PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTOS E NUTRIÇÃO
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ADRIANA ANICETO

AVALIAÇÃO DE BEBIDAS DE FRUTAS COM ÁGUA DE COCO PROCESSADAS POR TRATAMENTO TÉRMICO E ALTA PRESSÃO HIDROSTÁTICA

EVALUATION OF FRUIT BEVERAGES WITH COCONUT WATER PROCESSED BY THERMAL TREATMENT AND HIGH HYDROSTATIC PRESSURE

Rio de Janeiro
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Orientador: Dr. Anderson Junger Teodoro.
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EPÍGRAFE

Se você quiser ir rápido, vá sozinho

Se você quiser ir longe, vá acompanhado

(Provérbio africano)

RESUMO

A mistura de frutas nas bebidas prontas para beber tem surgido como uma maneira de melhorar as características sensoriais, nutricionais e funcionais. A água de coco é uma tendência versátil na indústria e vem crescendo economicamente por seu caráter funcional se torna uma alternativa como substituto da água no desenvolvimento de bebida. Frutas como goiaba e melancia são ricas em licopeno e tradicionalmente conhecidas pela população se tornan possibilidades para compor uma bebida de fruta com água de coco. A busca por manter as propriedades nutricionais e bioativas das bebidas tem levado as indústrias a desenvolver soluções tecnológicas que mantenham o perfil da fruta. O tratamento térmico é um método de conservação muito utilizado e apresenta alguns impactos nos produtos, principalmente sensorial. Dessa forma a alta pressão hidrostática tem se apresentado como uma solução para preservar as características sensoriais e nutricionais das bebidas. Nesse sentido, o objetivo principal desse estudo é avaliar bebidas de frutas com água de coco processadas por tratamento térmico e alta pressão hidrostática. Para tal, o presente trabalho foi dividido em cinco capítulos. No primeiro capítulo, foi realizado um levantamento bibliográfico sobre a água de coco, cultivo e características, as propriedades nutricionais e os benefícios para a saúde, utilizando as bases de dados eletrônicas Scielo, Scopus, Repositório Alice da EMBRAPA, Periódicos CAPES e Google acadêmico aplicando os descritores, água de coco, nutrição, composição, saúde, benefícios à saúde, economia, pasteurização, ultra pressão, ultrassom, ozônio. No segundo capítulo, foi desenvolvido bebidas de frutas (melancia e goiaba) com água de coco e suco de maçã para adoçamento natural e realizada a caracterização físico-química e de propriedades sensoriais. O planejamento fatorial 2^2 utilizado no desenvolvimento das bebidas e análises físico-química e sensorial foi performado. De acordo com os resultados da análise sensorial e metodologia de superfície de resposta, verificou-se que as fórmulas potencialmente otimizadas contêm em sua composição 40g de fruta, 30g de água de coco e 30g de suco de maçã por 100 ml de bebida. No terceiro capítulo, foi observado o efeito do tratamento térmico e vida de prateleira nas propriedades físico-químicas e capacidade antioxidante de bebidas de fruta com água de coco. O impacto das tecnologias de tratamento térmico convencional nas características das bebidas foi avaliado aplicando diferentes condições de tratamento térmico 124-126ºC e 104-109ºC por 17s. A vida útil sob refrigeração (4ºC) foi monitorada por 180 dias. Ambos os tratamentos nos parâmetros físico-químicos tiveram baixo impacto resultantes do processamento e durante todo o período de armazenamento. A aparência visual mostrou decantação da polpa de melancia e escurecimento da bebida de goiaba. A capacidade antioxidante determinada por diferentes métodos resultou em retenção da atividade antioxidante para as duas bebidas de frutas. A goiaba apresentou maior rendimento de polpa em relação à melancia, 86 e 62%, respectivamente. A bebida de goiaba com água de coco (1915,06$\pm$100,74 mg EAG/100g) apresentou cerca de 25 vezes mais teor de compostos fenólicos totais do que a bebida de melancia com água de coco (81,53$\pm$4,31 mg EAG/100g) e maior capacidade antioxidante em ambas as condições de processo. No quarto capítulo, foi avaliado o efeito da tecnologia de alta pressão hidrostática nas características das bebidas de fruta com água de coco e um planejamento fatorial 2^2 foi utilizado para avaliar as pressões e os tempos de processo. As diferentes condições de processo não afetaram significativamente ($p>0,05$) as características físico-químicas, demonstrando estabilização do pH, sólidos solúveis totais e acidez. A maior diferença de cor em relação ao controle (bebidas não processadas), foram observadas nas bebidas de melancia e, no caso da bebida de goiaba, as menores diferenças ocorreram na menor pressão e tempo (200MPa/4min.). As diferentes pressões e tempos mantiveram o teor de compostos fenólicos totais em ambos as bebidas. Na avaliação da capacidade antioxidante, não foi observada diferença significativa para o método FRAP na melancia e o método TEAC na goiaba sob diferentes condições, e o método DPPH foi o que obteve as maiores médias de atividade antioxidante, 11,15±0,00 e 47,56±4,59 µmol Trolox/ g para melancia e goiaba, respectivamente. Nos resultados de carotenoides, observou-se um aumento no teor de carotenoides totais, β-caroteno e licopeno na maior pressão e tempo de processo. Os resultados da análise de variância (ANOVA) e do efeito padronizado indicam que a variação de pressão (MPa) e tempo (minutos) não influencia os resultados de carotenoides totais, β-caroteno e licopeno. No quinto capítulo foi comparado os efeitos da alta pressão hidrostática (HHP) com o tratamento térmico (HT) nas bebidas de frutas com água de coco. O impacto das tecnologias de processamento foi avaliado pela aplicação de dois tratamentos HHP a 200 MPa/4min. e 400 MPa/12 min. e HT 124-126ºC e 104-109ºC por 17s. Todos os tratamentos garantiram estabilidade microbiológica. Além disso, os parâmetros físico-químicos não foram significativamente diferentes considerando as diferentes condições de processo. Em relação aos parâmetros de cor, a diferença de cor (ΔE) em relação ao controle (bebidas não processadas) maior no HT em relação ao HHP. Em relação ao teor de compostos fenólicos totais, houve aumento nas amostras com goiaba e diminuição nas amostras com melancia no HT, sendo que as amostras submetidas ao HHP tiveram maior retenção. Na capacidade antioxidante considerando apenas os métodos DPPH, FRAP e TEAC, todos os tratamentos tiveram retenção de quantidades. Na avaliação dos carotenoides, as amostras com HT apresentaram diminuição no teor de carotenoides totais, β-caroteno e licopeno, já nas amostras submetidas ao HHP houve retenção desses compostos. As bebidas de frutas com água de coco submetidas a alta pressão hidrostática é uma alternativa para produtos com apelo funcional, porém avaliação de impacto financeiro e posicionamento do produto no mercado deve ser avaliado para definir a melhor tecnologia a ser aplicada.

Palavras-chave: Água de coco; bebida de fruta; tratamento térmico; alta pressão hidrostática; compostos bioativos.

ABSTRACT

The mixture of fruits in ready-to-drink beverages has emerged as a way to improve sensory, nutritional and functional characteristics. Coconut water is a versatile trend in the industry and has been growing economically due to its functional character, becoming an alternative as a substitute for water in the development of beverages. Fruits like guava and watermelon are rich in lycopene and traditionally known by the population, they become possibilities to compose a fruit drink with coconut water. The quest to maintain the nutritional and bioactive properties of beverages has led industries to develop technological solutions that maintain the fruit's profile. Heat treatment is a widely used conservation method and has some impacts on products, mainly sensory. Thus, high hydrostatic pressure has been presented as a solution to preserve the sensory and nutritional characteristics of beverages. In this sense, the main objective of this study is to evaluate fruit beverages with coconut water processed by heat treatment and high hydrostatic pressure. To this end, this work was divided into five chapters. In the first chapter, a bibliographical survey was carried out on coconut water, cultivation and characteristics, nutritional properties and health benefits, using the electronic databases Scielo, Scopus, Alice Repository of EMBRAPA, Periodicals CAPES and academic Google applying the descriptors, coconut water, nutrition, composition, health, health benefits, economy, pasteurization, ultra-pressure, ultrasound, ozone. In the second chapter, fruit beverages (watermelon and guava) with coconut water and apple juice for natural sweetening were developed and the physical-chemical characterization and sensory properties were performed. The 2^2 factorial design used in the development of beverages and physicochemical and sensory analysis was performed. According to the results of the sensorial analysis and response surface methodology, it was verified that the potentially optimized formulas contain in their composition 40g of fruit, 30g of coconut water and 30g of apple juice per 100 ml of beverage. In the third chapter, the effect of heat treatment and shelf life on the physicochemical properties and antioxidant capacity of fruit beverages with coconut water was observed. The impact of conventional heat treatment technologies on beverage characteristics was evaluated by applying different heat treatment conditions 124-126°C and 104-109°C for 17s. Shelf life under refrigeration (4ºC) was monitored for 180 days. Both treatments on physicochemical parameters had low impact resulting from processing and throughout the storage period. The visual appearance showed decanting of the watermelon pulp and darkening of the guava beverage. Antioxidant capacity determined by different methods resulted in retention of antioxidant activity for both fruit beverages. Guava had the highest pulp yield compared to watermelon, 86 and 62%, respectively. The guava drink with coconut water (1915.06±100.74 mg EAG/100g) had about 25 times more content of total phenolic compounds than the watermelon drink with coconut water (81.53±4.31 mg EAG/100g) and higher antioxidant capacity in both process conditions. In the fourth chapter, the effect of high hydrostatic pressure technology on the characteristics of fruit beverages with coconut water was evaluated and a 2^2 factorial design was used to evaluate pressures and process times. The different process conditions did not significantly affect ($p>0.05$) the physicochemical characteristics, demonstrating stabilization of pH, total soluble solids and acidity. The highest color difference in relation to the control (non-processed beverages) was observed in the watermelon beverages and, in the case of the guava beverage, the lowest differences occurred in the lowest pressure and time (200MPa/4min.). The different pressures and times maintained the content of total phenolic compounds in both beverages. In the evaluation of the antioxidant capacity, no significant difference was observed for the FRAP method in watermelon and the TEAC method in guava under different conditions, and the DPPH method was the one that obtained the highest averages of antioxidant activity, 11.15±0.00 and 47.56±4.59 µmol Trolox/g for watermelon and guava, respectively. In the results of carotenoids, an increase in the content of total carotenoids, β-carotene and lycopene was observed in the higher pressure and process time. The results of the analysis of variance (ANOVA) and the standardized effect indicate that the variation of pressure (MPa) and time (minutes) does not influence the results of total carotenoids, β-carotene and lycopene. In the fifth chapter, the effects of high hydrostatic pressure (HHP) with heat treatment (HT) on fruit beverages with coconut water were compared. The impact of processing technologies was assessed by applying two HHP treatments at 200 MPa/4min. and 400 MPa/12 min. and HT 124-126ºC and 104-109ºC for 17s. All treatments guaranteed microbiological stability. Furthermore, the physicochemical parameters were not significantly different considering the different process conditions. Regarding the color parameters, the difference in color (ΔE) in relation to the control (non-processed beverages) is higher in HT than in HHP. Regarding the content of total phenolic compounds, there was an increase in the samples with guava and a decrease in the samples with watermelon in the HT, and the samples submitted to the HHP had higher retention. In the antioxidant capacity considering only the DPPH, FRAP and TEAC methods, all treatments had retention of quantities. In the evaluation of carotenoids, the samples with HT showed a decrease in the content of total carotenoids, β-carotene and lycopene, whereas in the samples submitted to HHP there was retention of these compounds. Fruit beverages with coconut water subjected to high hydrostatic pressure is an alternative for products with functional appeal, but an assessment of the financial impact and positioning of the product in the market must be evaluated to define the best technology to be applied.

Keywords: Coconut water; Fruit beverage; Heat treatment; High hydrostatic pressure; Bioactive compounds.

SUMÁRIO

CAPÍTULO II

DEVELOPMENT OF FRUIT BEVERAGES WITH COCONUT WATER: PHYSICOCHEMICAL CHARACTERIZATION, ANTIOXIDANT POTENTIAL AND SENSORY PROPERTIES

CAPÍTULO III

EFFECT OF HEAT TREATMENT AND SHELF LIFE ON PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT CAPACITY OF FRUIT BEVERAGES WITH COCONUT WATER

CAPÍTULO IV

EFFECT OF THE APPLICATION OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY ON CHARACTERISTICS OF FRUIT BEVERAGES WITH COCONUT WATER

CAPÍTULO V

COMPARISON OF HEAT TREATMENT AND HIGH HYDROSTATIC PRESSURE ON CHARACTERISTICS OF FRUIT BEVERAGES WITH COCONUT WATER

INTRODUÇÃO

INTRODUÇÃO

Os efeitos promovidos pelas frutas à saúde têm sido atribuídos à sua composição que inclui nutrientes essenciais e compostos bioativos. Estratégias para aumentar a disponibilidade, acessibilidade e prazo de validade fornecerão mais opções e oportunidades para as pessoas atenderem às recomendações diárias de ingestão. A susceptibilidade e o alto teor de umidade das frutas frescas as tornam altamente perecíveis, limitando sua disponibilidade. Tal circunstância cria demanda por formas alternativas de disponibilizar as frutas, como bebidas prontas para beber (TADAPANENI *et al*., 2014).

As bebidas de frutas são facilmente digeríveis, altamente refrescantes, saciam a sede, apetitosas e nutricionalmente muito superiores a muitas bebidas artificiais e gaseificadas (MOAZZEM; SIKDER; ZZAMAN, 2019).

A água de coco pode ser utilizada para acrescentar valor aos produtos e seu uso pode substituir a água natural na formulação de néctares. Suas propriedades nutricionais e terapêuticas, é uma solução natural, rica em sais minerais, açúcares e aminoácidos essenciais e o sabor característico da água de coco, ajuda a mascarar o sabor conferido por outros ingredientes (VIEIRA *et al*., 2014) (CHAUHAN *et al*., 2012).

A produção de água de coco no Brasil em 2020 foi de aproximadamente 170 milhões de litros, um aumento de 8% em relação a 2019. Isso reflete a tendência de aumento do consumo dessa categoria (ABIR – Associação Brasileira das Indústrias de Refrigerantes e de Bebidas Não Alcoólicas, 2022).

Muitas frutas com características sensoriais e nutricionais podem ter um efeito sinérgico sobre os atributos da água de coco, como goiaba vermelha (*Psidium guajava* L.) e melancia vermelha (*Citrullus lanatus*). A goiaba é muitas vezes reconhecida como "superfruta" que tem uma importância nutricional considerável em termos de vitaminas A e C com sementes que são ricas em ácidos graxos poli-insaturados ômega-3, ômega-6 e principalmente fibra alimentar, riboflavina, bem como como em proteínas e sais minerais (M. KADAM *et al.*, 2012). A melancia há muito tempo é identificada como uma boa fonte de licopeno e, recentemente, o suco de melancia também ganhou atenção como uma bebida funcional para exercícios, pois contém eletrólitos e uma quantidade significativa do aminoácido citrulina, que demonstrou ter efeitos ergogênicos (MILCZAREK *et al*., 2020).

A formulação de bebidas mistas de frutas, na forma “pronta para beber”, pode ser utilizada com o intuito de melhorar as características nutricionais de determinados sucos, pela complementação de nutrientes fornecidos por diferentes frutas (CARVALHO et al., 2005) (VIEIRA et al., 2014). Em relação a sucos e néctares, foi verificado uma variação anual de produção 4,7% quando comparado os anos de 2020 e 2021. Em termos de consumo per capita do mercado brasileiro de néctares, foi consumido cerca de 8,43 litros por habitante no ano de 2021 um aumento de 3,9% quando comparado a 2020. Mostrando que néctares é uma alternativa para o consumo de mistura de frutas. Apesar da variedade de bebidas de frutas com sabores agradáveis e alto potencial de comercialização, são poucos os produtos comerciais que utilizam misturas de frutas para sinergia de sabor, nutricional e funcional.

Há expectativas que as propriedades antioxidantes dos sucos de frutas frescas sejam impactadas por vários fatores. A trituração de frutas, clarificação, filtração e pasteurização por calor durante a produção de suco podem alterar as propriedades antioxidantes de sucos ou bebidas de frutas (JUMLAH *et al*., 2016).

A inovação desempenha um papel importante como ferramenta para melhorar a competitividade da indústria de alimentos. A indústria de sucos de frutas é um dos setores de alimentos que mais investiu na implementação de novas tecnologias, como as não térmicas (MARTINS *et al*., 2019).

O aumento do interesse dos consumidores por alimentos de alta qualidade com atributos sensoriais e sem aditivos levou ao desenvolvimento de tecnologias de processamento de alimentos não térmicos como alternativa ao tratamento térmico convencional. Portanto, uma estratégia importante no desenvolvimento de produtos pressurizados é aumentar a aceitabilidade dos produtos, manter os sabores originais, reduzir aditivos e aumentar os aspectos nutricionais (HUANG *et al*., 2017).

