis-(3 ethylbenzthiazoline-6-sulfonic acid], 2,2′-azobis(2-methylpropanimidamide) dihydrochloride (AAPH), sodium fluorescein, (all-*E*)-β-carotene, (all-*E*)-β cryptoxanthin, gallic acid, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, ellagic acid, catechin, epicatechin, epigallocatechin gallate, luteolin, quercetin, quercetin 3 glucoside, rutin, kaempferol, and myricetin, were purchased from Sigma-Aldrich® (St Louis, USA) and standards of ascorbic acid were acquired from Neon Comercial© (São Paulo, Brazil). (all-*E*)-α-carotene and zeaxanthin were donated by Fluka Analytical® (Buchs, Switzerland), and (all-*E*)-Lutein wasobtained from Indofine Chemical Company® (New Jersey, USA). Cartridge strata SPE $C_{18,}$ Phenomenex® from Allcrom (São Paulo, Brazil). Water was purified by Milli-Q® system Integral 10 (Millipore, Milford, MA, EUA). The samples and solvents were filtered through Millipore membranes (Millex LCR 0.45 μm, 13 mm).

2.2 Material

Feijoa residue (*Acca sellowiana* (O. Berg) Burret) comes from juice production, according to Schmidt et al., (2021). After the production of fruit juice, the residue was packed in plastic vacuum packaging (FASTVAC®/F200 flash) and stored in a freezer at -18 °C until use.

2.2.1 Preparation of feijoa flour

For the elaboration of the flour was used the methodology proposed by Almeida et al., (2020) with some modifications. Feijoa residues were thawed under refrigeration overnight. Afterward, bleaching was performed at 100 °C for 2 minutes, followed by cooling with water and ice. The residues were manually cut into small pieces of 2 x 2 cm and dried in an oven with forced air circulation (DeLeo, B4AFD, Brazil) at 55 °C for 16 hours. After drying, the peel was ground in a knife mill (Solab, model SL – 31, Brazil), and the powder obtained was sieved in a 35 mesh. The flour was then vacuum- packed in plastic bags and stored under refrigeration at 5 °C for further analysis.

2.3 Methods

2.3.1 Physicochemical analyses

All analyzes of the proximate composition of the flour were performed according to AOAC procedures (AOAC, 2012). Moisture was determined gravimetrically in a forced air oven at 105 °C (DeLeo, Porto Alegre, Brazil), and ash was quantified by muffle incineration at 550 °C (Linn High Therm, Elektro, Eschenfelden, Germany). Protein content was determined by the Kjeldahl method, using a conversion factor of nitrogen to wheat flour protein of 5.70. Lipid concentrate was measured by Soxhlet.

Carbohydrate content was calculated by the difference between the total percentage of moisture, ash, protein, lipid, and total fiber. The total energy value was calculated using the nutrient conversion factors: 4 kcal.g^{-1} of protein, 4 kcal.g^{-1} of carbohydrate, and 9 kcal.g^{-1} of lipid content. Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid) (dry weight).

Total dietary fiber and insoluble dietary fiber were determined by the enzymatic gravimetric method, according to the AOAC. Total dietary fiber was determined based on dry matter. For enzymatic digestion, samples were heated to 100 °C with α-amylase and incubated at 60 °C with protease and amyloglucosidase enzymes. Soluble dietary fiber was calculated by the difference between the total and insoluble fractions. All measurements were performed in triplicate.

The water activity (a_w) was measured in a portable water activity meter device Pre AquaLab Water Activity Analyzer (Meter Group Inc, WA, USA). The pH was determined by a potentiometric method with a pH meter (Quimis, model Q400AS, Brazil). Acidity was determined by titration with NaOH (0.1 N) (Instituto Adolfo Lutz, 2008). All measurements were performed in triplicate.

2.3.2 Functional properties

The solubility index was determined according to Cano-Chauca et al., (2005). Distilled water (50 ml) was added to an amount of 0.5 g of sample in a centrifuge tube and the mixture was homogenized by vortexing (Multifunctional vortex K40-1020, Kasvi) for 1 minute and then centrifuged (Himac CR21 GIII, Hitachi Koki Co., Tokyo, Japan) at 3000 g for 5 minutes. A 25 ml aliquot was removed from the supernatant and

transferred to a previously tared porcelain dish and then stored in an oven at 105 °C for 24 hours. Solubility was calculated by weight difference.

The oil and water retention capacities were calculated according to the methodology of Fernández-López et al., (2009). A volume of 30 ml of water/sunflower oil was added to 1 gram of the sample. The suspension was vortexed for 1 minute and allowed to stand at room temperature for 24 hours. It was then centrifuged at 3000 g for 20 minutes, the supernatant removed and the residue weighed.

2.3.3 Color parameters

Flour color was determined by colorimeter (CR-300, Minolta Co. Ltd., Osaka, Japan) operating with illuminant C, and CIELAB color parameters. The parameters used were *L** (luminosity), *a** (ranging from green to red) and *b** (ranging from blue to yellow). A white disk was used as the default (L0*: 91.52; a0*: -0.94; b0*: -1.12). The total color differences (ΔE) of the samples were calculated using the following formula (Eq. 1

Eq. (1) $\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$

Onde:

$\Delta L^* = L^* - L_0^*$, $\Delta a^* = a^* - a_0^*$ e $\Delta b^* = b^* - b_0^*$, onde L_0^*, a_0^* e b_0^* a are the values of the standard color L*, a* e b*.

The Hue and Chroma angle values indicate the tone of the sample (0 or 360 = red, 90 = yellow, 180 = green, and 270 = blue) and the intensity of the color saturation, respectively. They were calculated using Equations (2) and (3):

Eq. (2) $Hue = tan^{-1}(b^*/a^*)$

Eq. (3) $Chroma = [(a^*)^2 + (b^*)^2]^{1/2}$

Where: a* and b* are parameters of the CIELAB system.

2.3.4 Ascorbic acid

The determination of vitamin C was based on the methodology proposed by Rosa et al., (2007) with some modifications. Each 2g sample was homogenized in an Ultra-Turrax® (IKA, T25 digital) with 20 mL of 0.05 M 96% sulfuric acid for 1 min, centrifuged (Himac CR21 GIII, Hitachi Koki Co., Tokyo, Japan) at 25,400 g for 15 minutes and then filtered through a Teflon hydrophilic filter unit. Samples were analyzed by HPLC using

a Waters Alliance 2695® chromatograph (Milford, USA) connected to a diode array detector (DAD 2996). A C18 polymeric column (250 mm x 4.6 mm i.d., 5 µm) was used. The mobile phase of 0.05 M sulfuric acid, with a flow of 1.0 mL.min^{-1}, injection volume of 10 µL and a wavelength of 254 nm. Vitamin C was quantified by injecting the ascorbic acid standard on the day of analysis.

2.3.5 Bioactive compounds

Carotenoids

The exhaustive extract of carotenoids was prepared according to Rodriguez-Amaya (2001). The principal steps were: extraction of pigments with 3g of sample and 30 mL of acetone homogenized for 1 minute in Ultra-turrax (IKA®, T25 digital 1/min x 1000); and saponification with 10% KOH methanol overnight at room temperature. After alkali removal, the extract was concentrated in a rotary evaporator (Q334.2, Quimis®, Diadema, São Paulo, Brazil) (T<25 °C), dried in a nitrogen flow and stored in a freezer (-18 °C) for further quantification by high performance liquid chromatography (HPLC). For analysis, the concentrated extract was diluted in tert-methyl-butyl ether (MTBE), placed in ultrasound (Unique, model USC 1400) for 15 minutes and filtered through a filter (Millex LCR 0.45 µm, 13 mm) for further injection into the chromatograph.