As tecnologias de tratamento térmico continuam sendo centrais na indústria de alimentos, mas podem afetar a aparência, os sabores e o valor nutricional dos alimentos, e não necessariamente atendem à demanda da sociedade moderna por alimentos naturais, frescos e esteticamente atraentes. A tecnologia de alta pressão oferece para os fabricantes de alimentos desenvolverem novos alimentos com uma vida útil prolongada, mantendo as propriedades sensoriais e os valores nutricionais. Essas características normalmente não podem ser alcançadas usando a tecnologia de tratamento térmico e, portanto, a tecnologia emergente de alta pressão atende melhor a demanda do consumidor por novos alimentos seguros, saudáveis e contendo menos aditivos (HUANG *et al*., 2017).

Os consumidores parecem estar mais interessados em alimentos naturais e menos processados, como sucos frescos ou prensados a frio. No caso de processo de alta pressão, a falta de conhecimento sobre essa tecnologia reforça a necessidade de mais informações para aumentar a confiança e o conhecimento dos consumidores brasileiros (MARTINS *et al.*, 2019). Uma das estratégias das indústrias é desenvolver produtos com implementação de novas tecnologias, que proporcionam uma série de benefícios em termos de segurança alimentar, extensão da vida útil e aumento da qualidade nutricional e sensorial.

O estudo sobre desenvolvimento de produtos seguros, nutricionalmente atrativos e o impacto das tecnologias de tratamento térmico e alta pressão hidrostática é extremamente importante para trazer informação para a indústria e academia. Com esse intuito o objetivo desse estudo é avaliar bebidas de frutas com água de coco processadas por tratamento térmico e alta pressão hidrostática.

O presente trabalho segue as normas da tese no formato de artigo definido pelo Programa de Pós-Graduação em Alimentos e Nutrição em 14 de maio de 2019.

Assim esta tese, está dividida em 5 capítulos:

(I) Artigo de revisão bibliográfica: "Coconut water: production, nutritional properties and health benefits."

(II) Artigo original que contempla resultados e discussão dos experimentos: "Development of fruit beverages with coconut water: physicochemical characterization, antioxidant potential and sensory properties."

(III) Artigo original que contempla resultados e discussão dos experimentos: "Effect of heat treatment and shelf life on physicochemical properties and antioxidant capacity of fruit beverages with coconut water"

(IV) Artigo original que contempla resultados e discussão dos experimentos: "Effect of the application of high hydrostatic pressure technology on characteristics of fruit beverages with coconut water"

(V) Artigo original que contempla resultados e discussão dos experimentos: "Comparison of heat treatment and high hydrostatic pressure on characteristics of fruit beverages with coconut water"

CAPÍTULO I

COCONUT WATER: PRODUCTION, NUTRITIONAL PROPERTIES AND HEALTH BENEFITS

ÁGUA DE COCO: PRODUÇÃO, PROPRIEDADES NUTRICIONAIS E BENEFÍCIOS À SAÚDE

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ABSTRACT

Coconut water (Cocos nucifera L.) is an undiluted, non-fermented beverage obtained from the liquid part of the coconut fruit. It is a versatile product in the industry and has been growing economically due to its functional character. Objective: to identify in the scientific literature different aspects of coconut water production and its nutritional characteristics applied to health. Methodology: A narrative review of the literature was carried out, using Scielo, Scopus, Alice da EMBRAPA Repository, Periodicals CAPES and Google Scholar as electronic databases, applying the descriptors, coconut water, nutrition, composition, health, health benefits, economy, pasteurization, ultrapressure, ultrasound, ozone and their respective names in English, considering the Boolean operators "OR" and "AND" to combine subjects. Titles were screened, followed by abstracts, considering the following eligibility criteria: full text available in English and/or Portuguese; paid and/or free access; dissertations and theses, narrative, systematic review, observational and longitudinal studies, including clinical trials. Results: non-thermal processing methods such as: use of ozone, ultrasound and ultraviolet light were effective in maintaining the shelf life of the beverage, however ultrapressure showed changes in physical functionality and / or changes in the color of protein-rich foods. The presence of phytohormones, vitamins and amino acids was detected, which are responsible for the antioxidant property of the product, as well as the beneficial effects on health. Conclusion: New studies are proposed to evaluate the effects of coconut water on human health, as it is a drink with market potential and accessible to the consumer.

Keywords: Coconut water. Production. Health.

RESUMO

A água de coco (*Cocos nucifera* L.) é uma bebida não diluída, não fermentada, obtida da parte líquida do fruto do coqueiro. É um produto versátil na indústria e vem crescendo economicamente por seu caráter funcional. **Objetivo:** identificar na literatura científica diferentes aspectos da produção da água de coco e suas características nutricionais aplicadas à saúde. **Metodologia:** realizou-se uma revisão narrativa da literatura, utilizando as bases de dados eletrônicas Scielo, Scopus, Repositório Alice da EMBRAPA, Periódicos CAPES e Google acadêmico aplicando os descritores, água de coco, nutrição, composição, saúde, benefícios à saúde, economia, pasteurização, ultra pressão, ultrassom, ozônio e seus respectivos nomes em inglês, considerando os operadores booleanos "OR" e "AND" para combinação dos assuntos. Foi feita uma triagem dos títulos e em seguida, resumos, considerando os seguintes critérios de elegibilidade: texto integral disponível em inglês e/ou português; acesso pago e/ou gratuito; dissertações e teses, revisão narrativa, sistemática, estudos observacionais e longitudinais, incluindo ensaios clínicos**. Resultados**: os métodos de processamento não térmicos como: ozonização, ultrassom e luz ultravioleta foram eficazes em manter o tempo de vida útil da bebida, porém a ultrapressão apresentou alterações na funcionalidade física e / ou mudanças na cor de alimentos ricos em proteínas. Foi detectado a presença de fitohormônios, vitaminas e aminoácidos que são responsáveis pela propriedade antioxidante do produto, assim como os efeitos benéficos à saúde.**Conclusão**: Propõe-se novos estudos para avaliar os efeitos da água de coco na saúde humana, pois é uma bebida com potencial de mercado e acessível ao consumidor.

Palavras-chave: Água de coco. Produção. Saúde.

1. INTRODUCTION

According to Brazilian legislation, the product “coconut water” is defined as the undiluted, non-fermented beverage obtained from the liquid part of the coconut fruit (*Cocos nucifera* L.), through an appropriate technological process (1).

It has become a fashionable drink, known for its nutritional properties and a good source of vitamin C and several important minerals, in particular potassium (250 mg/100g) and manganese (0.142 mg/100g) (2). The highest concentrations of trace elements found were manganese, which makes coconut water a natural source of this element, covering almost the entirety of the daily reference intake of manganese recommended by the European Food Safety Agency (3 mg/day) (3).

Brazil is the fifth largest producer of coconut, with a share of 4.5% of the total world. Over the last decade, the annual growth of the activity was 0.8% of the harvested area and 0.1% of the world coconut production (4). Coconut palm is being cultivated in almost all of Brazil, whose current area is 187.5 thousand ha with production of 1.6 billion fruits. In the Northeast region are concentrated, 80.9% of the country's coconut harvested area and 73.5% of its production (5).

Coconut food products have been gathering supporters due to the availability of numerous forms of presentation, brands on the market, pleasant characteristics to the taste, digestive disorders issues, such as: gastrointestinal problems of intolerance and allergy to lactose and gluten. Another audience that has emerged in the sector are vegan and vegetarian consumers who base their diet on the consumption of vegetables, nuts and cereals and seek dairy-free products (without milk from animal sources), in addition to high performance athletes who use coconut as an electrolyte replenisher (6).

In addition to using fresh fruit for food (both for water and pulp), the fruit peel can be transformed into ropes, rugs, hats and vehicle backrests; and oil, in addition to its use in food, is a raw material for margarine, glycerol, cosmetics, synthetic detergent, soap, candles and airplane brake fluids (7)

Coconut water, in its natural form, is a refreshing and nutritious drink and is part of the diet of people in general, in addition to having several beneficial effects on human health. It has a unique composition and can be used as an intravenous hydration. It has antidiabetic, antihyperlipidemic and beneficial effects on the digestive system (8).

Evidence points out that coconut water contains folate, phytohormones, cytokines, auxins and several other bioactive compounds that are of medicinal importance and have promising potential in improving human (9).

Thus, taking into account that coconut water is a product that has been growing economically over the years and has an appeal for being a beverage with functional and nutritional characteristics, the present study aimed to review the scientific literature on the different aspects of coconut water production and its nutritional characteristics applied to health.

2. METODOLOGY

The present study will present a narrative review of the literature. For the elaboration, a consultation was carried out in the electronic databases Scielo, Scopus, Repository Alice da EMBRAPA, CAPES Periodicals and Google academic using the descriptors, coconut water, nutrition, composition, health, health benefits, economy, pasteurization, ultrapressure, ultrasound, ozone and their respective names in English, being considered Boolean operators "OR" and "AND" for combination of subjects.

A narrative review concerning the nutritional and technological aspects of vegetable oils with a predominance of medium-chain triacylglycerols used as a basis only scientific articles searched in electronic databases such as: Scopus, Scielo, Science Direct, Web of Science and Online Library Wiley (10). The criteria adopted for the selection of articles included publications in the English language, preferably between the years 2015 to 2021, relevant and recent research in the nutritional and technological area on the use of medium-chain fatty acids.

The selection of studies was initially carried out by screening the titles and later reading the abstracts, considering the following criteria: full text available in English and/or Portuguese; paid and/or free access; dissertations and theses, narrative review, systematic, observational and longitudinal studies, including clinical trials. With regard to exclusion criteria, abstracts of any order were rejected; works presented in congresses, symposia and included in proceedings; graduation studies assignments research outside the defined language. During the research, a lower time limit was not established for the search for articles. At the end of the search, the studies that met the inclusion criteria were added to a spreadsheet to facilitate data management.

3. RESULTS AND DISCUSSION

3.1. COCONUT CHARACTERISTICS AND CULTIVATION

Data provided by FAOSTAT (11) (2021) report that the world area harvested with coconut is 11.8 million hectares, producing 62.9 million tons. Only Indonesia, Philippines and India occupy 73.0% of this area and participate with 74.1% of world production.

The sale of coconut water throughout Brazil in 2020 was 21.9 tons, almost the same amount as the previous year. However, revenue was higher due to the 19.6% increase in price, possibly because of the increase in production costs related to imported inputs (5).

The COVID-19 pandemic in 2019/2020 affected sales of packaged coconut water worldwide, especially in the US and EU, where the market is better established. The high demand scenario with a shortage of coconuts sold at a higher price went through a crisis during the pandemic period, in which supply did not meet demand (4).

Among the varieties of the species *Cocos nucifera* L., the dwarf coconut tree is used mainly in the production of coconut water because it has superior sensory characteristics compared to other cultivars. The giant coconut tree is the most used in the fresh consumption of dried pulp, as well as in industrial use, to obtain derivatives such as coconut milk, from grated coconut. Meanwhile, the hybrid coconut tree (dwarf x giant) has been recommended for its dual aptitude based on parental characteristics, that is, to produce coconut water and dry coconut depending on the physiological maturity of the fruit at harvest (6).

The giant coconut tree reaches between 20 and 30 meters in height, and can produce up to 80 fruits a year, with an economic life of 60 to 70 years. The dwarf coconut tree reaches up to 12 meters in height and has a useful life between 30 and 40 years. The main advantage of the dwarf variety is that the beginning of average production takes place two to three years after planting, while the production of the giant variety takes five to seven years (7). Table 1 shows the differences between the dwarf, giant and hybrid cultivars.

Table 1. Characteristics of coconut cultivars.

Characteristics/Cultivars	Dwarf	Hybrid	Giant
Beginning of flowering (years)	2 to 3	3 to 4	5 to 7
Lifespan (years)	30 to 40	50 to 60	60 to 70
Fruit size	Small	Medium/Large	Large
Growth	Slow	Intermediary	Fast
Size (height)	10 to 12 m	20 m	20 to 30 m
Production (fruits/years)	150 to 200	130 to 150	60 to 80
Average fruit weight	900 g	1200 g	1400 g
Destination	Water	Water/Agroindustry/Cooking	Agroindustry/Cooking

Source: APROCOCO [12] (2020).

A coconut consists of three layers of husk, namely: exocarp, mesocarp and endocarp. The outermost layer, normally smooth with a greenish color, is called the exocarp. The middle layer is the fibrous bark, or mesocarp, that surrounds the hard woody layer called the endocarp. Inside the endocarp are the solid endosperm, which consists of coconut pulp, and the liquid endosperm, formed by coconut water. A soft coconut is one with a maturity of 6 to 9 months and its water is either consumed directly or processed into a variety of beverages (13). In figure 1 it is possible to observe three types of cultivars of red dwarf coconut, yellow dwarf and green dwarf, which is the most used to obtain coconut water.

The action of food enzymes, such as polyphenol oxidases (PPO) and peroxidases (POD), plays a key role in decreasing the storage time and market potential of coconut water. The catalytic activities of both enzymes have many unpleasant effects on coconut water, even during cold storage. Likewise, pink color formation, browning, off-notes and odors are certain questionable outcomes of catalytic functions and microbial activity (15).

Figure 1 - Detail for the color of the epicarp (outer part) which represents one of the differences between Dwarf Red, Dwarf Yellow and Dwarf Verde cultivars (from left to right in the photo).

Source: Embrapa [14] (2018).

Soft coconut water is highly sensitive to microbial attack within hours of its extraction from the fruit, which in turn results in the loss of nutritional components as well as a shortened shelf life (16). In this regard, certain substances inherent in coconut water, such as antimicrobial peptides and lauric acid, have been reported to act as an inhibitor of the growth of microbial strains (17).

Therefore, it is essential to preserve the sensory and nutritional quality of the drink. Thermal processing methods are the most used preservation techniques for coconut water. However, these treatments decrease the general acceptance of the product and, at the same time, reduce its qualities (18). For this reason, the industry has invested in non-thermal processing methods that aim to extend the shelf life of coconut water, but without causing sensory changes.

3.2. THERMAL PROCESSING METHODS

Thermal processing is the most commonly used approach to the manufacture and preservation of fruit and vegetable products. It includes several methods, which can be applied to solid or liquid foods (60 ◦C–200 ◦C), such as: steaming, cooking, boiling, roasting, microwaves, and the most common ones such as pasteurization and sterilization (19).

3.2.1. Pasteurization

Pasteurization conditions are designed to inactivate the most heat-resistant, non-spore-forming pathogens and spoilage bacteria (20). Pasteurization processes can be categorized as low temperature, long time (LTLT; 62–65 °C for at least 30 min) or high temperature, short time (HTST; 72–82 °C for 15–30 s) pasteurization followed by rapid cooling to less than 10°C (21). One of the issues that has drawn the attention of industries concerns the appearance of a pinkish or brownish color in coconut water subjected to refrigerated storage at a temperature of 3 ^{o}C to 5 ^{o}C, since, normally, a product treated by a HTST-type heat treatment undergoes oxidation reactions or sugar caramelization during the heating phase (22).

A study (22) showed that the HTST thermal treatment allowed a shelf life of 30 days for coconut water without preservatives and under refrigeration at a controlled temperature of 4 °C (± 1 °C), which is established as a reference temperature for the conservation of products refrigerated foods on a commercial scale. However, it was observed that this temperature indication is not properly followed when the product is displayed on supermarket and store shelves and points of sale.

3.2.2. Sterilization

Sterilization is a heat treatment that aims to produce a commercially sterile product with an extended shelf life. This can be achieved by vessel sterilization (110–116 °C for 20–30 min) or ultra-high temperature (UHT) treatment (135–145 °C for 1–10 s) (23). Container sterilization inactivates some enzymes, results in browning due to Maillard reaction and cooked flavor (20). UHT treatment results in fewer chemical changes and a poor cooked taste, but some bacterial proteases or lipases can survive the process.

The selection of UHT processing time-temperature combinations should be based on the inactivation of heat-resistant spores with the least possible undesirable changes in physicochemical and sensory properties, as well as retention of nutritional values (24,25).

3.3. NON-THERMAL TECHNOLOGIES

Non-thermal technologies applied to coconut water are researched to address inherent limitations of conventional thermal processing. While it is agreed that thermal processing is effective in causing enzyme inactivation and providing antimicrobial effects, the demand for preserving the sensory and nutritional properties of products after treatment makes it necessary to implement emerging non-thermal technologies. Emerging food processing technologies combined with non-thermal technologies find application in the preservation of coconut water and are in the line of commercialization (26).

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3.3.1. Ozone

Recognition of ozone as a GRAS (generally recognized as safe) substance in 1997, followed by FDA approval as an antimicrobial agent for direct use in food in 2001, has broadened the applications of ozone in the food industry (27). Studies have shown that ozonation can be a good alternative as a non-thermal application technology, as it is relatively simple, fast and low cost, with efficient oxidizing action on microorganisms and enzymes. This action is associated with both its molecular shape and the hydroxyl, hydroperoxide and superoxide radicals generated by its decomposition (28,29).

A Study (30) showed that the physicochemical and nutritional properties of coconut water were less affected by the treatment with ozone added nisin, being microbiologically safe for 3 weeks under refrigerated conditions when compared to coconut water treated with ultrasound and nisina, in which there were significantly greater losses of nutritional and functional content of coconut water. The greatest losses in ascorbic acid (62.58%), total sugars

(31.81%), phenolic content (44.27%) and flavonoids (48.04%) were observed probably due to the production of free radicals that can degrade polyphenols, flavonoids, ascorbic acids, and sugars, oxidizing them.

3.3.2. High Pressure

High pressure processing, also known as high hydrostatic pressure, is a non-thermal food processing method that submits food (liquid or solid) to pressures between 50 and 1000 MPa (31).

During the high pressure treatment of certain protein-rich foods, resulting changes in physical functionality and/or changes in the color of the raw product occur that are significantly smaller than those experienced using conventional thermal processing techniques (32).

A study in which high pressure processing was applied to fresh coconut water for microbial stability and quality monitoring during cold storage showed the applicability of high pressure of 500 MPa at 5 min. In addition, the shelf life of the samples was extended to 25 days at 4 °C with a substantial delay in the loss of nutritional characteristics, such as: total amino acids, proteins, sugar, ascorbic acid, phenols and antioxidant capacity (33).

However, some disadvantages, such as: high capital investment for equipment, pressure conditions must be applied to the product properly, as inadequate values can retain microorganisms and enzymes and in order to guarantee the quality of the product, it must be transported at low temperatures (17,33,34).

3.3.3. Ultrasound

Over the years, ultrasound technology has gained prominence for its potential use in the preservation of fruit juices. Studies show that high-intensity ultrasound can inactivate the main microorganisms and enzyme responsible for the deterioration reactions of products from vegetable source (35,36). Therefore, improvements in quality attributes and greater retention of bioactive compounds and nutrients are described in the literature.

One study (15) evaluated the effect of ultrasound on the inactivation and sensitization of the enzyme peroxidase (POD) in coconut water. The application of ultrasound at an acoustic density of 286 W/L, frequency of 20 kHz, at 25 °C for 30 minutes achieved 27% inactivation of the POD enzyme. Ultrasound as a pre-treatment showed a positive result in reducing the thermal resistance of the POD, allowing shorter times and/or temperatures in subsequent thermal processing.

Despite this, equipment is necessary, which has a high cost, and must be of high power to ensure maximum inactivation of enzymes and microorganisms (18,37).

3.3.4. Ultraviolet Light

Ultraviolet light is a low-cost, non-thermal processing option for liquid foods used successfully to disinfect drinking water and to pasteurize fruit and vegetable juices. UVC rays between 200 and 280 nm are considered germicidal due to the formation of thymine dimers in the DNA of pathogens, causing the termination of replication, transcription and translation of the bacterial gene and resulting in the inactivation of the pathogen (38).

A study performed in a continuous flow spiral UV reactor showed that treatment with UVC emitting 254 nm did not significantly change the physicochemical properties of soft coconut water, indicating aroma and flavor retention. Therefore, the natural properties of coconut water were not disturbed by UVC pasteurization (38).

An investigation (39) achieved 94 and 93% inactivation of polyphenoloxidase and peroxidase, respectively, using a fluence level of 400 mJ/cm2, in addition to showing no changes in essential amino acids. This inactivation is sufficient to prevent the pink discoloration caused by enzymatic browning in soft coconut water.

However, exposure to ultraviolet light for prolonged periods can damage the eyes and skin. In addition, it is a method that has low penetration power in food and it is difficult to predict the disinfection rate (39–41).

3.4 NUTRITIONAL COMPOSITION AND PHYSICOCHEMICAL CHARACTERISTICS

The chemical composition of coconut water can vary according to the post-harvest, such as the packaging, transport and storage conditions of the coconut, soil composition and the degree of maturation (42). Although both young and mature coconut water have equal nutritional values, for the most part, young coconut water is preferred by consumers as a health drink, whether consumed directly after collection or consumed after processing in various packaged beverages (43). Table 2 presents physicochemical nutritional data present in green coconut water.

The main sugars present in coconut water are fructose, glucose and sucrose and represent the largest fraction of soluble solids. However, during maturation, there is an increase in sucrose (non-reducing sugar) followed by a decrease in fructose and glucose (reducing sugars) contents. These changes can be explained due to sucrose formation at the expense of the glucose-fructose bond (42).

Table 2. Physicochemical and nutritional characterization of green coconut water.

Parameters	References				
	Tan et. al. [42](2014)	Mahayothee et al. [43] (2016)	Kailaku et. Al.[56] (2017)	Seow et. Al.[57] (2017)	Camargo Pardo et. Al.[58] (2015)
pH	4.78 ± 0.13	5.58 ± 0.12	5.60	5.17	5.01 – 5.94
Titratable Acidity (g/100mL)	0.089 ± 0.004	0.05 ± 0.01	-	-	-
Total Soluble Solids (ºBrix)	5.60 ± 0.14	7.6 ± 0.21	-	6.0	5.00 – 5.40
Phenolic Compounds (mg GAE/L)	54.00 ± 3.135	-	-	62.56	-
K (mg/100mL)	220.94 ± 0.32	-	184.05	372.10	-
Na (mg/100mL)	7.61 ± 0.041	-	2.07	2.51	-
Ca (mg/100mL)	8.75 ± 0.045	-	-	-	-
Glucose (mg/100mL)	35.43 ± 0.51	-	2.72	-	1.886 – 2.159
Fructose (g/100mL)	39.04 ± 0.824	-	2.71	-	1.847 – 2.379
Sucrose (g/100mL)	0.85 ± 0.01	-	0.64	-	-

In terms of the constituents of coconut water, minerals come in second in terms of quantity. Data indicates that they represent about 0.4 to 1% of the composition of coconut water, being enough amount to characterize an isotonic drink (42,44). The presence of six main minerals was detected: potassium (K), sodium (Na), calcium (Ca), zinc (Zn), iron (Fe) and magnesium (Mg). Potassium is the most abundant mineral in coconut water, followed by sodium, calcium and magnesium. However, iron and zinc are present in relatively low concentrations (45).

Regarding the profile of fatty acids, the short-chain ones increase during coconut maturation, while the long-chain ones decrease. Literature data show that lauric acid (C12:0) combined with myristic acid (C14:0) represent 70-75% of the percentage of total free fatty acids available in coconut water (46).

While solid endosperm contains high concentrations of fatty acids, with lauric acid (C12:0) being the highest concentration as maturation progresses (47), liquid endosperm contains many sugars (e.g. sucrose, glucose and fructose), sugar alcohols (e.g. sorbitol and mannitol), inorganic ions (e.g. K+, Na+, P+ and Mg++), vitamins (e.g. C, B3, B5 and niacin), lipids (mainly saturated fatty acids), amino acids (eg aminobutyric acid, glutamic acid and lysine), organic acids (eg malic acid and pyridoline) and phytohormones such as cytokinins and auxins (48).

The concentration of total protein and nitrogen increases as the coconut matures. The predominant proteins are globulin, albumin, glutelin and prolamin. Although coconut water is not a good source of protein, it does contain most of the amino acids (49).

The active ingredients in coconut water include secondary plant metabolites such as: phytohormones, vitamins and amino acids. Among these metabolites, we can mention coumarin, 4-hydroxycoumarin, coumaric acid, ferulic acid, glucoside, procyanidin, shikimic acid and quinic acid which are known to demonstrate antioxidant and hepatoprotective effects in experimental settings (50,51).

The pH and turbidity of coconut water increase as the coconut matures while the titratable acidity decreases with the ripening of the fruit. The decrease in titratable acidity may be due to the reduction in the amount of organic acids and ascorbic acid present in the water with maturity (45).

Acidity is an important parameter, as it is used as a sensory indicator, due to its influence on the flavor and aroma of foods (52). Generally, it is common to find in the literature the acidity of coconut water being expressed in citric acid and malic acid (53).

Total soluble solids are directly related to the sweetness and manifestation of flavor in coconut water and are usually represented by the sugars and organic acids naturally present in its composition (54). Total soluble solids content has been used more as a quality criterion than the point of harvest, since the characteristics of coconut water are influenced by seasonality (55).

3.5 HEALTH BENEFITS

3.5.1. Isotonic Natural Drink and its Effect

A study comparing the use of coconut water, an orange drink and water in physical exercise practitioners in hot conditions showed that previous consumption of coconut water resulted in lower urine production, indicating greater hydration capacity, not caused gastrointestinal discomfort, despite its composition, in addition to promoting an improvement in subsequent exercise capacity (59).

Coconut water helps with hydration, with an impact similar to pure water, in addition to enabling a decrease in heart rate in situations of extreme exercise (60).

3.5.2. Antioxidant and Anti-inflammatory Effect

Coconut water is sterile while it remains inside the fruit. It is made up of organic and inorganic compounds that play a vital role in supporting the human body's antioxidant system (9). In addition, it contains a large amount of micronutrients, such as inorganic ions and vitamins, which increase the body's natural antioxidant system. These micronutrients act directly on the body by quenching free radicals, which can damage cells, or they can indirectly increase the production of antioxidant enzymes (such as superoxide dismutase, catalase, and glutathione peroxidase) promoting the removal of harmful radicals (61).

Data collected from a study using the HPLC-MS/MS method to identify the main phenolic compounds in green dwarf coconut water and evaluate their effects on oxidative stress parameters and ethanol-induced liver damage in Wistar rats, demonstrated the presence of chlorogenic acid, caffeic acid, methyl caffeate, quercetin and ferulic acid isomers in green dwarf coconut water. In addition, the data showed that the consumption of coconut water and caffeic acid, at the concentrations used, has a hepatoprotective effect, confirmed by the values of gammaglutamyltranspeptidase and alananine aminotransferase (62).

Another study evaluating the effects of coconut water in the prevention of myocardial infarction showed that it exerted significant antiperoxidative activities in rats administered isoproterenol hydrochloride, with its antioxidant property being superior to that of streptokinase, which is a potent antithrombotic. Part of this oxidative stress reduction effect refers to the presence of L-arginine, potassium, Mg, Ca, vitamin C and polyphenols (63).

A study (51) evaluated the antioxidant protection efficacy of coconut water in experimental rats induced by thermal stress and revealed the presence of polyphenolic compounds, namely, 4-hydroxycoumarin, 6-geranylnaringenin, ferulic acid, 4-O-glucoside and p-acid. coumaric, as well as four essential amino acids, namely L-phenylalanine, DL-tyrosine, D-tryptophan and L-isoleucine. These compounds act synergistically, being responsible for the prevention of systemic inflammation, mainly phenylalanine and isoleucine, which inhibit drug-induced inflammation in several experimental model systems, and with antioxidant potential, mainly polyphenolic compounds, indicating their direct involvement as a scavenger of free radicals.

3.5.3. Hepatoprotective Effect

One study showed that coconut water inhibits hepatocyte inflammation by reducing IL-1β-induced Tnf and Il6 transcript expression, while increasing acute phase protein (Serpine1 transcript) and antioxidant (HMOX1 protein) expression in primary hepatocytes mouse (64). Such data suggest that coconut water may favorably alter the antioxidant defenses and inflammatory response of hepatocytes as a component of its protective effects.

These findings are consistent with previous studies demonstrating that coconut water protects hepatocytes against H2O2-mediated oxidative damage and testes against heat-induced damage (50,51).

3.5.4. Impact on Diabetes

Animal experiments have reported that coconut water can reduce blood glucose levels, regulate carbohydrate metabolism, and improve antioxidant capacity. Several studies have reported that coconut water can relieve kidney damage caused by diabetes (63,65–67).

Another animal study found that coconut water can lower blood glucose in alloxan-induced diabetic rats (68).

These results corroborate a study (69) that evaluated the effects of coconut water and glibenclamide in diabetic rats, showing the potential of coconut water to reduce glycemia and damage to the diabetic retina, but with no effect on the weight change caused by the disease. He speculates that this may be due to the antioxidant properties of coconut water.

4. FINAL CONSIDERATIONS

This review highlights the different aspects of coconut water production and how its nutritional composition can be applied for health benefits. It was possible to observe that coconut water is a fashionable drink that has been gaining the national and international market. However, it is a highly perishable product, and it is important to preserve the sensory, nutritional, and functional qualities of the drink. Non-thermal processing methods such as: use of ozone, ultrasound and ultraviolet light were effective in maintaining the shelf life of the beverage, however the ultrapressure method showed changes in physical functionality and/or changes in color of protein-rich foods. It was possible to evaluate the nutritional composition of coconut water, with special attention to the composition of minerals that guarantee the characteristic of an isotonic drink to the product. In addition, there is the presence of phytohormones, vitamins and amino acids that are responsible for the antioxidant properties of the product, as well as the beneficial effects on health.

Finally, further studies are suggested to evaluate the effects of coconut water on human health, as it is a beverage with market potential and accessible to consumers that could easily be used in the formulation of new products with functional appeal.

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CAPÍTULO II

DEVELOPMENT OF FRUIT BEVERAGES WITH COCONUT WATER: PHYSICOCHEMICAL CHARACTERIZATION, ANTIOXIDANT POTENTIAL AND SENSORY PROPERTIES

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Applied Research

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Short version of title (running head): Fruit beverages with coconut water

Choice of journal/topic : Journal of Food Science: Health, Nutrition, and Food

ABSTRACT

Fruit beverages blends have been consumed for the balance between nutrients and flavor. The coconut water to replace water is a more natural solution and an option for blending in the fruit beverages. The present study aimed to develop fruit beverages using coconut water as a base, guava, watermelon and was added apple juice as natural sweetener. Experimental design was used for the development of beverages, considering a factorial design 2^2, followed by the physicochemical and sensory analysis. The guava fruit was highlighted on antioxidant capacity results compared to the watermelon fruit, in the DPPH (86.0±2.5 and 11.7±4.5% of reduction, respectively) and in the TEAC (31.7±0.0µmol trolox/g 12.8±1.6µmol trolox/g, respectively). The physicochemical results of the blends revealed that the beverages with guava (0.5±0.0%) are more acidic than those with watermelon (0.2±0.0%), indicating the impact of the intrinsic characteristics of each fruit in the final composition. According to the results of the sensory analysis and response surface methodology, verified the potential optimized formulas contain in their composition 40g of fruit, 30g of coconut water and 30g of apple juice per 100 ml of beverage, this composition affected the sensory acceptance of the beverages. Our findings indicated the fruit beverages with coconut water have functional attributes, sensory acceptance and can be used as one more option for beverages category.

Keywords: Blending fruits, optimized formula, functionality, guava, watermelon

Practical Application:

In this work, the formulation of a guava (*Psidium guajava* L.) and watermelon (*Citrullus lanatus*) beverage with coconut water (*Cocos nucifera* L) can be optimized through an intelligent experimental design applied to the sensory data set. The quality of the beverage is measured by physical and chemical assays intended to predict the behavior of the ingredients during the manufacturing processes of final beverage. The presented study can be used as a reference for other similar beverages to achieve a complete process design from basic formulation optimization to thermal processing conditions.

1. INTRODUCTION

The fruit beverages are becoming popular drinks because of refreshing taste and having an equilibrium of vitamins, minerals, and other bioactive components (Dhar *et al*., 2021). In the development of new products, the commitment is to improve the sensory characteristics as well as the quality of nutrition, so the combination of fruits, vegetables and spices is a promising solution. This improvement can be attributed to the mix of two or more kinds of fruit juices, to enhance the vitamins, mineral and the nutritional value of the product (Subramani & Venilla, 2019).

Several factors contribute to the growing demand for coconut water beverage like freshness, low calories content and a natural source of electrolytes including sodium and potassium, fats, carbohydrates, proteins and other minerals (Raghubeer *et al*., 2020). Non-carbonated and carbonated coconut water beverages possess the characteristic flavor of coconut water, but this is slightly masked by the acid-sweetish taste imparted by the different additives (Chauhan *et al*., 2012).

Most coconut (*Cocos nucifera* L) producers are in Asia, with 86% of the world's production made in that continent. The top five coconut producers are: Indonesia (17.1 million tons), followed by Philippines (14.7 million tons), India (14.6 million tons), Sri Lanka (2.4 million tons) and Brazil (2.3 million tons) (Food and Agriculture Organization of United Nations, 2019). The production of coconut water in Brazil in 2020 was approximately 170 million liters, an increase of 8% compared to 2019. This reflects the trend of increased consumption in this category (ABIR – Associação Brasileira das Indústrias de Refrigerantes e de Bebidas Não Alcoólicas, 2021).

Water has been replaced by coconut water in the formulation of nectars, presenting a series of nutritional and therapeutic properties, being a natural solution. Multiple fruit juices can be included in coconut water beverages, especially those with high acidity, as they lower the pH of the drink, making it suitable for a milder heat treatment (Silva et al., 2006).

Many fruits with sensory and nutritional characteristics can have a synergistic effect on the attributes of coconut water, such as guava (*Psidium guajava L.)* and watermelon (*Citrullus lanatus*). Guava is recognized often as "super-fruits" which has a considerable nutritional importance in terms of vitamins A and C with seeds that are rich in omega-3, omega-6 poly-unsaturated fatty acids and especially dietary fiber, riboflavin, as well as in proteins, and mineral salts (M. Kadam *et al*., 2012).Watermelon has long been identified as a good source of lycopene and recently, watermelon juice has also gained attention as a functional beverage for exercise, since it contains electrolytes and a significant amount of the amino acid citrulline, which has been found to have ergogenic effects (Milczarek *et al*., 2020).