The analyzes were performed on an Agilent 1100 series chromatograph (Santa Clara, CA, USA), equipped with a quaternary solvent pumping system and a UV-Visible detector. The column used for carotenoids was a 250 mm x 4.6 mm ID, 3 µm, polymeric C30 reversed phase column (YMC, model CT99SO3-2546WT). The mobile phase was water/methanol/methyl tert-butyl ether (MTBE) starting at 5:90:5, reaching in 12 minutes 0:95:5, in 25 minutes, 0:89:11, 0:75: 25 at 40 minutes and finally 00:50:50 after a total of 60 minutes, with a flow rate of 1 mL.min^{-1} at 33 °C. The spectra were performed between 250 and 600 nm, and the chromatograms were transformed at a fixed wavelength of 450 nm for carotenoids.

Confirmation of the identified carotenoids was performed in the analysis by HPLC-DAD-MS/MS under the same conditions described by Rodrigues et al., (2013) with some modifications. The same column described above was used, , with a flow rate of 0.9 mL.min^{-1}, injection volume of 20 µL and oven temperature at 29 ºC. A linear gradient was used from the 95:5 (v/v) methanol-MTBE mixture as the mobile phase, reaching 70:30 over 30 min, followed by 50:50 over 20 min and maintaining this ratio for

10 min. At 62 minutes, the initial condition was restored and remained for another 5 minutes. Spectra were measured between 200 and 600 nm and chromatograms were processed at 450 nm.

The column eluate was injected directly into an atmospheric pressure chemical ionization (APCI) source, and the mass spectrometer parameters followed the same conditions previously described by De Rosso & Mercadante (2007). The mass spectrometer parameters were defined as positive mode, corona current 4,000 nA, source temperature at 450 ºC, N2 gas (White Martins SA, Porto Alegre, Brazil), chromatographic degree, with a temperature of 350 ºC and flow of 4 $L.min^{-1}$, nebulizer at 60 psi. MS/MS experiments were performed in automatic mode, with a fragmentation energy of 1.4 V. Mass spectra were acquired with a *m/z* scanning range from 100 to 2,200.

For this analysis was used a chromatograph (Shimadzu, Kyoto, Japan) equipped with two pumps (Shimadzu LC-20AD), an on-line degasser (Shimadzu® DGU-20A3R), a column oven (Shimadzu CTO-20A), diode array (Shimadzu SPDM20A) connected in series to a mass spectrometer with an atmospheric pressure chemical ionization source (APCI) and an ion capture analyzer (microOTOF-QIII model, Esquire 4000, Bruker Daltonics, Bremen, Germany).

Identification and quantification were performed by comparing the retention times of sample peaks and their respective standards under the same chromatographic conditions. Carotenoids were identified based on elution order, UV-Vis spectral characteristics [maximum absorption wavelength (λmax), spectral fine structure (% III/II), and maximum cis intensity (% AB/AII)] and mass spectrometry spectrum. In addition, these parameters were compared to data available in the literature. Chromatograms were processed at 450 nm. For quantification, a standard curve was constructed for carotenoids in the ranges shown in Table 1.

Table 1. Concentration ranges, correlation coefficient (R^2), limits of detection (LOD) and limits of quantification (LOQ) of carotenoid standards.

Bioactive Compound	Concentration range ($\mu g.mL^{-1}$)	Correlation coefficient (R^2)	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
Lutein	1-65	0.9991	6.90×10^{-3}	1.15×10^{-2}
Zexanthin	1-40	0.9997	9.56×10^{-2}	1.59×10^{-2}
Cryptoxanthin	3-100	0.9911	2.11×10^{-2}	3.51×10^{-2}
α-carotene	2-25	0.9934	1.97×10^{-2}	3.28×10^{-2}
β-carotene	5-50	0.9998	6.53×10^{-2}	10.89×10^{-2}
Lycopene	10-100	0.9977	7.0×10^{-3}	33.0×10^{-3}

2.3.6 Antioxidant capacity

ABTS assay

The determination of the total antioxidant capacity of the flour was performed by capturing the free radical ABTS according to the methodology used by Rufino et al., (2007). For the extract, 1 gram of sample and 20 mL of 50% methanol were homogenized in Ultra-Turrax® and left to rest for 60 minutes in a dark environment. Afterwards, the extract was centrifuged at 25,400 g for 15 minutes, and the supernatant was stored in a 50 mL amber flask. This process was repeated with 70% acetone replacing methanol. The flask was made up to 50 ml. From the obtained extract, test tubes were prepared, with at least three different dilutions, in triplicate. In a dark environment, an aliquot of 100 μL of each dilution of the extract was transferred to test tubes with 1.0 mL of the ABTS·+ radical, the tubes were homogenized in a shaker and the reading was performed at 734 nm in a spectrophotometer (Shimadzu® UV -1800) after 6 minutes of mixing.

ORAC assay

The extraction followed the methodology described by Rodrigues et al., (2013) with modifications. An exhaustive extraction for 2 minutes in the Ultra-turrax (IKA®, T25 digital 1/min x 1000) adding 20 mL of a mixture of methanol with distilled water (80:20%; v/v) to 0.4 g of the sample at room temperature was performed. Subsequently, the extract was centrifuged (Himac® CR21 GIII, Hitachi Koki Co., Tokyo, Japan) at 10,000 g for 10 minutes at 20°C and the supernatant transferred to an amber volumetric flask. This procedure was performed in triplicate and repeated until the absence of color and the supernatants combined.

The antioxidant capacity was determined by the ORAC assay (Huang et al., 2005). This method verifies the scavenging capacity of an antioxidant against a peroxyl radical induced by AAPH at 37 ºC. In each microplate well were added 25 uL of the extracts previously diluted in 75 mM potassium phosphate buffer and 150 uL of the fluorescein working solution (81nM). The plate was incubated for 10 min at 37°C, with the last 3 minutes under constant agitation. Afterward, 25 uL of the AAPH solution (152 mM) was added. To monitor the fluorescence decay, a fluorescence reader (Enspire 2300, Multimode Plate Reader, Perkin Elmer, USA) was used at 37°C for 90 minutes or until it reached less than 0.5% of the initial value. Excitation and emission wavelengths of 485 nm and 528 nm, respectively, were used. The area under the curve (AUC) was calculated

and the blank value (no antioxidant) was subtracted to obtain the net AUC. The AUC is given by the following equation (4):

$$\text{Eq. (4) } AUC = 1 + {}^{f1}/_{f0} + {}^{f2}/_{f0} + {}^{f3}/_{f0} + \cdots {}^{fn}/_{f0}$$

Where

fn = relative fluorescence in one read cycle (1 min)

$f0$ = fluorescence at time zero

A standard curve was made with Trolox (0 – 96 uM).