Despite the variety of fruit beverages with pleasant flavors and high marketing potential, there are few commercial products that use fruit blends for flavor, nutritional and functional synergy. In this context, the present study aims to develop fruit beverages with coconut water, in "ready to drink" form, to obtain a sensory accepted formula, in addition to the physicochemical characterization of the fruits and the final beverage and analysis of the antioxidant capacity of guava and watermelon.

2. MATERIALS AND METHODS

2.1 Samples

Samples of commercial frozen fruit juices of watermelon, guava and apple were obtained from local suppliers and all samples were stored in the original packaging at -18ºC until the beverage's preparation. The coconut water was obtained from a Brazilian producer and stored in the original packaging at 4ºC, until the beverage's preparation.

2.2 Physicochemical characterization

All physicochemical analyses were performed in triplicate at room temperature according to the AOAC methodology (AOAC, 1995). The pH and titratable acidity (TA) were performed using an automatic titrator equipment - model 916 Ti-Touch - (Metrohm, Switzerland), calibrated with pH 4.0, 7.0 and 10 buffer solutions. TA was determined by titration with hydroxide of sodium (NaOH) 0.1N, using 1g of sample. The results are expressed as percentage of citric acid. The Total Soluble Solids (TSS) content was performed using a BS digital refractometer, model RFM340. Results are expressed in °Brix.

2.3 Content total of phenolics and antioxidant activity

2.3.1 Extraction

2.5g of the samples were added in 10ml of the extractor solutions: (I) water, (II) ethanol 50%, (III) acetone 70%, remained in agitation in ultraturrax for 2min and were submitted to centrifugation (Heraeus Multifuge X3FR Centrifuge Thermo Fisher Scientific, Waltham, MA, USA) for 15min. The supernatant was transferred to a 50ml volumetric flask and filled with distilled water. The extract obtained was used for total phenolic content, antioxidant capacity analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (TEAC).

2.3.2 Determination of the Total Phenolic Content

Total phenolic content was determined by the Folin–Ciocalteau method (Singleton, V. L.; Orthofer, 1999). Extracts (1.0ml) were added with 2.5ml of Folin–Ciocalteau reagent and 2 ml of 4% sodium carbonate solution. The solution was incubated in a dark ambient for 2h. The absorbance was measured at 760nm in the spectrophotometer (Shimadzu UV-2700, Shimadzu Corporation, NAKAGYO-KU KYOTO, Japan) and the results are expressed as gram of gallic acid equivalents (GAE) per 100 grams of sample using a gallic acid (2.5–50 µg/µl) standard curve.

2.3.3 TEAC assay

TEAC assay was performed following the procedure proposed by Thaipong *et al*. 2006. TEAC assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cations were produced by reacting potassium persulfate. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70±0.02 at 734nm using a spectrophotometer (Shimadzu UV-2700). Aliquots of volumes of samples (10, 25 and 50µL) were used to subtract a final volume of 3ml in each reading. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard curve. Results are expressed in µmol Trolox equivalent per gram of sample.

2.3.4 DPPH (free radical scavenging) assay

The antioxidant capacity using DPPH assay was determined according to the method based on the quantification of free radical-scavenging by (Brand-Wiliams, W.; Cuvelier, M.E.; Berset, 1995). The samples were added to react with stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol solution. The reduction of DPPH radical was measured by reading the absorbance at 515nm using a spectrophotometer (Shimadzu UV-2700) at 30 minutes of reaction. The antioxidant capacity is expressed as the concentration of antioxidant required to reduce the original content of free radicals by 50% (EC50) and values are expressed as% of reduction.

2.4 Experimental design

The beverages were developed according to factorial design 2^2 (Table 1), using the following independent variables: concentration of coconut water in g/100g (X_1), concentration of guava or watermelon juice in g/100g (X_2), apple juice in g/100g (X_3). The dependent variables were the sensory attributes: appearance, aroma, flavor, consistence, and overall liking. A study conducted by L. M. R. de Souza *et al*. (2021) used the 2^2 factorial experimental design technique to evaluate the efficiency of the foam layer drying process of the umbu pulp (*Spondias tuberosa*) to verify that the beating time, temperature and the interaction of both did not significantly influence the response variables within the studied planning matrix.

Table 1. Experimental design for independent variables: watermelon and coconut water beverages and guava and coconut water beverages (uncoded and coded values)

Samples	Uncoded and coded values		
	Coconut Water (g/100g)	**Guava pulp/Watermelon juice (*) (g/100g)**	**Apple Juice (**) (g/100g)**
1	41.7(+1)	5 (-1)	30
2	20 (-1)	27.8 (+1)	30
3	60 (+ 1.44)	10 (0)	30
4	30 (0)	2.8 (-1.44)	30
5	30 (0)	10 (0)	30
6	30 (0)	10 (0)	30
7	20 (-1)	5 (-1)	30
8	41.7(+1)	27.8 (+1)	30
9	30 (0)	10 (0)	30
10	11.2 (-1.44)	10 (0)	30
11	30 (0)	40 (+1.44)	30
12	30 (0)	10 (0)	30

(*) Watermelon juice at 66ºBrix diluted in the proportion of 1: 8.8 (**) Apple juice at 70ºBrix diluted in the proportion of 1: 6.7
1: minimum level; +1: maximum level; -1.44: minimum axial point; +1.44: maximum axial point; 0: center point

The fruit pulp content used in experimental design was determined in accordance with Brazilian legislation for beverages establishing the mixture of two or more juices or fruit pulp (Brasil, 2009). Apple juice is being added at a fixed concentration to all samples to help sweeten it naturally, without the need to add sugars or artificial sweeteners.

2.5 Beverage preparation

The components of the formulation were weighed and mixed in suitable equipment until complete homogenenisation. The beverages were packaged in sanitized plastic packaging and stored under refrigeration at 7-10ºC until the sensory tests. The preparation and storage of beverages were conducted at the Technical Laboratory and Dietetics at the Federal University of the State of Rio de Janeiro, following the Good Manufacturing Practices.

2.6 Sensory analysis

The acceptance test was performed in a sensory laboratory of the Federal University of State of Rio de Janeiro using individual cabins under artificial lighting. Consumers were chosen at random from different age and gender groups, recruited by interest and frequency of consumption, and their availability to return after one week for finishing the evaluation. Eighty-eight consumers evaluated twelve samples of watermelon beverages with coconut water the evaluation was carried out in two blocks of six samples. 103 consumers evaluated twelve samples of guava beverages with coconut water and the evaluation was performed in two blocks of six samples. The samples were distributed in a monadic way, using a balanced complete block design. Each panelist received approximately 30mL of refrigerated (7ºC) product sample, in disposable plastic cups coded with random three-digit numbers (MacFIE, 1989). Biscuit and water were provided for evaluators to clean and rinse the palate between samples. Measuring the sensory attributes of appearance, aroma, flavor, consistence and overall liking, a 9 points hedonic scale was used (9=like extremely; 1=dislike extremely). The expressions were converted to numerical values and analyzed. The research protocol of the study was approved by the Ethics Committee of the Federal University of State of Rio de Janeiro (number 39693914.8.0000.5285).

2.7 Statistical analysis

The physicochemical characterization and antioxidant capacity were submitted to analysis of variance (ANOVA) and the means were compared using Tukey's test at a 5% probability level, using the GraphPad Prism 4.0. The results are expressed as the mean+standard deviation.

The experimental design and Analysis of variance (ANOVA) with 95% reliability were performed using Statistica® v.14 (TIBCO SOFTWARE INC., 2020). P-values lower than 0.05 were considered significant. The results are expressed as the mean + standard deviation.

3. RESULTS AND DISCUSSION

3.1 Physicochemical analysis of fruit juices and coconut water

The results of physicochemical analysis of the coconut water, guava pulp, watermelon and apple juice are shown in Table 2.

Table 2 Physicochemical characterization of watermelon juice, guava pulp, coconut water and apple juice

Parameter	Watermelon	Guava	Coconut water	Apple Juice
Total Soluble Solids (ºBrix)	65.8±0.1[a]	9.5±0.4[b]	5.9±0.0[c]	70.4±0.0[d]
Titratable acidity (%)	0.8±0.0[a]	0.7±0.0[b]	0.2±0.0[c]	1.3±0.1[d]
pH	4.9±0.0[a]	3.8±0.0[b]	5.1±0.0[c]	3.9±0.0[b]

Mean+SD parameter values. Different letters in same line indicate the values are statistically significant ($p<0.05$) by Tukey test

The quality of the beverage is measured by physical and chemical assays intended to predict the behavior of the ingredients during the manufacturing processes of final beverage. The results of TSS between watermelon and apple juice, 65.8±0.1 and 70.4±0.0ºBrix, respectively, are higher compared to coconut water and guava pulp, as these juices are in concentrated form. During the beverage preparation process, they were diluted to the original concentration. A study carried out by M. Kadam *et al*. (2012) observed a TSS result of 7.0-7.5ºBrix in guava pulp and Rojas-Barquera, (2009) obtained 8.6±0.6ºBrix in regional red guava. For watermelon concentrate juice was observed in other study a result of 65.5±0.0ºBrix (Milczarek *et al*., 2020). In the case of coconut water, a study showed the results in different stages of maturation and in the ninth month of maturation, it was observed a result between 4.0 to 8.5ºBrix (Carvalho *et al*., 2006).

Regarding TA, the results obtained revealed apple juice (1.3±0.1%) has higher acidity than other fruits. This is due to the intrinsic characteristics of each fruit and apple juice is in concentrated form. Watermelon juice, even in concentrated form, has a higher pH (4.9) compared to guava pulp (3.8) that is in its original concentration. M. Kadam *et al.* (2012) observed a range from 0.29 to 0.34 $g.100g^{-1}$ for guava fresh pulp, other study observed for red guava a mean of 0.6±0.2% (Thuaytong & Anprung, (2011) , this result is similar to observed in our study. Carvalho *et al*. (2006) observed in Coconut water: Nutritional, functional, and processing properties study a range from 0.4 to $0.9g.100g^{-1}$ in a coconut water. The significant difference in titratable acidity observed between the studies can be explained by the growing conditions and the degree of ripeness of the fruit.

3.2 Total Phenolic and antioxidant activity guava pulp and watermelon juice

For total phenolic compounds and antioxidant capacity in DPPH and TEAC methods, three different extractive agents were used: water (I), ethanol 50% (II) and acetone 70% (III). Figure 1 shows the antioxidant capacity and total phenolic compounds of watermelon juice and guava pulp.

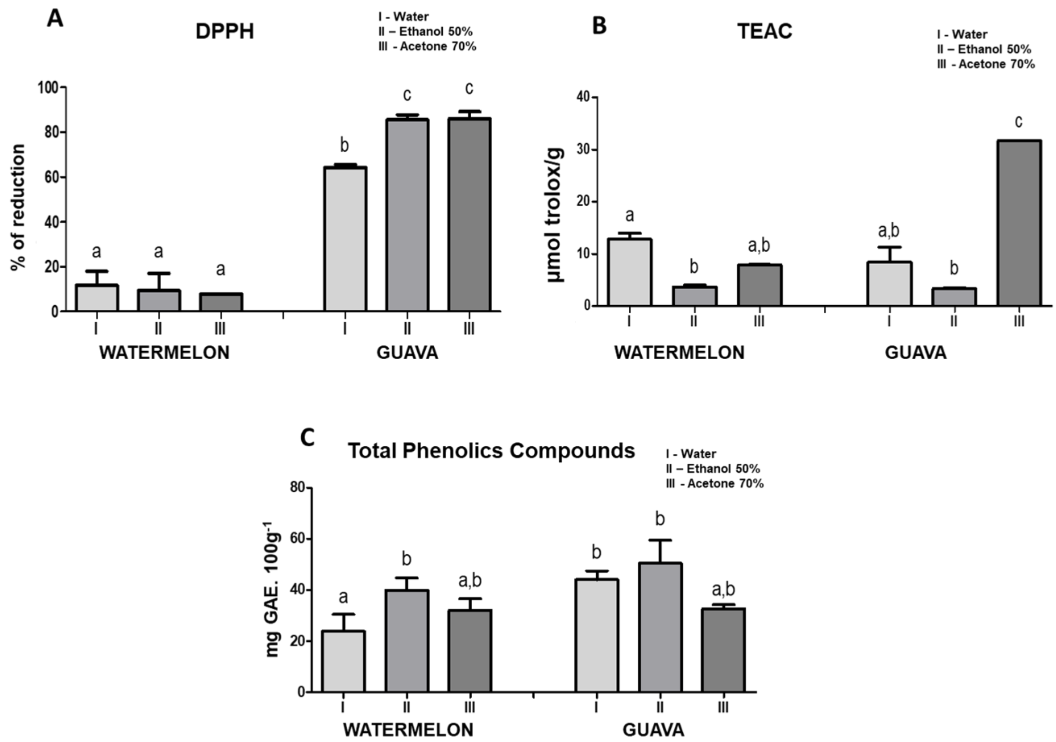

Figure 1 Antioxidant capacity of watermelon juice and guava pulp Mean+SD. (A) DPPH method, (B) TEAC Method and (C) Total Phenolics Compounds. Different letters in same line indicate the values are statistically significant ($p<0.05$) by Tukey test

Figure 1A shows, in the DPPH method there was no significant difference ($p>0.05$) between the extractive agents for watermelon juice. In the case of guava pulp, ethanol 50% and acetone 70% showed significant higher results when compared to water as an extractive agent. Considering the DPPH method, guava showed the highest values of percentage of reduction when compared to watermelon, 86.0±2.5% of reduction, and 11.7±4.5% of reduction, respectively. Neglo *et al.* (2021) observed in their study on comparative antioxidant of the peels, rind, pulp and seeds of watermelon a result of 33.0±4.5% of reduction in pulp of watermelon fruit.

In the TEAC method, shown in Figure 1B, the water as an extractive agent had a higher extraction result in relation to ethanol 50% and acetone 70% in watermelon juice, whereas in guava pulp, the higher extraction was obtained by acetone 70% as extractive agent. Guava pulp obtained the highest content (31.7±0.0µmol trolox/g) compared to watermelon juice (12.8±1.6µmol trolox/g). A previous study observed 31.9±2.5µmol trolox/g of antioxidant capacity in guava juice in a dry weight basis (Fu *et al*., 2016).

Evaluating the results from total phenolic compounds, shown in Figure 1C, it was observed that ethanol 50% had a higher extraction result for watermelon and guava compared to the other extractive agents. The guava pulp had the highest results, but with no significant difference ($p>0.05$), obtaining 50.5±7.3 mgGAE.100g^{-1} content, and watermelon (39.9±3.9 mgGAE.100g^{-1}). Neglo *et al.* (2021), compared the total phenolics compounds between watermelon pulp (0.01±0.00 mgGAE/g) and watermelon seed (0.04±0.00 mgGAE/g), the seed contained higher total phenolics compounds. Total phenolics compounds of guava from different regions of Brazil showed a range between 158.0 to 447.0 mgGAE.100g^{-1}(Corrêa *et al*., 2011).

3.3 Sensory analysis

The acceptance sensory results of watermelon and coconut beverages and guava and coconut water beverages for the attributes appearance, aroma, flavor, consistence, and overall liking are presented in Table 3.

Table 3. Sensory analysis results for dependent variables (attributes) for watermelon beverage with coconut water and guava beverage with coconut water

	Watermelon beverage with coconut water					Guava beverage with coconut water				
	Sensory attributes					Sensory attributes				
Samples	**Appearance**	**Aroma**	**Flavor**	**Consistence**	**Overall liking**	**Appearance**	**Aroma**	**Flavor**	**Consistence**	**Overall liking**
1	5.3±1.8	5.8±1.5	5.4±2.0	7.0±1.6	5.7±1.7	5.2±2.1	5.7±2.0	5.7±2.1	6.5±2.0	5.8±2.0
2	7.5±1.4	6.2±1.5	6.4±1.8	7.2±1.3	6.6±1.6	8.0±1.2	7.8±1.2	7.1±1.7	7.7±1.2	7.5±1.5
3	6.5±1.7	6.1±1.5	6.1±2.2	7.1±1.6	6.4±1.8	6.5±1.9	6.7±1.8	6.6±2.2	6.9±1.9	6.8±1.8
4	4.8±2.2	5.7±1.5	5.1±1.9	6.8±1.6	5.3±1.7	4.2±2.2	5.8±2.0	5.5±2.1	6.1±2.2	5.4±2.0
5	6.6±1.5	6.0±1.5	5.8±1.9	6.8±1.6	6.1±1.6	6.5±1.7	6.6±1.6	6.1±1.9	7.0±1.9	6.3±1.6
6	6.3±1.7	6.1±1.4	6.1±1.8	7.3±1.4	6.3±1.7	6.5±1.8	6.9±1.6	6.5±1.8	7.2±1.7	6.7±1.6
7	4.7±2.0	5.5±1.4	5.2±2.0	6.4±1.9	5.4±1.7	4.5±1.9	5.7±1.8	5.0±2.0	6.2±2.0	5.2±1.8
8	7.6±1.3	6.5±1.5	6.7±1.9	7.2±1.5	6.9±1.4	8.2±0.9	7.6±1.3	7.2±1.9	7.8±1.4	7.6±1.5
9	5.9±1.7	6.0±1.5	6.0±1.9	6.8±1.7	6.1±1.7	6.4±1.6	6.3±1.4	6.2±1.8	6.5±1.7	6.3±1.6
10	5.5±1.7	5.9±1.4	5.2±2.0	6.5±1.8	5.5±1.7	6.3±1.6	6.4±1.5	5.3±1.8	6.2±1.9	5.7±1.7
11	7.9±1.1	6.6±1.5	7.0±1.8	7.6±1.3	7.3±1.5	8.3±1.2	7.9±1.5	7.4±1.9	7.9±1.5	7.7±1.7
12	5.5±1.8	5.9±1.4	5.7±1.7	6.8±1.6	5.9±1.6	6.4±1.8	6.3±1.7	6.2±1.9	6.5±1.7	6.4±1.7

The highest mean in all attributes was observed in the sample eleven. This sample has the highest amount of watermelon /guava juice (40g/100g), concluding the higher juice content obtains the higher acceptance of the beverage. The appearance attribute showed the highest acceptance compared to the other attributes in sample eleven, for watermelon beverage with coconut water (7.9 ± 1.0) and ($8.3+1.2$) for guava beverage with coconut water. The intense color of the fruits can influence the evaluation of the beverages, since both have a red/pink color.