2.4 Statistical analysis

All analyses were evaluated in triplicate and the results were expressed as mean ± standard deviation. Statistical analysis was performed using ANOVA, with a comparison test of the means by the Tukey test at the level of 5% of significance; thus, in all cases, significant differences were considered when $p \leq 0.05$. All statistical analyzes were performed using Statistica 12.0 software (Statsoft Inc., Tulsa, USA).

3. RESULTS AND DISCUSSION

For the processing of flour, the residue of the feijoa was obtained from the production of fruit juices (Figure 1). The process lasted for 16 hours, with an initial moisture content of 79.23% and a final moisture content of 8.03%.

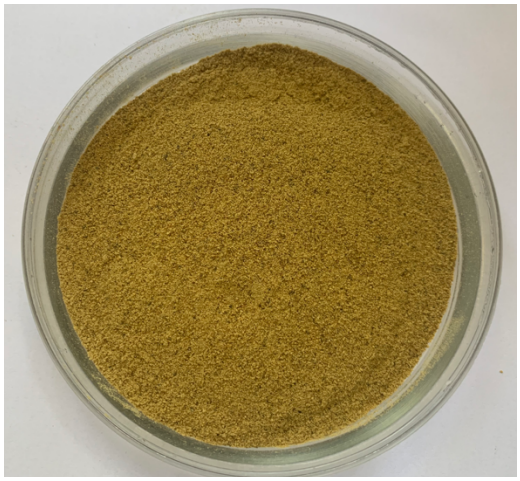

Figure 1. Feijoa residue flour dried in an oven with forced air circulation at 55 °C for 16 hours

The proximate composition of feijoa flour is presented in Table 2. The flour has a moisture content of 8.03%, within the parameters for commercialization of this product, in which the moisture must be up to 15% (Brasil, 2005). The flour showed to be low in carbohydrates (35%) when compared to other traditional flours such as wheat, corn, and flours from other fruits such as *Opuntia monocantha*, orange passion, *Prosopis nigra* and mutamba (Assis et al., 2019; Dick et al., 2020; Reis et al., 2020). Almeida et al. (2020) evaluated feijoa flour from the epicarp and mesocarp and reported similar ash values (2%). However, a lower content of lipids (1.5%) and proteins (2.27%) was found by the author when compared to the present work (3.22 and 4.43 for lipids and protein, respectively). This result may be because the flour of the residue of this study, in addition to the epicarp and mesocarp, included seeds.

Table 2. Characterization physicochemical and technological of feijoa waste flour

Parameters	Results
Moisture (%)	8.03 ± 0.14
Ash (%)	2.21 ± 0.11
Proteins (%)	4.43 ± 0.15
Lipids (%)	3.22 ± 0.14
Total Fiber (%)	54.88 ± 0.40
Insoluble Fiber (%)	49.17 ± 1.57
Soluble Fiber (%)	5.71 ± 0.34
Total Carbohydrates (%)[a]	35.25 ± 0.26
Kcal	187.76 ± 1.57
A_w	0.3650 ± 0.04
pH	2.87 ± 0.01
Total Titratable Acidity (per cent of acid citric)	10.74 ± 0.60
Solubility index (%)	27.69 ± 0.25
WHC (water/g DW)	2.61 ± 0.65
OHC (g oil/g DW)	2.90 ± 0.04
*L**	39.53 ± 0.22
*a**	2.76 ± 0.01
*b**	18.56 ± 0.06
*C**	18.76 ± 0.06
° Hue	81.53 ± 0.01

Values are the mean of 3 replications (± SD).
Centesimal composition (g/100g DW)
All measurements are on dry weight (DW) basis, except the moisture.
[a]Calculated by the equation: total carbohydrate = 100 – (protein (DW) + lipid (DW) + ash (DW)).
*L** brightness, *a** redness/greenness, *b** yellowness/blueness, *C** chroma, hue angle.

The results showed that the residue flour contains high levels of total fiber (54%), with the insoluble fraction corresponding to almost 50% of the total and the soluble fraction presenting a smaller proportion, around 5%. Insoluble fibers improve the functioning of the gastrointestinal tract, promote gastrointestinal peristalsis, increase fecal volume and have the effect of accelerating defecation time. (Atzler et al., 2021).

The recommended intake of dietary fiber (DF) is between 25 and 35 g/d (World Health Organization, 2003), and DF has a positive effect on health since its consumption has been related to decreased incidence of several diseases. According to the European Parliament Regulation (EC) No. 1924/2006 and BRASIL (2012), feijoa flour can be considered a food with high fiber content and can be used to enrich foods, once 50g of flour is already reaches the daily recommendation for total dietary fiber. Furthermore, high levels of intake of fiber-rich foods are associated with to protect from the risk of cancer and cardiovascular diseases (Garcia-Amezquita et al., 2018; Ruel et al., 2014).

In addition to its physiological role for health, the incorporation of dietary fiber in foods alters the textural, rheological, nutritional and sensory properties of food products. They can be used as functional ingredients to modify viscosity, prevent syneresis, decrease calories and as a fat substitute, being used in several food categories, such as bakery, beverages, confectionery, dairy products, frozen dairy products, meats, pasta and soups (Sharma et al., 2017).

The Table 2 presents some technological properties of the flour from the residue of the feijoa. The parameters of pH and water activity (a_w) are important for the conservation of food products. Microbiological deterioration is minimal in foods with water activity below 0.5 and acidic pH (Crizel et al., 2013; López-Vargas et al., 2013). The a_w found for the flour was 0.3650, and the pH was 2.8, which indicates, therefore, a relatively high stability of the flour. A similar result was observed by Assis et al. (2019) for flour obtained from mutamba fruit and dried at 65°C (a_w = 0.391).

The water holding capacity (WHC) represents the amount of water that remains bound to the hydrated fiber after the application of an external force (pressure or centrifugation), being affected by the particle size, porosity, hydrophobicity and measurement temperature (Chantaro et al., 2008; López-Vargas et al., 2013). The flour from the feijoa residue showed a water retention capacity of 2.61 g water/g DW (Table 2). The WHC obtained in our study is similar to the finding by Almeida et al., (2020) (2.65 g/g) and wheat bran (2.7 g/g). It is noteworthy that the WHC value obtained in this research was higher than those reported for corn flour (1.42 g/g), rice flour (1.29 g/g),

corn starch (1.34 g/g), and flour from the residues of lemon, orange and apple juice extraction (1.6–2.3 g/g) (Martínez & Gómez, 2017). Nonetheless, the WHC was low compared to carrot residue (13–22 g/g), Opuntia monocantha flour (7.03 g/g), orange passion peel (6.8 to 7.8 g/g), pomegranate pomace (4.5–4.9 g/g) and soybean hulls (3.5–4.0 g/g) (Chantaro et al., 2008; Dick et al., 2020; Figuerola et al., 2005; Reis et al., 2020).

Oil retention capacity (OHC) is a technological property related to the chemical structure of plant polysaccharides that depends on the surface properties, thickness and hydrophobic nature of the fiber particle (López-Vargas et al., 2013). Oil holding capacity is an important functional property because oil plays a significant role in increasing the mouthfeel of foods and also in preventing fat and flavor loss during cooking (Pușcaș et al., 2020). The analysis revealed that the OHC of the flour was 2.90 g oil/g DW (Table 2), a result superior to that found for the flour from the residues of the extraction of lemon, orange, and apple juice (1.6 to 2.3 g/g) and wheat bran (1.6 g/g) and similar to that found for orange passion peel (2.8 to 2.9 g/g) (Figuerola et al., 2005; Reis et al., 2020). The result of solubility obtained in this work (27,7%) was similar to orange by-products (28.9 %) and red pitaya peel flour (30%) and lower than pineapple and papaya wastes (55-60%) (Crizel et al., 2016).