The sample four of guava beverage with coconut water showed the lowest acceptance in the appearance attribute. This is the sample containing the lowest guava pulp content in the formulation (2.8g/100g). It means that the lower the amount of fruit, the lower the acceptance of the beverage in this attribute. A study carried out to evaluate the acceptance of beverages on soy extract and coconut water blended with umbu (*Spondias tuberosa*) pulp concluded the samples with the lowest acceptance were those containing the lowest amounts of umbu juice (Neto *et al.*, 2016).

In a study on the development of a beverage with coconut water and acerola (*Malpighia emarginata* D. C.), the highest sensory acceptance (flavor and overall liking) was obtained in the beverage that contained the highest amount of fruit and sugar (30% of acerola juice and added sucrose up to 12 °Brix) (Lima *et al.*, 2008).

Table 4 shows the ANOVA test and the respective p values to the sensory attributes of appearance, aroma, flavor, consistence and overall liking for watermelon beverage with coconut water. Observing the p values, the watermelon constituent in the linear term had a significant effect ($p<0.05$) on all attributes. This means, the variation of watermelon content in the samples has a significant impact on the acceptance of the beverage. Regarding the coconut water constituent in the linear term, the p values had a significant effect ($p<0.05$) in the aroma ($p=0.03$), flavor ($p=0.02$) and overall liking ($p=0.01$) attributes. In other words, the variation of coconut water content in the samples has a significant impact in these attributes. In the interaction between coconut water and watermelon in linear term, there was no significant effect ($p>0.05$) for all attributes.

Table 4. P value of sensory acceptance for watermelon beverage with coconut water

Constituents	Sensory attributes				
	Appearance	**Aroma**	**Flavor**	**Consistence**	**Overall liking**
Coconut water (L)	0.17	0.03	0.02	0.08	0.01
Coconut water (Q)	0.52	0.33	0.15	0.13	0.22
Watermelon (L)	0.00	0.00	0.00	0.02	0.00
Watermelon (Q)	0.04	0.15	0.07	0.90	0.16
Interaction between Coconut water (L) by Watermelon (L)	0.43	0.72	0.79	0.15	0.58

(L) – Linear Term; (Q) - Quadratic term

Considering the beverages developed with guava and coconut water, the table 5 shows the ANOVA test and the respective p values in relation to the sensory attributes of appearance, aroma, flavor, consistence and overall liking. The p values showed the guava constituent had a significant effect ($p<0.05$) on all attributes, except for consistence in quadratic term ($p=0.08$). This means, the variation of guava content in the samples has a significant impact on the acceptance of the beverage. Regarding the coconut water constituent in linear term, the p value had a significant effect ($p<0.05$) on the flavor ($p=0.01$) and overall liking ($p=0.02$) attributes. Thus, the variation of coconut water content in the samples has a significant impact in these attributes. In the interaction between coconut water and guava in linear term, there was no significant effect ($p>0.05$) for all attributes.

Table 5. P value of sensory acceptance for guava beverage with coconut water

Constituents	Sensory attributes				
	Appearance	**Aroma**	**Flavor**	**Consistence**	**Overall liking**
Coconut water (L)	0.55	0.95	0.01	0.16	0.02
Coconut water (Q)	0.94	0.89	0.06	0.18	0.11
Guava (L)	0.00	0.00	0.00	0.00	0.00
Guava (Q)	0.00	0.05	0.01	0.08	0.00
Interaction between Coconut water (L) by Guava (L)	0.64	0.50	0.13	0.51	0.13

Aniceto *et al.* (2021) used the p values to evaluate the significant effect of the constituents present in the murici (*Byrsonima Crassifolia* (L.) Kunth) and taperebá (*Spondias*

Mombin L.) mixed beverages. Significant effects ($p<0.05$) were reported for sugar ($p=0.00$) and murici ($p=0.03$ and $p=0.01$) for flavor and overall liking attributes, respectively.

3.4 Beverage Optimization

Another way to visualize the impact of constituents on the acceptance of fruit beverages with coconut water is through the standardized estimated effect of the constituents watermelon and coconut water and guava and coconut water as well in the linear and quadratic terms and their interactions for the overall liking attribute is shown in figure 2. In the Pareto chart of standardized effect, it can be observed which factors and interactions were or were not statistically significant and most relevant in order of importance. The bars that go beyond the region to the right of the vertical where $p=0.05$ is indicated (5% of significance) represent the parameters that have a significant effect on the evaluated response. The linear terms of the watermelon and coconut water are statically significant ($p<0.05$) for overall liking attribute, according to figure 2A. This means, the linear term watermelon is the most important constituent on the effect of acceptance in this attribute, as evaluated in the p-value analysis.

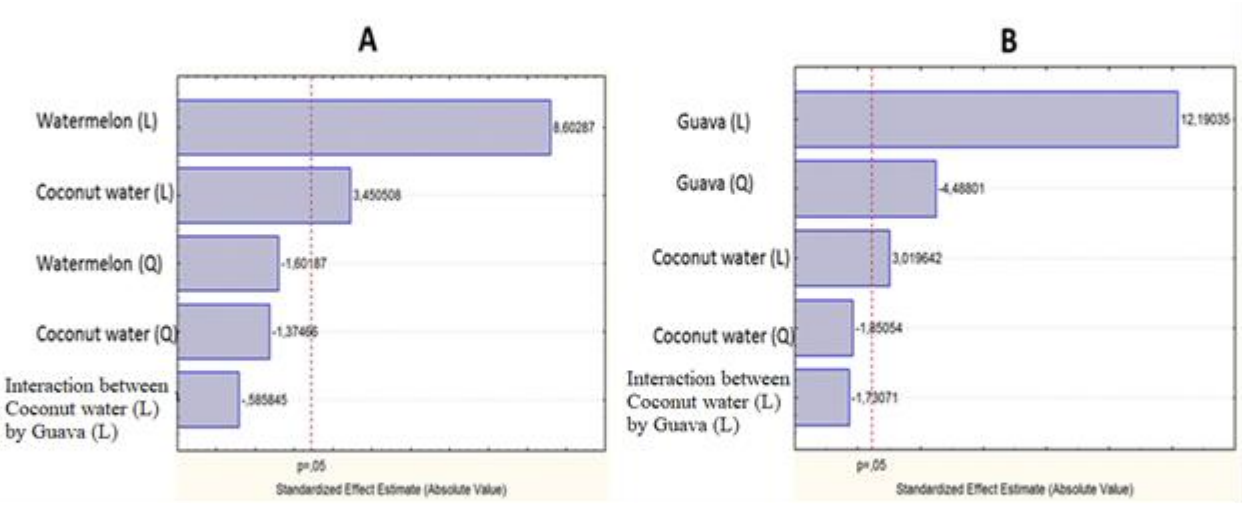

Figure 2 Pareto chart of standardized effect for overall liking attribute. (**A**) Watermelon beverage with coconut water. **(B**) Guava beverage and coconut water

Figure 2B shows the independent variables guava (in linear and quadratic terms) and coconut water (in linear terms are statically significant ($p<0.05$) in the acceptance of the attribute overall liking. In other words, the guava in both terms (linear ant quadratic) are important on the effect of acceptance in this attribute.

For the optimization of the beverage formulas, the response surface graph was performed. Figure 3 shows the response surface graph of the guava-blended beverage and watermelon blended beverage in the overall liking attribute.

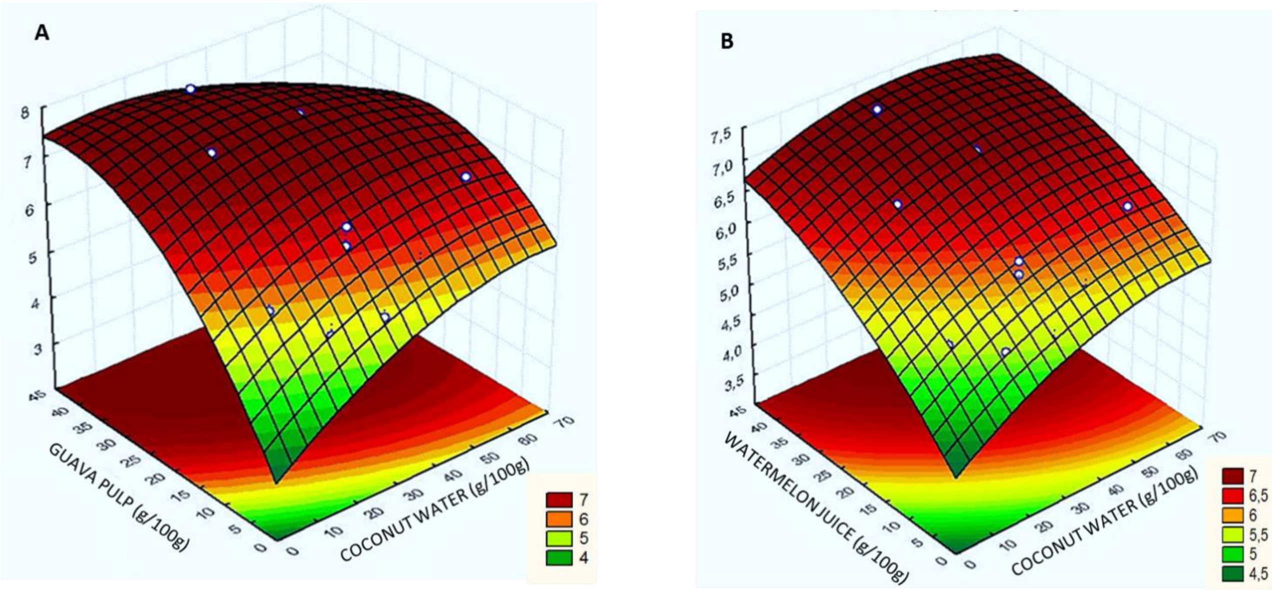

Figure 3 Response surface for the overall liking attribute. (**A**) Guava beverages with coconut water an apple juice content (30g/100g) and (**B**) Watermelon beverages with coconut water an apple juice content (30 g/100g).

Figure 3A demonstrated the highest acceptance of the overall liking attribute is comprised in the guava pulp concentration between 27.8 and 40g/100g in the final beverage. The same was observed in figure 3 B, the highest acceptance are those with the highest concentrations of watermelon juice.

The lowest acceptance for overall liking attribute is related to the lowest concentration of fruit in the formulation (2.8 and 5g/100g). This means, the lower the fruit content is the lower the acceptance of the beverage. In a study on litchi based mixed fruit beverage it was used the D-optimal mixture design with three different variables (the amount of litchi juice, coconut water and lemon juice) and concluded the proportion of litchi juice, coconut water and lemon juice were optimized in the ratio of 72:27:1, to form a blended beverage with acceptable sensory attributes (Jayachandran *et al*., 2015).

The coconut water content showed no impact on beverage acceptance, it was observed the highest acceptance in the overall liking attribute was in sample eleven, the beverage contains

30g/100g of coconut water in the formulation, while sample four had the lowest acceptance for the same attribute and also contains 30g/100g of coconut water in the formulation.

According to the results obtained the optimized formula of fruit beverages with coconut water contain in their composition 40g/100g of fruit, 30g/100g of coconut water and 30g/100g of apple juice.

V. R. de Souza *et al.* (2020) determined the optimal formulations of fruit-based beverages murici and tapereba, the ideal values of each independent variable for fruit-based beverage murici were as follows: 40% fruit pulp, 15% sugar and 45% water, and 32.5% pulp, 12% sugar, and 55% water for taperebá.

3.5 Physicochemical analysis of optimized beverages

The physicochemical analysis of the optimized beverages is shown in Table 6. The TSS results demonstrated there no statistical difference (p>0.05) between watermelon beverage with coconut water (8.5±0.0) and guava beverage with coconut water (8.8±0.0). This means, there is similarity of the two formulas in solids content.

A study on coconut water and concentrated cashew juice beverage blended obtained results between 11 and 12ºBrix among the five formulas developed. However, according to the results, the formulations presented values higher than the fixed value for the beverage (11°Brix) and concluded that the evaporation of water from the product during the heat treatment may have caused a higher concentration of soluble solids content at the end of the processing (Carvalho *et al.*, 2005).

Table 6 Physicochemical characterization of fruit beverages with coconut water optimized formulas

Parameter	Watermelon beverage with coconut water	Guava beverage with coconut water
Total Soluble Solids (ºBrix)	8.5 ± 0.0^{a}	8.8 ± 0.0^{a}
Titratable acidity (%)	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}
pH	4.7 ± 0.0^{a}	4.1 ± 0.0^{b}

Mean±SD parameter values. Different letters in same line indicate the values are statistically significant (p<0.05) by Tukey test

Assessing the titratable acidity results, guava beverage with coconut water had the highest results (0.5±0.0% and pH=4.1) compared to the watermelon beverage with coconut

(0.2 ± 0.0% and pH=4.7) . The result can be explained, considering the guava pulp is more acidic than the watermelon juice, even with equivalent amounts in the final beverages. The acidity of the guava pulp impacted the final beverage. Shigematsu *et al.* (2019) in their study of a mixed beverage of guava and coconut water observed a pH between 4.12 and 4.15, which suggests the similarity with the beverage developed in this study. Another study on development of umbu and umbu-caja (*Spondia spp*) beverages with coconut water observed a higher result of acidity for samples with a higher amount of juice (45% of umbu juice and 35% umbu-caja juice), with umbu-caja ($0.7 \pm 0.1 g.100ml^{-1}$) being the fruit that obtained the highest acidity results compared to umbu ($0.7 \pm 0.1 g.100ml^{-1}$) (Vieira *et al*., 2014).

4. CONCLUSION

Guava pulp and watermelon juice can be considered sources of phenolic compounds. The guava fruit presented the highest antioxidant activity results compared to watermelon, in two different methods TEAC and DPPH. The guava beverage with coconut water had a significant difference ($p<0.05$) in titratable acidity, demonstrating the fruit can impact significantly the parameters of the final beverage. In the response surface analysis, the fruit beverages with coconut water are well accepted, especially in the samples with the highest amounts of juice, and the fruit beverages developed with guava fruit presented a slightly higher acceptance compared to watermelon. In this way, fruits beverages with coconut water can be used as a new way for consuming a nutritional and tasty beverage. Further studies considering nutritional claims and impacts during industrial processes should be addressed to assess the applicability of these products as commercial functional beverages.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author

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CAPÍTULO III

EFFECT OF HEAT TREATMENT AND SHELF LIFE ON PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT CAPACITY OF FRUIT BEVERAGES WITH COCONUT WATER

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ABSTRACT

Fruit beverages are sources of nutrients and phytochemicals important for the diet and heat treatment is a technique widely used as a method of preservation and increase of shelf-life in beverages. The effect of heat treatment on the physicochemical and nutritional characteristics of fruit beverages with coconut water was the aim of this study. The impact of conventional heat treatment technologies on beverage characteristics was evaluated by applying different heat treatment conditions 124-126°C and 104-109°C for 17s. Shelf life under refrigeration (4ºC) was monitored for 180 days. Both treatments on physicochemical parameters had low impact resulting from processing and throughout the storage period. The visual appearance showed decanting of the watermelon pulp and browning of the guava beverage. Total phenolic compounds observed a decrease in watermelon beverage and an increase in guava beverage. Antioxidant capacity determined by DPPH, TEAC, FRAP and ORAC methods resulted in retention under both treatment conditions for the two fruit beverages. Guava had the highest pulp yield compared to watermelon, 86 and 62%, respectively. The guava beverage with coconut water (1915.06±100.74 mg EAG/100g) had about 25 times more content of total phenolic compounds than the watermelon beverage with coconut water (81.53±4.31 mg EAG/100g) and higher antioxidant capacity in both process conditions. Regarding the results evaluated can be concluded the heat treatment showed a low impact on the physicochemical and nutritional characteristics and can be used as a preservation method for fruit beverages.