Fiber-rich ingredients can be used for economic purposes as well as for their functional and technological properties, such as water holding capacity, oil-holding capacity, swelling capacity, gel-forming capacity, and texture of food products (Pathania & Kaur, 2022).

The color parameters of the flour presented a moderate luminosity, and the parameters b^* and C^* of the flour were similar to those presented for the feijoa juice (Schmidt et al., 2021), characteristic of dark yellow color (Table 2; Fig. 1). The Hue angle (81), in the first quadrant of the CIELab color chart, indicates the color characteristic in yellow.

Table 3 presents the carotenoid composition of the flour from the feijoa residue. The consumption of carotenoid pigments has been proposed to confer health benefits, such as reducing the risk of developing chronic degenerative diseases attributed to their role as antioxidants (Vargas-Murga et al., 2016). Five individual carotenoids were identified: lutein, zeaxanthin, cryptoxanthin, β-carotene and α-carotene. The total carotenoid content in the flour was determined to be 586 $\mu g.100g^{-1}$ DW, corresponding to 14% lutein, 19% β-carotene, 31% zeaxanthin, 23% cryptoxanthin, and 11% α-carotene. In a previous study with edible fruit pulp and feijoa juice, β-carotene was the main

carotenoid found, followed by lutein, and α-carotene was not found in the feijoa juice (Schmidt et al., 2022, Schmidt et al., 2019).

The total carotenoid content found in the residue was higher than that found for the edible pulp (322 µg/100 g) (Schmidt et al., 2019). However, the total carotenoid content was lower when compared to other flours such as *opuntia monocantha* flour (9,500 µg.100 g^{-1}), orange passion fruit peel flour (16,614 µg.100 g^{-1}) (Dick et al., 2020; Reis et al., 2020).

The vitamin C teor found in the residue was 27 mg.100g^{-1} (Table 4). This value is six times higher than that found for fruit juice (Schmidt et al., 2022) and amounts to around 40% of the Recommended Daily Intake (DRIS, 2011). When compared with other fruit flours, the feijoa residue flour presented higher contents than the chaña fruit flour with 19.5 mg.100g^{-1} (Costamagna et al., 2013). Considered an important antioxidant in food, vitamin C performs primordial functions of the human organism reducing, for example, susceptibility to infections and acting on the healing and iron absorption (Cunha-Santos et al., 2019).

Bioactive compounds are consumed in small amounts in the diet but are essential for health, since they play several roles in the body. Due to their antioxidant activity, these compounds can capture reactive oxygen species (ROS) and protect cells against oxidative damage (Biazotto et al., 2019).

The antioxidant activity of feijoa waste flour was determined by two different methods (ABTS and ORAC) and are presented in Table 4. The ORAC method measures the capacity of an antioxidant to protect the disodium fluorescein from oxidation catalysed by •$ROO^{–}$ radicals, produced by the addition of the radical initiator α,α′-azodiisobutyramidine dihydrochloride (AAPH).

Table 3. Chromatographic, UV–visible, and mass spectrometric characteristics and content of carotenoids of feijoa peel flour obtained by HPLC-DAD/APCI-MS2.

Peak[a]	Carotenoid	t_R (min)[b]	λ_{max} (nm)[c]	% III/II	% A_B/A_{II}	[M + H]$^+$ (*m/z*)	Fragment ions (*m/z*)	Concentration (μg.100g^{-1})
1	all-*trans*-lutein	12.8	420, 442, 472	61.5	ND	569	551 [M+H-18]$^+$, 533 [M+H-18-18]$^+$, 495 [M+H-18-56]$^+$, 477 [M+H-92]$^+$	84.40 ± 3.76
2	all-*trans*-zeaxanthin	17.3	425, 450, 476	25	ND	569	551 [M+H-18]$^+$, 533 [M+H-18-18]$^+$, 463 [M+H-106]$^+$	184.76 ± 10.37
3	13-cis-β- cryptoxanthin	22.3	335, 414, 443, 470	NC	38%	553	535 [M+H-18]$^+$, 461 [M+H-92]$^+$	136.25 ± 11.08
4	all-*trans*-α- carotene	30.6	421, 441, 472	50	ND	537	457 [M+H-80]$^+$, 444 [M-92]$^+$, 413 [M+H-124]$^+$	68.53 ± 9.01
5	all-*trans*-β- carotene	35.0	421, 451, 476	25	ND	537	457 [M+H-80]$^+$, 444 [M-92]$^+$, 413 [M+H-124]$^+$	112.39 ± 6.37
	Total Carotenoids	-	-	-	-	-	-	586.33 ± 30.00

ND: not detected; NC: not calculated.
[a] Numbered according to the chromatogram shown in Fig. S1.
[b] Retention time on C30 column.
[c] Linear gradient of methanol and MTBE.

The ABTS assay assesses the elimination of radicals by antioxidants that involve electron transfer, followed by the donation of protons. For antioxidant capacity measured with $ABTS^{\bullet+}$ radical, the result was 520 μM Trolox.g^{-1} and for the ORAC assay the result was 1324 μM Trolox.g^{-1}. Comparing these results with those of other waste flours, such as pineapple crown flour (ORAC= 45 μM Trolox.g^{-1} and ABTS= 427 μM Trolox.g^{-1}) and banana peel flour (ORAC= 435 μM Trolox.g^{-1} and ABTS= 242.2 μM Trolox.g^{-1}), it is observed that the antioxidant activity of the flour from the feijoa residue was superior (Brito et al., 2021; Rebello et al., 2014). The antioxidant activity measured by the ORAC assay found in the feijoa residue flour was approximately 10x greater than that of the fruit juice (Schmidt et al., 2022).

The values obtained by the different methods used for the determination of antioxidant activity, one can observe the highest value for the ORAC method. This fact may indicate that the compounds present in the flour act more efficiently by the mechanism of hydrogen atom transfer than electron transfer for cation radical ABTS (Rebello et al., 2014).

Table 4. Antioxidant capacity and acid ascorbic concentration of feijoa peel flour

Parameters	**Results**
ABTS radical (μM Trolox/g)	520.39 ± 38.38
ORAC assay - (μM Trolox/g)	1324.30 ± 67.25
Acid ascorbic (mg.$100g^{-1}$)	27.67 ± 1.28

Values are the mean of 3 replications (± SD).

The flour produced from the residue of extracting the juice of the feijoa proved to be a good alternative as an ingredient for functional foods, due to its nutritional characteristics, such as fiber, carotenoids, ascorbic acid, and antioxidant capacity. Therefore, flour from feijoa residue may have potential for use in various food products such as bread, cakes, cookies, and cereal bars, improving their nutritional qualities. In addition to their functionality and nutrition, fruit processing by-products also have the advantage of being gluten and lactose-free, which makes them potentially ideal ingredients for a variety of bakery and confectionery products (O'Shea et al., 2015).

Furthermore, the industrial use of native fruit by-products would not only contribute to new businesses, but would also provide alternative uses and, consequently, the proper disposal of large amounts of waste generated by the agricultural and food industries.

4. CONCLUSION

The flour produced from the residue of feijoa juice proved to be a suitable ingredient for the enrichment of food products or food supplements since it presented high levels of dietary fiber (50%), with insoluble fiber being the main constituent. The flour developed had low carbohydrate and lipid content, low moisture content and water activity, desirable characteristics for this type of product, which contribute to greater stability during storage.

As for the bioactive compounds, the feijoa residue flour presented zeaxanthin and cryptoxanthin as the main carotenoids, followed by β-carotene. The residue flour showed an excellent concentration of vitamin C, making up almost half of the daily consumption, as well as its antioxidant activity evidenced by the qualitative analysis.

Therefore, it is suggested that this residue can be used as an ingredient in different food formulations, which can contribute to a diversified diet and innovation in the development of new products, such as partial replacement of flour in food manufacturing, and collaborating to reduce of agro-industrial waste.

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CAPACIDADE ANTIOXIDANTE DOS SUCOS DE GOIABA SERRANA (*ACCA SELLOWIANA*) DURANTE O ARMAZENAMENTO

** formatado de acordo as normas do Congresso*

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1. INTRODUÇÃO

A goiaba serrana (*Acca sellowiana*), espécie pertencente à família Myrtaceae, é uma fruta nativa da região Sul do Brasil. A fruta possui casca tom verde independente do seu grau de maturação, o mesocarpo branco, com polpa suculenta em torno das sementes e pouco arenosa perto da casca. A polpa é doce, ácida e aromática. No entanto, a fruta apresenta a casca amarga, e por isto, esta não é geralmente consumida com a polpa. Tem sido relatado que a goiaba serrana possui várias atividades biológicas, como antimicrobiana e antioxidante. A atividade antioxidante da polpa da goiaba serrana, se deve principalmente aos polifenóis, que habitualmente conferem um gosto adstringente e, por vezes amargo (HAMINIUK *et al.*, 2011; SCHMIDT, 2018; WESTON, 2010).

Além do consumo *in natura*, a goiaba serrana pode ser processada e utilizada na produção de alimentos e bebidas. A fruta é usada para fazer muffin, pão, chocolate, doces, sorvete, iogurte, geleia, smoothie, vinho e suco. O suco da goiaba serrana, mostrou-se ser uma boa fonte de vitamina C e de compostos bioativos, principalmente compostos fenólicos (SCHMIDT *et al.*, 2022). O objetivo deste trabalho foi avaliar a capacidade antioxidante do suco da goiaba serrana e sua estabilidade por 90 dias.

2. MATERIAL E MÉTODOS

2.1 Produção dos sucos

Os frutos foram selecionados visualmente, lavados em água corrente e higienizados por imersão em solução clorada (200 $mg.L^{-1}$ por 15 min). Para o suco controle, que foi o suco fresco (F), após a lavagem, os frutos foram despolpados e homogeneizados em máquina de despolpamento vertical (DES-20, Braesi, Caxias do Sul, RS, Brasil). Em seguida, o suco foi filtrado em tecido de organza e transferido para garrafas de vidro de 100 mL, armazenadas sob atmosfera de nitrogênio e posteriormente fechadas com tampa de rosca.

Para o suco tratado com enzima e pasteurizado (P), após a limpeza, os frutos foram branqueados em banho-maria (Dubnoff NT 232 – Novatecnica®, Piracicaba, Brasil) a 80°C por 2,5 minutos e resfriados a 10°C. Em seguida, foi aplicada a enzima Pectinex Ultra Clear® (Novozymes, Espanha) (10 $U.mL^{-1}$), a 50 °C por 40 min, sob agitação e resfriada a 20 °C. Os frutos foram despolpados e homogeneizados em máquina de despolpamento vertical. Em seguida, adicionou-se novamente a enzima Pectinex Ultra Clear (5 $U.mL^{-1}$), a 50°C por 40 min, sob agitação, seguido de resfriamento a 20°C. Na primeira adição enzimática, antes do despolpamento, o objetivo foi aumentar o rendimento de extração do caldo. Na segunda adição, após o despolpamento, o objetivo principal foi clarificar o suco. Sucessivamente, o suco foi filtrado em tecido de organza, engarrafado (garrafas de vidro de 100 mL), pasteurizado (1 min a 90°C), seguido de resfriamento a 20°C. Os sucos foram armazenados sob atmosfera de nitrogênio e depois fechados.

Para o suco tratado com enzimas, microfiltrado e pasteurizado (MP), foi realizado o mesmo procedimento do item anterior, porém, com a adição do processo de microfiltração. A clarificação enzimática foi utilizada como pré-tratamento para remoção dos sólidos em suspensão. Após a etapa de filtração em tecido de organza, o suco passou

por microfiltração. Para a microfiltração foi utilizada uma membrana de poliamida (PAM Selective Membranes, Rio de Janeiro, RJ, Brasil) com diâmetro de poro de 0,4 μm e área de filtração de 0,7 m². Condições fixas de 20 ± 2 °C e 300 kPa foram aplicadas durante o experimento. Após a MF, o suco foi pasteurizado (1 min a 90°C) e todas as etapas após a pasteurização foram realizadas da mesma forma descrita anteriormente para o SP.

Para o suco in natura (F), as garrafas foram armazenadas sob refrigeração a 4 ºC por um dia e analisadas em triplicata. Os sucos SP e SM foram armazenados a 20 ºC por 90 dias. Todas as amostras foram armazenadas em uma câmera de demanda bioquímica de oxigênio (DBO) sob fotoperíodo de 12 horas (ciclo claro/escuro), para mimetizar o tipo de armazenamento utilizado em o mercado local. A umidade relativa e a temperatura da câmara foram monitoradas (50,7 ± 3,5% e 4,7 ± 0,5°C, respectivamente) para o suco fresco (65,3 ± 4,6% e 20,7 ± 0,3°C, respectivamente) e para o suco pasteurizado e microfiltrado e pasteurizado. Estes dois tratamentos foram analisados em triplicata aos 1, 7, 14, 21, 30, 60 e 90 dias de armazenamento. Uma alíquota de todos os tratamentos foi coletada para análise físico-química. O suco restante foi armazenado em tubos Falcon de 15 e 50 mL e congelado (-18 ºC), e para a realização das análises, as amostras foram descongeladas antes do uso a 4 ºC durante uma noite.

O suco fresco foi tomado como amostra de referência para estudar o efeito dos diferentes tratamentos sobre os parâmetros analisados

2.1 Capacidade antioxidante

Método ABTS

A determinação da capacidade antioxidante total dos sucos foi realizada pela atividade sequestrante de radicais $ABTS^{\bullet+}$ de acordo com a metodologia proposta por RUFINO *et al*., (2007). O suco (1,5 mL) foi extraído com 20 mL de metanol a 50% e homogeneizado em Ultra-Turrax e a amostra foi deixada em repouso por 60 min no escuro. O extrato foi centrifugado por 15 min, a 25.400 g e o sobrenadante foi transferido para um balão volumétrico âmbar de 50 mL. Este procedimento foi repetido com acetona 70% em vez de metanol. Tubos de ensaio com três diferentes diluições foram preparados em triplicata a partir do extrato obtido. Uma alíquota de 100 μL de cada extrato foi transferida para tubos de ensaio com 1,0 mL do $ABTS^{\bullet+}$. Os tubos foram homogeneizados e após 6

minutos de mistura foi realizada a leitura em 734 nm em espectrofotômetro (Shimadzu UV -1800).