KEYWORDS: Fruit beverages; Heat treatment; Shelf life; Pphysicochemical and nutritional characteristics

1. INTRODUCTION

Beverages have also been developed utilizing coconut water. Coconut water beverages possess the characteristic flavor of coconut water, but this is slightly masked by the acid-sweetish taste imparted by the different additives. Both products were found to be highly acceptable and preferred to other artificial fruit drinks (CHAUHAN et al., 2012). Coconut water is not a common fruit juice. Its low acidity combined with well-balanced sugar content and isotonic mineral composition makes it a potential rehydration and sport drink (PRADES *et al.*, 2012).

Fruits, vegetables, and beverages are source phenolic compounds in the human diet. The phenolic composition in fruits is usually influenced by factors of genetic and environmental nature but may be modified during processing and storage due to oxidative reactions. Studies demonstrated an enhancement of the phenolic compounds and antioxidant activity during heat treatment, as a reaction to the stress induced by temperature. However, in some other cases heat processing may partially destroy them or significantly reduce their bioavailability, thus decreasing the beneficial health effects (GUINE; BARROCA, 2014).

Food processing is one of the major means to produce safe foods for consumer's use. Most of the food products in stores are produced in either one or more than one type of food processing technologies (YETENAYET; HOSAHALLI, 2015). One of the major attributes affecting the shelf life of fruit products are processing treatments and conditions. Different processing methodologies including thermal and non-thermal techniques have great impact on product shelf life during storage(SATTAR et al., 2020).

Thermal preservation is the traditional and most used preservation technique even in today's industries. Thermal techniques like pasteurization and sterilization are used to increase the shelf life of the product(LAMO et al., 2019). Thermal processing operations are conventionally classified according to the intensity of heat used: pasteurization (65–85∘C), sterilization (110–121∘C), and ultrahigh temperature (UHT) treatment (140–160∘C). The process chosen depends upon pH, microbial load, and desired shelf life(AAMIR et al., 2013).

Study demonstrated that quality attributes of fresh juice were better maintained in thermal pasteurization than fresh juice during the shelf life. This indicates that pasteurization preserved the valuable attributes of the juice. One the contrary, the sensory characteristics of the thermally pasteurized juice was more adversely affected, although the application of heat gave the juice a longer shelf life (RABIE et al., 2015).

The aim of this study is evaluating the impact of heat treatment on physicochemical characterization and antioxidant activity of fruit beverages with coconut water as well as shelf life.

2. MATERIALS AND METHODS

2.1 RAW MATERIALS

Pink Guava (*Psidium guajava* L.) and red pulp watermelon (*Citrullus lanatus*) fruits were purchased at the local market in Rio de Janeiro/RJ, stored and kept under refrigeration until processing.

The apple juice concentrate, pasteurized and frozen, was supplied by a company specialized in concentrated juices, stored and kept under freezing (-18ºC) until the moment of use.

Frozen coconut water was purchased at the local market in Rio de Janeiro/RJ, stored and kept under freezing (-18ºC) until use.

2.2 PROCESSING OF GUAVA AND WATERMELON PULP

The fruits were sanitized by immersion in 200 ppm sodium hypochlorite for 20 minutes. After sanitization, the guavas were cut, and injured parts were removed and separated. The watermelon was cut, its rinds and seeds were removed and separated.

The pulps were obtained by grinding in Thermomix ™ (Vorwerk) – max. 3L - until complete pulp formation (without pieces), after which they were subjected to a sieve to separate the seeds. The pulps obtained were stored in sanitized recipients and kept under refrigeration until the use. The fruits, seeds, rinds and pulps were weighed to evaluate the processing yield.

2.3 BEVERAGE FORMULATION

The formulation of fruit beverages with coconut water was defined according to a previous study (Chapter I). The formula chosen for application in this study was the one identified in the response surface methodology as the optimized formula. According to the results obtained, it was concluded that the optimized formulas of watermelon with coconut water and guava with coconut water contain 40g of fruit, 30g of coconut water and 30g of apple juice per 100 ml of beverage in their composition.

The ingredients were homogenized (5 min.) under constant stirring and the resulting beverage was kept under refrigeration at 4 ± 2°C until the moment of heat treatment process.

2.4 HEAT TREATMENT PROCESS

The processing of fruit beverages with coconut water by heat treatment was carried out in HTST - "high temperature, short time" equipment - model HT 122 (OWVE) with a flow capacity of 10 liters/h and a maximum temperature of 165ºC. (Figure 1)

Figure 1. Heat Treatment Equipment – HTST. Model HT 122

The time and temperature process parameters were obtained through thermal calculations carried out through information on equipment restriction, beverage composition, final pH of the beverages, microbiological characteristics, and storage conditions during commercialization (ambient or refrigerated).

According to the pH results of guava beverage with coconut water (pH = 4.25) watermelon beverage with coconut water (pH = 4.46, after acidification with citric acid), the equipment holding time restriction (17 seconds) and the storage conditions to which the product would be subjected (ambient and refrigerated), the thermal treatment conditions of fruit beverages with coconut water were calculated.

To define the parameters of the heat treatment, the target microorganisms and the process conditions were identified, through the heat resistance of bacteria important to thermally processed foods which can be seen in the adapted table 1. (FOOD AND DRUG ADMINISTRATION, 2010).

Table 1. Heat Resistance of Bacteria Important to Thermally Processed Foods

Food Class	Microorganism type	Microorganism (s)	Reference Temperature (ºC)	D-Value (*) (minutes)	Z-Value (**) (ºC)
Acid foods and acidified foods (pH 4.0 -4.6)	Thermophiles (spores)	*B.coagulans* (facultatively mesophilicc))	121.1	0.01 -0.07	7.8-10
	Mesophiles (spores)	*B. polymyxa e B. macerans*	100	0.10-0.50	6.7-8.9
		Butyric anaerobes (*C. pasteurianum*)	100	0.10-0.50	6.7-8.9
		B. licheniformis	93.3	4.5	15

* Time required to destroy 90% of vegetative cells or spores at a given temperature.

** Increase in the number of degrees (from a given starting temperature) that results in a 90 percent reduction in the D value

The process parameters were defined through the equation 1. (BERTO; BERTELI, 2015)

$$TL.min = 10^{(Tg-Tref)/z} \quad \text{Eq. 1}$$

Where: TL = Lethal Process Rate, Tg = Guard temperature, Tref = reference temperature,
z = Increase in the number of degrees (from a given initial temperature) that results in a 90 percent reduction in the D value.

The beverages were processed according to the parameters (time and temperature), observed in table 2.

Table 2. Time and temperature parameters of fruit beverages with coconut water uncoded and coded

Product	Temperature (ºC)			Holding time
	Min.	Target	Máx.	seconds
G0 – Guava + Coconut water (Control)	-	-	-	-
M0 - Watermelon + Coconut Water (Control)	-	-	-	-
G1 – Guava + Coconut water	124	125	126	17
M1 – Watermelon + Coconut Water	124	125	126	17
G2 - Guava + Coconut water	104	105	106	17
M2 - Watermelon + Coconut Water	107	108	109	17

After heat treatment, the beverages were filled in 300 ml PET bottles, sealed, capped and the bottles inverted for 3 minutes. After the inversion time, they were immersed in an ice bath. The samples were removed from the ice bath after 20 minutes and stored at 4 ± 2°C until the analysis was performed. The heat treatment process were carried out at the Coca-Cola Brasil Pilot Plant, Rio de Janeiro.

2.5 PHYSICOCHEMICAL CHARACTERIZATION, YIELD ANALYSIS AND STORAGE EVALUATION

The pH, titratable acidity (TA) and total soluble solids (TSS) content of the different processing of fruit beverages with coconut water were determined. The pH and TA were determined using an automatic titrator equipment - model 916 Ti-Touch - (Metrohm, Switzerland), calibrated with pH 4.0, 7.0 and 10 buffer solutions. TA was determined by titration with hydroxide of sodium (NaOH) 0.1 N, using 1g of sample. The results were expressed as percentage of citric acid. The TSS content was determined at room temperature using a BS digital refractometer, model RFM340 and the results were expressed in °Brix. The physicochemical analyzes of pH, TA and TSS were performed according to AOAC (2010).

Yield analysis was weighed on fruit, peels, seeds after processing, pulps after processing. The process loss was calculated through the difference between the total fruit and the sum of the peel, seed and pulp.

The samples submitted for storage evaluation were stored under refrigeration at 4 ± 2°C until the moment of analysis. pH, titratable acidity (% citric acid), total soluble solids and appearance were performed. In the appearance evaluation, color change and sedimentation were analyzed.

2.6 ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS

2.6.1 Determination of total phenolic compounds

The analysis of total phenolic compounds was performed according to the Folin-Ciocalteau spectrophotometric method (SINGLETON, V. L.; ORTHOFER, 1999). The determination was performed using aliquots of the obtained extracts, which were later pipetted and added with the Folin-Ciocalteau reagent. The mixture was left to stand for 3 to 8 minutes. Then 4% sodium carbonate was added and kept for 2 hours, protected from light. After this period, the reading was performed with absorbance of 750nm using spectrophotometer (SpectraMax i3X, Molecular Devices). The results were compared with the standard gallic acid curve and expressed in mg of gallic acid (GAE) per 100g of sample.

2.6.2 DPPH radical scavenging activity Assay

The measurement of DPPH radical scavenging activity was performed according to an adaptation of the methodology described by BRAND-WILIAMS, W.; CUVELIER, M.E.; BERSET (1995).To evaluate the antioxidant activity, samples at three concentrations in triplicate were added for reaction with the stable radical DPPH in a methanol solution. In the radical form, DPPH has a characteristic absorption at 515 nm, which disappears after reduction by hydrogen stripped from an antioxidant compound. The radical reduction of DPPH was measured by reading the absorbance at 515 nm at 30 minutes of reaction using spectrophotometer (SpectraMax i3X, Molecular Devices). Trolox (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) standard was used. Antioxidant activity was expressed in μmol of trolox per gram of sample.

2.6.3 TEAC Assay

The TEAC method (2,2'-azino-bis 3-ethylbenzothiazolin 6-sulfonic acid) was adapted from the method described by RUFINO, MARIA S M, (2007).The ABTS•+ radical is formed by a chemical reaction with potassium persulfate in a stoichiometric ratio of 1:0.5. Once formed, the ABTS•+ radical is diluted in ethanol until obtaining an absorbance measurement of 0.70 (± 0.02) at a wavelength of 734 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). Three different volumes of beverage samples, in triplicate, reacted with the ABTS•+ radical for 6 min. A standard curve with Trolox solutions and the antioxidant activity were expressed in TEAC, Trolox equivalent antioxidant capacity (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) in μmol of trolox per gram of sample.

2.6.4 FRAP Assay

The FRAP assay was done according to ABREU *et al*. (2019). The FRAP reagent was prepared using 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. 2.7 ml of FRAP reagent at 37ºC was added with 90 µl of sample extract and 270 µl of distilled water. After 30 min in warm bath, the readings of samples were taken at 595 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). The ferrous sulfate was used to the standard curve and the results were expressed µM ferrous sulfate per gram of sample

2.6.5 ORAC (oxygen radical absorbance capacity) assay

The ORAC adapted method by PRIOR et al. (2003) aims to measure the ability of the antioxidant to scavenge peroxyl radicals that were generated by a radical source, AAPH (2,2`-azobis(2-amidinopropane) dihydrochloride), at 37ºC. For this, the peroxyl radical generated by the reaction with atmospheric oxygen, reacts with fluorescein to form a non-fluorescent product, which can be measured by spectrophotometry with maximum fluorescence emission at 485 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). Known concentrations of trolox were used to generate a standard curve. The antioxidant activity of the substances was determined through the difference between the area under the curve (AUC) of the sample subtracted by the AUC of the blank, measured by the decay of fluorescence with the addition of the antioxidant substance over time. This assay expresses the result in μmol trolox equivalents per gram of sample.

2.7 STATISTICAL ANALYSIS

The physicochemical characterization and the antioxidant capacity were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at a 5%

probability level and p-values lower than 0.05 were considered significant. GraphPad Prism 5.0 was used. Results were expressed as mean ± standard deviation

3. RESULTS AND DISCUSSIONS

3.1 GUAVA AND WATERMELON PULP FRUIT YIELD

The yield analysis of watermelon and guava fruits after pulping processing can be observed through figures 2A and 2B where guava (86%) has a higher percentage of pulp quantity when compared to watermelon (62%). This result indicates the differences between the fruits and the use of the fruit according to the morphological characteristics of each fruit species. It was observed this characteristic in the percentage of watermelon and guava rind, 30% and 5% respectively.

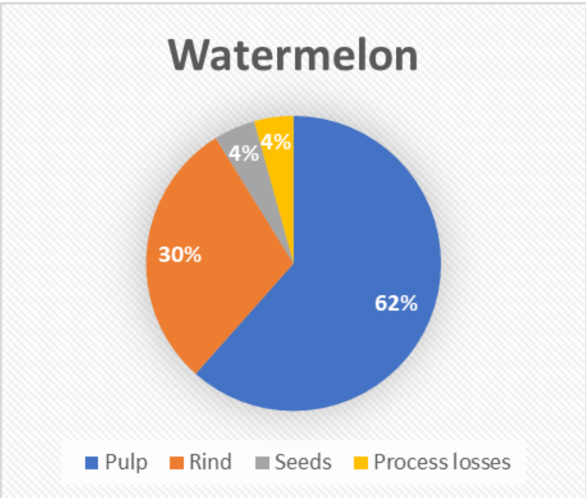

Figure 2A. Watermelon Process Yield

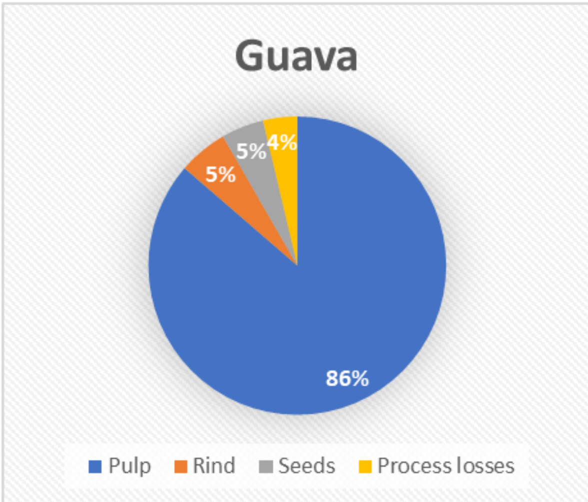

Figure 2B. Guava Process Yield

Other studies have evaluated fruit yield, as was the case of the study entitled Morphological characterization of organic guava fruits, which observed that guava fruits presented attributes favorable to industrial use, such as high pulp yield (83.47%), as well as for fresh consumption (DUTRA et al., 2018). The observed yield is very similar to that found in our study, which indicates that even in different studies, places and periods, guava presents standardized yield values. In the study conducted by MACHADO CAMPOS et al. (2013) , on the quantification of genetic divergence between guava accessions using the wardmlm strategy, the results obtained from the yield of 8 different groups of guavas analyzed, it was observed that the yield of guava pulp can vary between 50.31 and 83.52%.

In the case of watermelon, it was verified that the yield of watermelon pulp in the study on the quality of fruits of different varieties of watermelon from Mossoró, varied from 42.05 and 58.46%, considering the cultivars Charleston Gray and Fairfax, respectively, which showed that the pulp yield is associated not only with the average thickness of the fruit rind, but also with the fruit mass (DA et al., 2010).

3.2 PHYSICOCHEMICAL CHARACTERIZATION

3.2.1 Physicochemical characterization of fruit pulp

The physicochemical analyzes of the fruits after pulping, coconut water and concentrated apple juice, used in the preparation of fruit beverages with coconut water can be observed in table 3.

The results showed that there was a significant difference ($p<0.05$) in all analyzes in the different fruits, indicating that the intrinsic characteristics of each fruit impact the result presented. The values of TSS and TA of apple juice, 70.24±0.009ºBrix and 1.27±0.008% respectively, are highest compared to the other fruits, as this juice is in concentrated form. During the beverage preparation process, it was diluted to the original concentration.

Table 3. Physicochemical characterization of fruits used in the beverages

Parameters	Watermelon	Guava	Coconut Water	Apple Juice
TSS (ºBrix)	$8.15^{a}\pm0.005$	$8.34^{b}\pm0.017$	$6.88^{c}\pm0.009$	$70.24^{d}\pm0.009$
TA% citric acid	$0.20^{a}\pm0.019$	$0.91^{b}\pm0.045$	$0.10^{c}\pm0.026$	$1.27^{d}\pm0.008$
pH	$5.78^{a}\pm0.005$	$3.98^{b}\pm0.011$	$5.22^{c}\pm0.007$	$3.74^{d}\pm0.005$

Different letters in same line indicate that the values are statistically significant ($p<0.05$) by Tukey test

A study on guava products quality observed a variation of TSS ranged from 7.0-7.5 ºBrix in fresh pulp (M. KADAM; KAUSHIK; KUMAR, 2012). The values are lower than those found in our study. In the study developed by ROJAS-BARQUERA, (2009) , showed a value of for pink guava 8.6±0,6 ºBrix, this result is within the significant difference of our study. MARTÍNEZ-SÁNCHEZ et al. (2017)observed in the functional watermelon juice study a value of TSS 7.30 ± 0.10 Brix. In the case of coconut water, a study showed the results in different stages of maturation and in the ninth month of maturation, it was observed a result between 4.00 to 8.50ºBrix (CARVALHO et al., 2006).