Método de capacidade de absorção de radicais de oxigênio (ORAC)

A atividade antioxidante dos extratos também foi determinada pelo método ORAC (HUANG; BOXIN; PRIOR, 2005). Para a determinação da concentração ótima do extrato para análise, foram realizados testes preliminares com concentrações conhecidas variando de 200 a 1000 $mg.L^{-1}$ (diluído em tampão fosfato de potássio); a concentração ótima foi determinada em 800 $mg.L^{-1.}$ Em seguida, as amostras foram homogeneizadas no vórtex (Multifunctional vortex K40-1020, Kasvi) por 1 minuto, seguido de Ultrassom (Unique, modelo USC 1400A) por 3 minutos. Uma alíquota de 25 µL dos extratos ou Trolox previamente diluído em tampão fosfato de potássio (75 mM) foi combinado com 150 µL da solução de trabalho de fluoresceína (81 nM) em cada poço de uma microplaca. Após incubação a 37 ºC por 7 min, com os últimos 3 min sob agitação constante, foram adicionados 25 µL da solução de AAPH (152 mM). Um leitor de fluorescência (EnSpire 2300 Multimode Plate Reader, Perkin Elmer, Waltham, MA, EUA) foi usado para monitorar o decaimento de fluorescência a 37 ºC por 80 min. Foram utilizados comprimentos de onda de excitação e emissão de 485 e 528 nm, respectivamente. Os valores ORAC foram calculados por uma equação de regressão entre as soluções Trolox (0–96 µM) e a área sob a curva (AUC) do decaimento da fluoresceína. Trolox foi usado como controle positivo (8 µM) e esta análise foi realizada em triplicata. A AUC foi calculada pela Eq. (5):

$$AUC = 1 + {}^{f1}/_{f0} + {}^{f2}/_{f0} + {}^{f3}/_{f0} + \cdots {}^{fn}/_{f0} \quad (5)$$

Onde: fn = fluorescência em um ciclo de leitura (1 min) e f0 = fluorescência em tempo zero.

3. RESULTADOS

Os sucos foram avaliados quanto à capacidade antioxidante pelos ensaios ABTS e ORAC e os resultados podem ser vistos na Tabela 1. O ensaio ABTS avalia a eliminação de radicais por antioxidantes que envolvem transferência de elétrons, seguida da doação de prótons. Para a capacidade antioxidante medida com radical $ABTS^{\bullet+}$, não houve diferença significativa entre os três tratamentos (dia 1). Esses resultados sugerem que os

processos, térmico, enzimático e microfiltração, aos quais os sucos foram submetidos, não diminuíram a capacidade antioxidante avaliada pelo ensaio radical ABTS•$^{+}$. No tratamento com P, a capacidade antioxidante medida pelo ABTS permaneceu constante durante 60 dias de armazenamento. Observa-se que no 60º dia, houve um aumento que persistiu até o 90º dia. No tratamento MP, a capacidade antioxidante permaneceu constante durante todo o período avaliado, sem diferença significativa. A atividade sequestradora de radicais ABTS•$^{+}$ dos sucos de P e MP variou de 1.852,62 a 2.931,68 e 1.921,06 a 2.196,24 μMol TE.100mL^{-1}, respectivamente, valores superiores aos do suco de açaí (*Euterpe oleracea*) (1843 μMol TE.100mL^{-1}) (OLIVEIRA. *et al.*, 2018).

O método ORAC mede a capacidade de um antioxidante para proteger a fluoresceína dissódica da oxidação catalisada por radicais •ROO$^{–}$, produzidos pela adição do iniciador radical α,α′-azodiisobutiramidina dicloridrato (AAPH). A capacidade antioxidante dos sucos de goiaba serrana foi sempre superior ao Trolox, um homólogo sintético da vitamina E, que é um antioxidante biológico com alta capacidade de captura de EROs (Figura 1). Para este ensaio, houve diferença significativa entre o suco fresco e os sucos tratados, o suco fresco apresentou maior capacidade antioxidante que os tratamentos P e MP. Apesar dessa perda, sabe-se que o tratamento é necessário e está associado à disponibilidade de um produto seguro para consumo.

Assim como para o ensaio do radical ABTS•$^{+}$, no ensaio ORAC, os sucos tratados (P e MP) não apresentaram diferenças significativas entre si no dia 1. Ao longo dos 90 dias, a capacidade antioxidante do suco P permaneceu constante. Por outro lado, para o tratamento MP houve redução da capacidade antioxidante neste período de armazenamento, no entanto, as maiores reduções ocorreram no 21º (1456 μMol TE.100mL^{-1}) e 90º (830 μMol TE.100mL^{-1}) dias , com reduções de aproximadamente 50 e 75%, respectivamente, em relação ao primeiro dia (3088 μMol TE.100mL^{-1}). Os valores de ORAC do suco in natura e dos sucos tratados no dia 1 foram superiores aos do suco de maçã (2800 μMol TE.100mL^{-1}), suco tropical (1300 μMol TE.100mL^{-1}), suco de cranberry (1600 μMol TE. 100mL^{-1}), suco de abacaxi (760 μMol TE.100mL^{-1}) e semelhante ao suco de toranja (3400 μMol TE.100mL^{-1}) (STOCKHAM *et al.*, 2011).

O ensaio ORAC é um dos métodos mais utilizados para estimar a capacidade antioxidante de alimentos, devido a sua relevância biológica para efeito antioxidante in vivo, uma vez que utiliza uma fonte de radical biologicamente relevante (HUANG; BOXIN; PRIOR, 2005; PRIOR *et al.*, 2003). Embora a atividade de eliminação de

radicais ABTS•$^{+}$ também tenha sido amplamente aplicada para avaliar a capacidade antioxidante de extratos de alimentos, o ensaio ABTS•$^{+}$ é mais confiável quando usado para comparar alterações no mesmo antioxidante durante o processamento ou armazenamento. Em tais aplicações, as limitações do teste permanecem, mas os componentes antioxidantes são constantes (SCHAICH; TIAN; XIE, 2015). Uma vez que foram observadas semelhanças na resposta nos ensaios ORAC e ABTS•$^{+}$ para os sucos de goiaba serrana, parece que tanto elétrons quanto prótons foram transferidos nesses dois ensaios, e os sucos são promissores na capacidade antioxidante.

4. CONCLUSÃO

Os resultados sugerem que os processos térmicos, enzimáticos e de microfiltração aos quais os sucos foram submetidos não diminuíram a capacidade antioxidante avaliada pelo ensaio radical ABTS•+. Por outro lado, para o ensaio ORAC, o suco fresco apresentou maior capacidade antioxidante do que os sucos tratados e ao longo de 90 dias, o tratamento microfiltrado e pasteurizado apresentou uma redução de aproximadamente 50-75%.

Tabela 1. Capacidade antioxidante pelos métodos ABTS e ORAC nos três sucos de goiaba serrana: suco fresco (F), suco clarificado por tratamento enzimático e pasteurizado (P), e, suco clarificado por tratamento enzimático, microfiltração e pasteurizado (MP) durante 90 dias de armazenamento

		Tempo de armazenamento (dias)						
Parâmetro	**Tratamento**	**D1**	**D7**	**D14**	**D21**	**D30**	**D60**	**D90**
ABTS	F	2111.95 ± 15.40^{A}	-	-	-	-	-	-
	P	1852.62 ± 185.11^{bA}	1865.35 ± 16.55^{bA}	2150.49 ± 225.47^{bA}	1820.75 ± 185.97^{bB}	1998.34 ± 150.94^{bA}	2813.76 ± 84.14^{aA}	2931.68 ± 133.01^{aA}
	MP	2091.98 ± 76.29^{abA}	1921.06 ± 101.22^{bA}	2039.70 ± 133.47^{abA}	2135.34 ± 60.67^{abA}	2196.24 ± 103.15^{aA}	2032.92 ± 44.94^{abB}	2102.43 ± 102.63^{abB}
ORAC	F	5302.45 ± 282.12^{A}	-	-	-	-	-	-
	P	3448.68 ± 572.90^{aB}	2679.67 ± 225.85^{aA}	2841.23 ± 265.50^{aA}	2714.12 ± 173.33^{aA}	3454.94 ± 377.88^{aA}	2916.76 ± 306.10^{aA}	2767.41 ± 15.92^{aA}
	MP	3088.07 ± 398.17^{aB}	2094.98 ± 200.87^{bB}	2159.52 ± 12.51^{bB}	1456.86 ± 222.62^{cB}	1674.19 ± 261.69^{bcB}	1375.56 ± 46.40^{cdB}	830.15 ± 40.85^{dB}

Diferentes letras maiúsculas sobrescritas na mesma coluna indicam diferença estatisticamente significativa entre os tratamentos para o mesmo composto pelo teste de Tukey ($p<0,05$). Diferentes letras minúsculas sobrescritas na mesma linha indicam diferença estatisticamente significativa para o mesmo tratamento ao longo do período de armazenamento pelo teste de Tukey ($p<0,05$). Os valores são a média de 3 repetições (± desvio padrão). ABTS e ORAC expressos em: $\mu Mol.100mL^{-1}$ equivalente de Trolox.

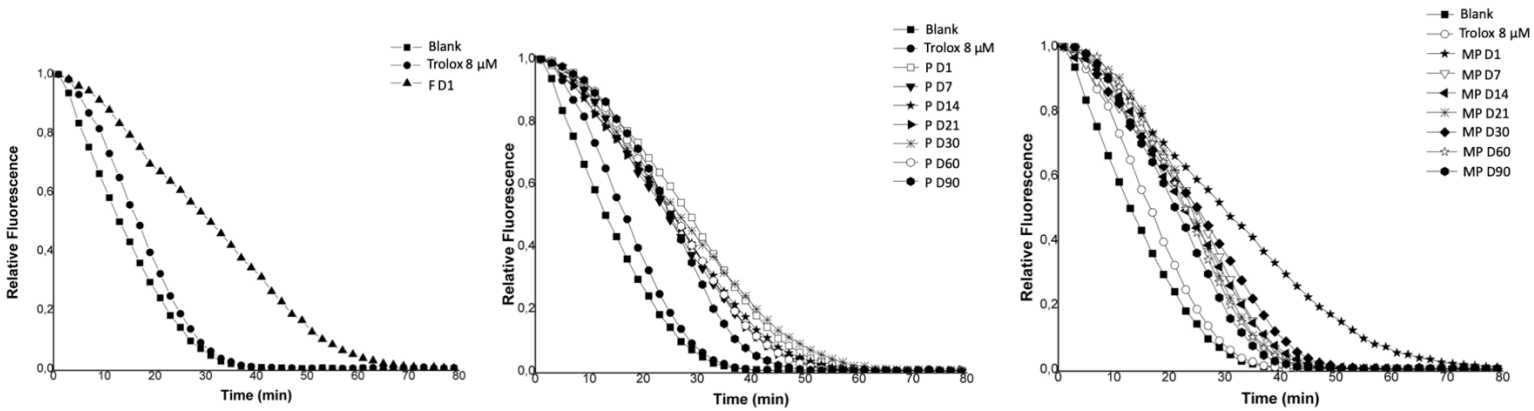

Figura 1. Capacidade antioxidante, pelo ensaio ORAC, do suco fresco (F), clarificado por tratamento enzimático e pasteurizado (P) e clarificado por tratamento enzimático, microfiltração e pasteurizado (MP), durante 90 dias de armazenamento em comparação com Trolox.

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CAPÍTULO 6

6 DISCUSSÃO GERAL

O consumo regular de produtos vegetais, como as frutas, tem sido amplamente recomendado e já foi associado a efeitos benéficos à saúde, como um menor risco para o desenvolvimento de doenças crônicas. A conscientização sobre a questão da saúde leva a um aumento no consumo de sucos de frutas naturais e outros produtos naturais como uma alternativa às bebidas e alimentos tradicionais.

O Brasil possui um grande número de espécies de frutas nativas, mas apenas uma pequena fração desse potencial é conhecida e utilizada. A goiaba serrana, uma fruta nativa e amplamente empregada em outros países, nos últimos anos tem sido estudada, devido ao seu sabor e aroma, e também à sua composição nutricional. No estado de Santa Catarina, a espécie tem sido cultivada, e tanto a fruta, como a flor, utilizada em diversos segmentos. Entretanto, a fruta possui uma vida útil bastante limitada, devido a sua composição enzimática, e este fato limita o consumo *in natura*. No entanto, o processamento da fruta é uma alternativa para agregar valor em diversos produtos alimentícios.

O presente trabalho foi desenvolvido com o objetivo de produzir e avaliar as etapas do processamento do suco integral da goiaba serrana (*Acca sellowiana*), e o resíduo originado da produção do suco, na forma de farinha, uma vez que a goiaba serrana possui elevado potencial de produção e consumo.

Ao considerar que para produção do suco integral de fruta, alguns fatores precisam ser considerados para uma melhor aparência visual, sabor, conservação e composição nutricional, diferentes enzimas, e concentrações foram empregadas na elaboração deste produto assim como, etapas do processamento foram avaliadas.

No **manuscrito 1** foram avaliadas diferentes enzimas, assim como suas concentrações, nas características físico-química do suco produzido. O rendimento, a cor, assim como o teor de acidez e sólidos solúveis apresentou diferenças significativas a depender da enzima e sua concentração utilizada. A partir dos resultados, foi possível identificar qual a melhor enzima, assim como sua concentração, para obter o suco da goiaba serrana. No **manuscrito 2**, dois sucos foram avaliados por três meses quanto aos parâmetros físico-químicos, teor de açúcares, vitamina C e carotenoides, assim como a identificação e quantificação dos compostos fenólicos foram determinados.

Apesar das três enzimas terem melhorado o parâmetro de cor consideravelmente, com o aumento da intensidade da cor amarela, da translucidez, e a diminuição da turbidez. No entanto, a aplicação das enzimas Lallzyme e Rohapect 10 L reduziram os teores de açúcares redutores, assim como de sólidos solúveis e é relatado em diversos estudos que a diminuição desta relação, implica em uma menor aceitação sensorial pelo consumidor. Além disso, os sucos tiveram um menor rendimento, quando utilizada estas enzimas.