Considering the acidity results, coconut water and watermelon presented the lower values 0.10±0.026 and 0.20±0.019% respectively, the fruits are defined as low acid due to its characteristics. A study revealed the physicochemical characteristics of different fruit pulps, pH and %TA values for watermelon pulp, 5.55±0.01 and 0.13±0.02%, respectively (OLUDEMI; AKANBI, 2013) . CARVALHO et al. (2006) observed that coconut water varied from 0.40 to 0.90%. Red guava evaluation conducted by a study observed pH (4.77) and TA (0.62±0.24%) values, this study compared the physicochemical properties of red and white guava, and there was also a significant difference between them(THUAYTONG; ANPRUNG, 2011).The significant difference ($p<0.05$) in acidity observed between the studies can be explained by the growing conditions and the degree of ripeness of the fruit

3.2.2 Physicochemical characterization of fruit beverages with coconut water during the storage

The figure 3 shows TSS of fruit beverages with coconut at different temperatures and during the storage.

The figure reveals that the G2 sample has no significant difference ($p>0.05$) during storage at 0, 90 and 180 days, with TSS results of 8.19±0.019, 8.18±0.017 and 8.18±0.014 ºBrix, respectively. In the case of G1 sample, the observation was different, there was significant difference ($p<0.05$) between the samples from 0 days (8.19±0.022 ºBrix) and 90 days (8.13±0.029 ºBrix), compared to the sample of 180 days (8.25±0.000 ºBrix). The TSS changes during storage may be attributed to a conversion of polysaccharides into simple sugars and a degradation of pectic substances in soluble solids. MOAZZEM; SIKDER; ZZAMAN, (2019) observed in their study that TSS of the samples gradually increased from the first day (16.30 to 16.50ºBrix) to the end of storage (16.78 to 18.25ºBrix) throughout the storage period.

Between G1 and G2 samples during the storage time, only the sample G1 in 180 days of shelf life showed a significant difference. Indicating that the temperature did not directly influence the TSS results.

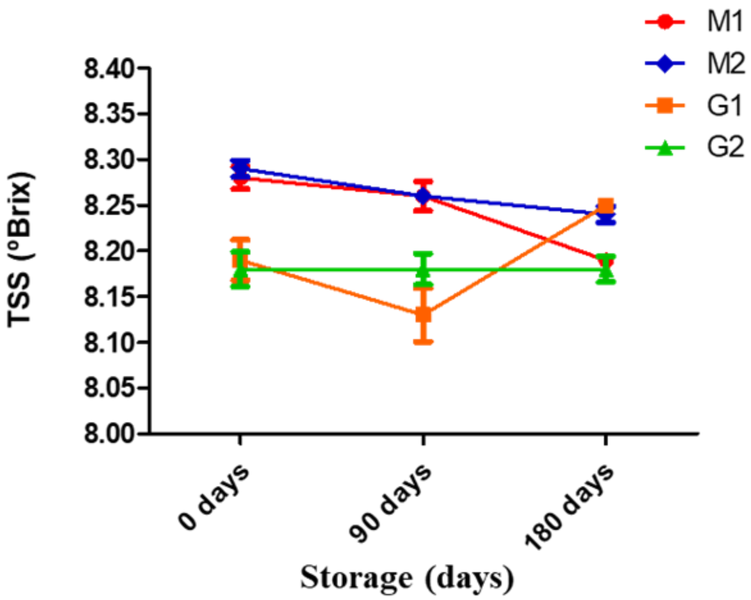

Figure 3. TSS values (ºBrix) Mean±SD of fruit beverages with coconut water at 0, 90 and 180 days of storage.

Regarding the TSS results of watermelon beverages with coconut water, the sample M1 (8.19±0.005 ºBrix) at 180 days showed a significant difference ($p<0.05$) between all conditions evaluated. During the entire process, from preparation to packaging, a decantation of the watermelon pulp was observed. SATTAR et al., (2020),observed an increase of TSS in their study, the total soluble solids of the fresh juice (13.3°Brix) were lower than the thermally pasteurized juice (13.7°Brix) throughout the 21 days of shelf life.

A study on pasteurization of guava juice concluded that TSS decreased with increase in pasteurization temperature and maximum TSS (12.5 ºBrix) was found at lowest pasteurization temperature range (75ºC/30 sec.) (LAMO et al., 2019).

The figure 4 shows TA of fruit beverages with coconut at different temperatures and during the shelf life.

The titratable acidity presented similar characteristics for the fruit beverages with coconut water during the storage time. A decrease in acidity values from time zero to 90 days and then an increase in acidity from 90 days to 180 days.

Similar condition was observed by in their study, the titratable acidity (%) of the samples gradually decreased from the first day (0.69–0.43%) to the end of storage (0.43–0.08%) throughout the storage period. A decrease in acidity during storage might be due to chemical interactions between organic constituents and enzymatic reactions (MOAZZEM; SIKDER; ZZAMAN, 2019).

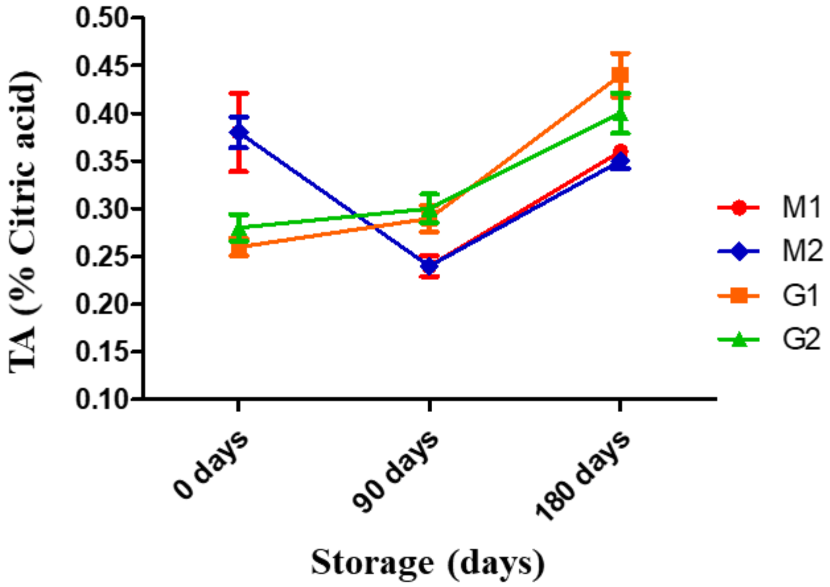

Figure 4. TA values (% citric acid) Mean±SD of fruit beverages with coconut water at 0, 90 and 180 days of storage.

In the case of watermelon beverages with coconut water, there was no significant difference (p>0.05) between the TA results comparing the different thermal process conditions for each storage period. The same condition was observed in guava beverages with coconut water. These results indicated that the different thermal process conditions did not impact the total acidity result, but the storage time influences the TA of the beverages.

Similar results were observed by RABIE et al., (2015). The titratable acidity of the pasteurized juice (1.40%) was lower than that of the fresh juice (1.66%) throughout the 21 days of shelf-life period.

Comparing the different fruits used in the study, guava beverages had higher acidity compared to watermelon beverages. The highest acidity was observed in sample G2 (0.59±0.014 %) at 0 days, in the case of watermelon the samples M1 (0.38±0.041%) and M2 (0.38±0.016%) at 0 days. The results indicate that the intrinsic characteristics of the fruits impact directly the result of the beverages.

A study identified the total acidity of whey guava beverage varied from 1.24 to 1.49 % and the pasteurizing temperatures and timings did not affect the acidity but during the storage period the acidity of whey guava beverage was increased slightly (SINGH Ȧ; SINGH Ȧ; BHATT Ȧ, 2014).

The figure 5 shows pH of fruit beverages with coconut at different temperatures and during the shelf life

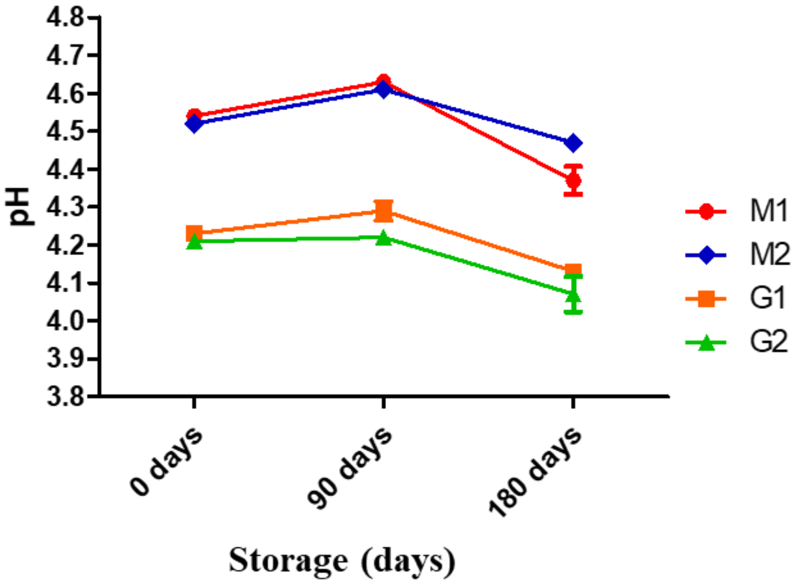

Figure 5. pH values Mean±SD of fruit beverages with coconut water at zero, 90 and 180 days of storage.

The pH results indicated that the samples of guava beverages with coconut water presented stability between the zero and 90 days of storage, that is, there were no significant differences ($p>0.05$) in the values obtained. Between 90 and 180 days there was a decrease in pH and the results had a significant difference ($p<0.05$), indicating that the beverages showed changes in the physicochemical characteristics compared to the fresh product. Among the different thermal process conditions, there was no significant difference ($p>0.05$) on pH results between samples G1 and G2.

Similar results were observed in other study of shelf life in wood apple beverages, the pH of the samples gradually decreased from the first day (5.43–4.88) to the end of storage (4.97–1.30) throughout the storage period. A decline in pH during storage was observed, which may be due to the action of citric and ascorbic acid on the sugar and protein component of the product (MOAZZEM; SIKDER; ZZAMAN, 2019).

In the case of watermelon beverages with coconut water, there was a significant difference ($p<0.05$) between zero, 90 days of storage and 180 days of storage. The pH had an increase from M1 (4.54±0.009) and M2 (4.52±0.002) at zero days to M1 (4.63±0.007) and M2 (4.61±0.015) with 90 days and a decrease with 180 days. Among the different thermal process conditions, there was significant difference ($p<0.05$) on pH results between samples M1 (4.37+0.036) and M2 (4.47+0.017) at 180 days of storage.

Comparing the different fruits used in the study, guava beverages had lower pH compared to watermelon beverages. The lowest pH was observed in sample G2 (4.07±0.046) at 180 days of storage, in the case of watermelon it was samples M1 (4.37±0.036) at 180 days of storage. The results indicate that the intrinsic characteristics of the fruits impact directly the result of the beverages.

A study observed that the pH of peach-based beverage showed significant changes in pH value among different storage time intervals. The storage results for pH parameter of pasteurized juice samples demonstrate that pH slightly decrease throughout the storage time as compared to fresh juice in which pH tends to increase when storage time exceed 20 days (SATTAR et al., 2020).

SINGH Ȧ; SINGH Ȧ; BHATT Ȧ, (2014) concluded in their study, the pH of whey guava beverage varied from 3.39 – 4.15 and there was not much difference among the samples and pasteurization temperatures and timings did not affect the pH of beverage but during the storage period the pH of whey guava was slightly decreased.

Considering the appearance of the product during the shelf life, figure 6 shows how the product behaves during the shelf life from a visual point of view. It was observed in Figure 6A, there was a decantation of the watermelon pulp during the storage of the product, being 180 days, which presents the highest decantation compared to 90 and 0 days. Figure 6B shows that the guava drink with coconut water showed a higher darkening with the heat treatment and along the storage, and a syneresis can be observed in the 180 days of shelf life. This evaluation is important to assess whether possible adjustments in the formula or process are necessary to avoid these defects that may compromise the purchase by the consumer.

A study revealed, the cloud loss in the citrus-maqui beverages provided a more intense and bright reddish color over storage time, which could result in a more visually attractive commercial beverage for potential consumer (SALAR et al., 2021).

Figure 6. Visual appearance of fruit beverages with coconut water at 0, 90 and 180 days of storage in different process conditions. (**A**) watermelon beverage with coconut water and (**B**) Guava beverage with coconut water.

According to CHEN, TANG (1998) , carotenoids may partially lose color after heat treatment, this may be attributed to the transformation of the trans to cis isomeric form. The emergence of other compounds resulting mainly from non-enzymatic browning produced by the "Maillard" reaction can increase the dark color of the juice during storage time (REMACHA, IBARZ, GINER, 1992).

3.3 ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS

3.3.1 Total phenolic compounds of fruits and fruit beverages with coconut water

The figure 7 shows the results of total phenolic compounds of fruits and fruit beverages with coconut water.

The results presented in Figure 7A indicated that guava pulp has a significant difference (p>0.05) compared to watermelon pulp and coconut water. Presenting an average value of 1516.28+313.04 mg GAE/100g.

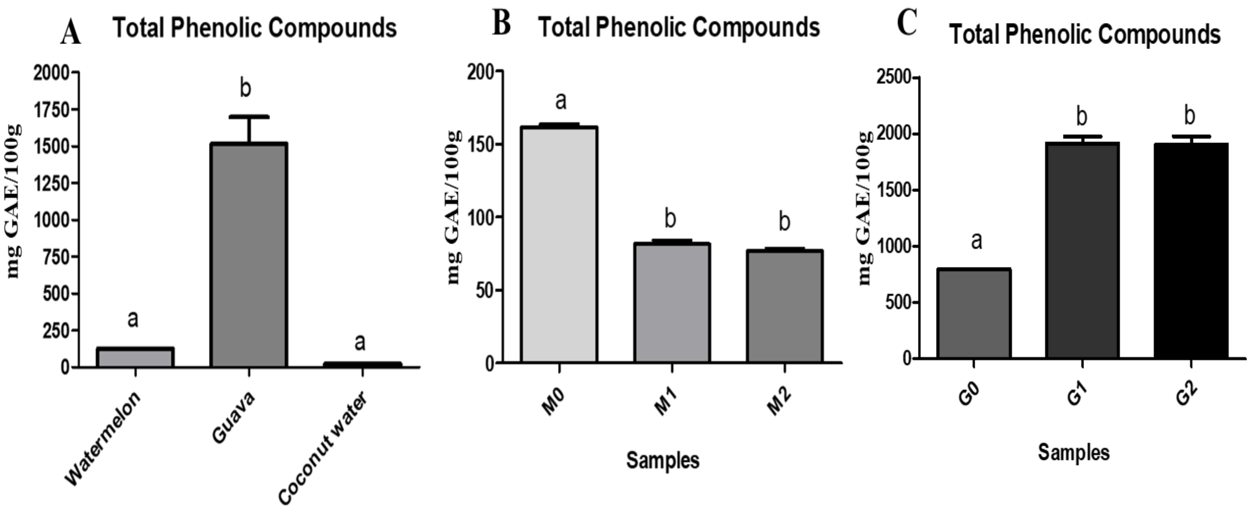

Figure 7. Values of Total Phenolic Compounds (Mean+SD) of fruits and fruit beverages with coconut water. (**A**) Fruits, (**B**) Watermelon beverages with coconut water and (**C**) Guava beverages with coconut water. Different letters indicate that the values are statistically significant (p<0.05) by Tukey test

In the samples of watermelon beverages with coconut water (figure 7B), it was observed that the sample without heat treatment M0 (161.47+4.10 mg GAE/100g) had the highest amount of total phenolic compounds compared to the heat treated samples, indicating that the temperature impacted the total phenolic compounds content. At the different temperatures M1(81.53+4.31 mg GAE/100g) and M2 (76.74+3.26 mg GAE/100g), no significant difference was observed in the amount of total phenolic compounds.

ADENEKAN MK; SOLOMON B; FADIMU GJ (2021) observed in the pasteurized canary melon juice ranged between 159.75 to 235.26mg/L while untreated sample had 242.27mg/L for phenolic compunds. Low phenolic content of the treated juice signifies that the pasteurization measures applied caused significant reduction in the phenolic content.

In the case of guava beverages with coconut water (figure 7C), the opposite was observed for the watermelon samples. The heat-treated samples obtained the highest amounts of total phenolic compounds compared to the sample without thermal process G0 (795.95+9.16 mg GAE/100g), indicating that for this fruit the temperature had a positive impact. At the different temperatures G1 (1915.06+100.74 mg GAE/100g) and G2 (1901.74+130.67 mg GAE/100g), no significant difference was observed in the amount of total phenolic compounds.

In the comparison between the fruits, the samples with guava showed significantly higher amounts in all process conditions (with and without heat treatment) than the samples with watermelon. It was concluded that guava has a superior profile of phenolic compounds than watermelon.

3.3.2 Antioxidant capacity of fruits and fruit beverages with coconut water

Figure 8 shows the antioxidant capacity of fruits in different analysis methods.