A enzima Pectinex® Ultra Clear obteve os melhores resultados para o suco da goiaba serrana, visto que, ela foi a mais eficiente na extração, o suco ficou mais translúcido, como observado pelo aumento do parâmetro de cor *L**, além do aumento da da intensidade da cor. Além disso, a enzima não reduziu os açúcares totais, sólidos solúveis e acidez presentes naturalmente no suco, e ainda, aumentou o rendimento do suco. Além disso foi observado que quanto maior concentração utilizada (1,5 e 10 $U.mL^{-1}$) na etapa anterior ao despolpamento, maior o rendimento da bebida produzida. O mesmo ocorreu em relação aos açúcares redutores, conforme o aumento da concentração enzimática, previamente a etapa de filtração, maior o teor encontrado para este parâmetro. A enzima na maior concentração ainda manteve o teor de sólidos solúveis e acidez. A amostra que se destacou (T17) foi submetida a microfiltração e como esperado, a clarificação do suco foi mais eficiente, deixando-o com aparência translúcida. Portanto, os parâmetros de cor melhoraram, no entanto, os açúcares redutores, sólidos solúveis e relação sólidos solúveis e acidez reduziram. Ainda, foi verificado que o suco *in natura* da goiaba serrana, sem o tratamento enzimático e microfiltração (suco controle) apresentou um modelo de fluido não newtoniano, enquanto, os sucos com tratamento enzimático e com microfiltração apresentaram um comportamento de fluido newtoniano. Este comportamento de fluido newtoniano, é melhor para indústria, visto que indica uma alta eficiência de mistura quando tal fluido é homogeneizado com um agitador mecânico. Além disso, o conhecimento da viscosidade pode contribuir para a otimização do processo, redução de custos com ingredientes e melhoria da consistência do produto.

O suco extraído com Pectinex® Ultra Clear na concentração de 10 $U.mL^{-1}$ (suco P) e o mesmo suco com a adição da etapa de microfiltração (suco MP) foram produzidos e armazenados por três meses em condições de simulação de ambiente de mercado. As características físico-químicas dos sucos obtidos sofreram ligeira alteração durante o armazenamento. O β-caroteno foi o principal carotenoide, seguido pela luteína e os resultados indicam que o armazenamento leva a uma diminuição na proporção de

carotenoides nos sucos tratados, de 25-37%, da mesma forma, uma redução no teor de vitamina C de 64-75% após 30 dias de armazenamento. No entanto, o suco de goiaba serrana, mostrou ter uma boa quantidade desta vitamina antes do armazenamento.

Um total de 44 compostos fenólicos foram identificados nos sucos da goiaba serrana e o isômero pedunculagina foi o principal composto identificado nos sucos tratados com enzimas. Alguns compostos fenólicos foram identificados pela primeira vez em produtos de goiaba serrana, como, corilagina, casuarinina, dilactona de ácido valoneico, glicose trisgalloil HHDP e ácido cafeoil chiquímico.

Quanto aos compostos fenólicos totais, observa-se que o suco microfiltrado teve uma redução superior no teor desses compostos bioativos, possivelmente devido a retenção desses compostos na membrana. Os sucos tratados com enzimas apresentaram uma quantidade muito maior de compostos fenólicos totais do que o suco fresco, o que indica que a preparação enzimática conseguiu extrair melhor estes compostos.

Os sucos processados de frutas originam uma quantidade expressiva de resíduos. No suco da goiaba serrana, esse resíduo foi de 36 a 70%, a depender, da enzima e concentração utilizada. A gestão desses resíduos está se tornando cada vez mais importante e, portanto, é interessante saber o que pode ser feito com os resíduos de produtos vegetais. Embora considerados produtos residuais, esses materiais ainda contêm uma abundância de nutrientes e compostos bioativos. No **manuscrito 3**, o resíduo da produção dos sucos foi utilizado para o desenvolvimento de farinha, e esta, avaliada quanto a caracterização físico-química, perfil de carotenoides e atividade antioxidante. A secagem do resíduo foi realizada em estufa de circulação de ar e moída em moinhos de facas para obtenção da farinha, e o teor de umidade e atividade de água ficaram dentro dos parâmetros recomendados para comercialização deste tipo de produto.

A farinha de resíduo da goiaba serrana apresentou baixo teor de lipídios e carboidratos, no entanto, ela possui um alto teor de fibra total, mais de 50% de sua composição. Diferentemente do suco, na farinha o principal carotenoide identificado foi a zeaxantina e β-criptoxantina. A farinha possui uma boa concentração de vitamina C, assim como uma forte capacidade antioxidante, demonstrados nos ensaios ORAC e ABTS.

Portanto, utilizar a fruta de forma integral, é possível, e assim pode-se agregar valor nutricional em diversos produtos alimentícios. Dessa forma, o uso integral da goiaba serrana pode contribuir para uma alimentação diversificada, assim como na inovação no

desenvolvimento de novos produtos, além de colaborar para a redução de resíduos agroindustriais.

Desenvolvimentos futuros nesta linha de pesquisa incluem análises sensoriais com o suco da goiaba serrana que dará resultados importantes. Além disso, incorporar a farinha do resíduo em produtos alimentícios, assim como, realizar testes sensoriais também pontos são importantes que podem ser estudados.

CAPÍTULO 7

7 CONCLUSÃO GERAL

Os resultados das análises da produção do suco e da casca da goiaba serrana, permitem concluir que a enzima Pectinex® Ultra Clear apresentou os melhores resultados para os parâmetros físico-químicos, sendo a mais eficaz para a extração do suco de goiaba serrana. A maior concentração utilizada no estudo (10 $U.mL^{-1}$) na etapa anterior à despolpação aumentou o rendimento, assim como a concentração de açúcares. A microfiltração melhorou os parâmetros de cor, tornando o suco translúcido e com a cor mais intensa, entretanto, reduziu o teor de sólidos solúveis, açúcares redutores e acidez. Os sucos de goiaba serrana tratados com enzima e com microfiltração apresentaram comportamento de fluido newtoniano.

Um total de 44 compostos fenólicos foram identificados e o isômero pedunculagina foi o principal composto identificado em sucos tratados com enzima. Os sucos tratados com enzima apresentaram uma quantidade muito maior de compostos fenólicos totais do que o suco fresco. O suco armazenado por 90 dias levou a uma diminuição nos carotenoides e vitamina C (64-75%). Nos sucos o β-caroteno foi o principal carotenoide, seguido pela luteína.

A farinha de resíduo da goiaba serrana apresentou baixo teor de lipídios e carboidratos e alto teor de fibra bruta, com a fibra insolúvel o principal constituinte. Na farinha os principais carotenoides foram a zeaxantina e a β-criptoxantina. A farinha do resíduo apresentou boa concentração de vitamina C, perfazendo quase metade do consumo diário, assim como forte capacidade antioxidante.

De modo geral, conforme os resultados obtidos, tanto o suco da goiaba serrana, quanto a farinha mostram-se promissores quanto a composição físico-química, nutricional e funcional. Ambos os produtos são boas alternativas à alimentos tradicionais, e podem ser consideradas para melhorar a dieta da população. Além do mais, a utilização de espécies nativas contribui para agro biodiversidade local.