In the evaluation of the antioxidant capacity in the different methods applied, the guava pulp presented the highest averages in all the evaluated methods. Being the DPPH method (figure 8A) the biggest difference between guava (21.46±0.10 µmol trolox/g) and watermelon (3.45±0.90 µmol trolox/g).

In the TEAC method, showed in the figure 8B, there was no significant difference between watermelon (15.09±0.37 µmol trolox/g) and coconut water (16.51±0.37 µmol trolox/g).

It was observed in the figure 8D, that coconut water (0.36±0.01 µmol Trolox eq/g) had the lowest average in the ORAC method, compared to other fruits. Followed by the FRAP method shown in Figure 8C

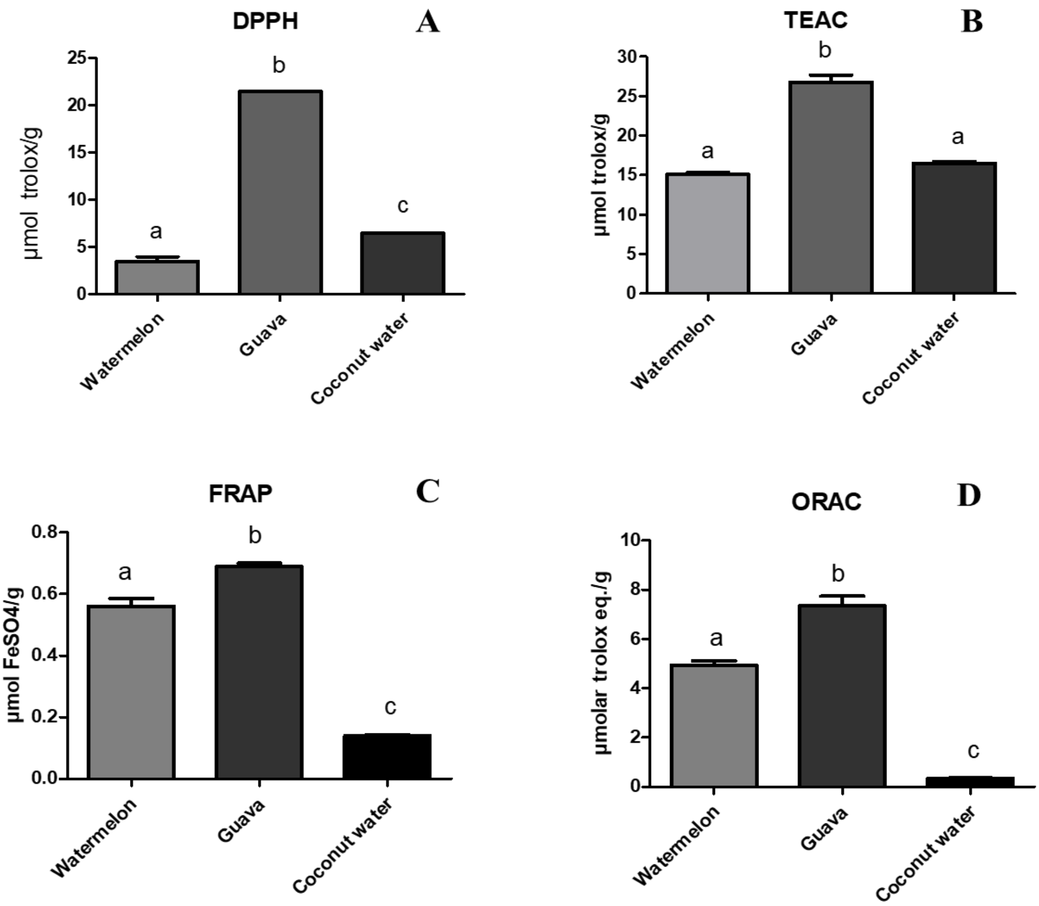

Figure 8. Values of antioxidant capacity (Mean±SD) of fruits. (**A**) DPPH methos, (**B**) TEAC method and (**C**) FRAP test and (**D**) ORAC Test. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

Figure 9 shows the antioxidant capacity of watermelon beverages in different analysis methods.

In watermelon beverages with coconut water, it was observed that there was no significant difference between the samples, that is, without heat treatment and with treatment under different temperature conditions in the TEAC (figure 9B) and ORAC (figure 9D) methods. Indicating that the temperature did not change the antioxidant activity of the samples in the two methods.

Different values were observed for TEAC method in other study for untreated canary melon juice was 80.76µmolTrolx/g while pasteurization at 80°C ranged between 60.42 and 78.42 µmolTrolx/g and at 90°C ranged between 53.25 and 64.63 µmolTrolx/g. The TEAC of treated sample decreased significantly after pasteurization (ADENEKAN MK; SOLOMON B; FADIMU GJ, 2021).

In the DPPH method (figure 9A) , the M1 (7.81±0.78 µmol trolox/g) sample obtained the highest antioxidant activity compared to the M0 (4.80±0.60 µmol trolox/g) and the M2 (5.96±0.40 µmol trolox/g) samples. Indicating that the higher the temperature, the antioxidant activity increase.

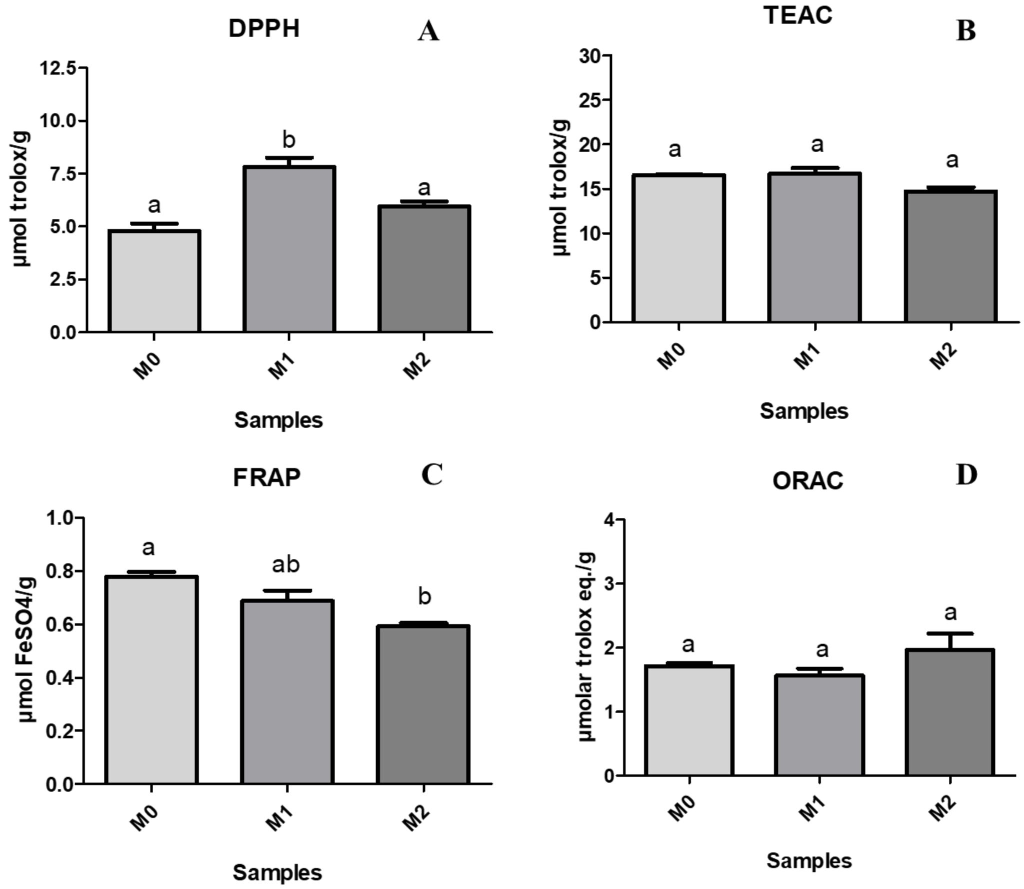

Figure 9. Values of antioxidant capacity (Mean±SD) of watermelon beverages with coconut water in different processes conditions. (**A**) DPPH methos, (**B**) TEAC method and (**C**) FRAP test and (**D**) ORAC Test. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

Figure 9 C, the FRAP method shows that there was no significant difference between sample M0 (0.79±0.03 µmol $FeSO_4$/g) and sample M1 (0.69±0.07 µmol $FeSO_4$/g) and that sample M1 had no significant difference compared to M2 (0.59±0.02 µmol $FeSO_4$/g). This indicates that in this methodology, the heat treatment M1 did not impact the antioxidant activity.

GUINE; BARROCA, (2014) observed antioxidant activity of pomegranate juices measured by different analytical methods, TEAC, DPPH and FRAP). The antioxidant capacity varied from 27.29 to 34.80 mmol Trolox/L and was significantly influenced by the heat treatment applied. The highest values were found in 80ºC and 95ºC treated juices, whereas the lowest ones were recorded in untreated samples.

Figure 10 shows the antioxidant capacity of guava beverages with coconut water in different analysis methods.

In guava beverages with coconut water, it was observed that there was no significant difference between the samples, that is, without heat treatment and with treatment under different temperature conditions in the, TEAC (figure 10B), FRAP (figure 10C) and ORAC (figure 10D) methods. Indicating that the temperature did not change the antioxidant activity of the samples in the three methods.

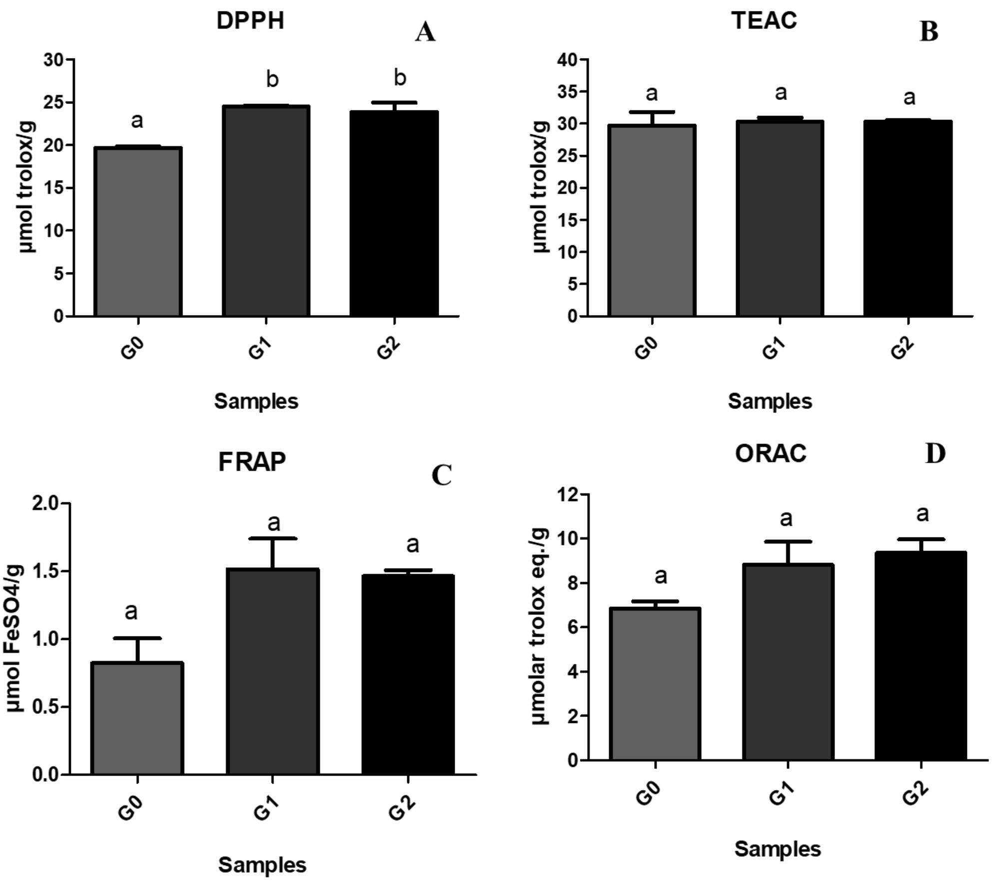

Figure 10. Values of antioxidant capacity (Mean+SD) of guava beverages with coconut water in different processes conditions. (**A**) DPPH methos, (**B**) TEAC method and (**C**) FRAP test and (**D**) ORAC Test. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

The trolox equivalent antioxidant capacity (TEAC) of the untreated canary melon juice was 80.76µmolTrolx/g while pasteurization at 80°C ranged between 60.42 and 78.42 µmolTrolx/g and at 90°C ranged between 53.25 and 64.63 µmolTrolx/g. The TEAC of treated sample decreased significantly after pasteurization (ADENEKAN MK; SOLOMON B; FADIMU GJ, 2021).

In the DPPH method (figure 10A) , the G0 (19.68±0.33 µmol trolox/g) sample obtained the lowest antioxidant activity compared to the G1 (24.53±0.17 µmol trolox/g) and the G2 (23.86±1.92 µmol trolox/g) samples. Indicating that the temperature impacts posivitely the antioxidant activity. There was no significant difference between temperatures.

A different result was observed for physalis juice, a reduction of antioxidant activity was identified after the heat treatment (90ºC/10min) from 488.75±22 to 416.87±1.07 µmol trolox/100g (RABIE et al., 2015). This result may indicate that different treatment conditions, such as HTST, may have a lower impact on the antioxidant activity of the beverages.

Comparing the antioxidant capacity of guava and watermelon, only in the FRAP method had no significant difference between the M0 (0.79±0.03 µmol $FeSO_4$/g) watermelon sample with the G0 (0.82±0.26 µmol $FeSO_4$/g) guava sample. Both of samples were not heat treated.

A study on the effect of heat treatment on tropical fruit juice, observed that there was no significant difference in antioxidant activity considering the TEAC and FRAP methods for non-thermal and pasteurized samples . Observing values of 8.66±1.58 and 8.93±0.58 µmol trolox/g, for non- thermal and pasteurized, respectively in the TEAC method. For the FRAP method was identified 21.30+1.99 and 22.02+2.29 µmol $FeSO_4$/g for non- thermal and pasteurized, respectively (WURLITZER et al., 2019).

4. CONCLUSION

Fruit beverages with coconut water may be associated with physicochemical and nutritional stability after heat treatment under both conditions. Quality attributes such as increase in titratable acidity and decrease in pH had expected changes during storage and in relation to visual appearance defects were observed such as browning in guava and decantation in watermelon. Heat treatment revealed that the amount of total phenolic compounds decreased in watermelon and increased in guava. There was a retention of the antioxidant capacity in the different analysis methods and in the two process conditions. The guava beverage with coconut water presented 25 times more total phenolic compounds when compared to watermelon and higher antioxande capacity in both heat treatment conditions. Guava is a fruit with a high pulp yield and great potential to be used as part of a functional beverage blend. The different conditions of heat treatment used did not show differences between them according to the results obtained, and any one of them can be used depending on the storage condition to which it will be submitted (ambient or refrigerated). The heat treatment showed a low impact on the physicochemical and nutritional characteristics and can be used as a preservation method for fruit beverages.

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CAPÍTULO IV

EFFECT OF THE APPLICATION OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY ON CHARACTERISTICS OF FRUIT BEVERAGES WITH COCONUT WATER

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ABSTRACT

High-pressure treatment of beverages offers a source of nutrients and health effective components. The present study aimed to evaluate the effect of high hydrostatic pressure on characteristics of fruit beverages with coconut water**.** A factorial design 2^2 was used to evaluate effect of processing conditions regarding pressures (200 to 400 MPa) and holding time (4 to min.). The different process conditions do not significantly affect ($p>0.05$) the physicochemical characteristics, demonstrating a stabilization of pH, total soluble solids, and acidity. The highest color difference was observed in watermelon beverage, with guava product presenting lower differences in the lower evaluate pressure and time (200MPa/4min.) comparing to the non-processed control. The different pressures and times retained the total phenolic compounds content for both fruits beverages. In the evaluation of antioxidant capacity, no significant difference was observed for the FRAP method in watermelon beverage and the TEAC method in guava beverage under different conditions and the DPPH method was the one that obtained the highest activity averages of 11.15±0.00 and 47.56±4.59 µmol trolox/g antioxidant for watermelon and guava, respectively. An increase in the total carotenoids content, β-carotene and lycopene was observed at the highest pressure and process time. The results of analysis of variance (ANOVA) and the standardized effect indicate that the variation of pressure (MPa) and time (minutes) does not influence the results of total carotenoids, β-carotene and lycopene For this reason, high hydrostatic pressure consists on an emerging technology that could not affect the phytochemical profile and may be considered an effective alternative in the functional beverage industry, as exemplified in the study.

Keywords: High hydrostatic pressure; Fruit; Beverage; Carotenoids

1. INTRODUCTION

The formulation of ready-to-drink beverages can be an alternative to improve the nutritional characteristics that various nutrients from different juices can complement. The mixture of fruits can provide several advantages, such as aroma, flavor, texture and nutritional characteristics that the combination of these fruits can contribute to the product, such as in the case of mixtures with coconut water and other fruits and vegetables (GEISEANNY FERNANDES DO AMARANTE MELO, 2016). Coconut water has been added as a basis for adding value in nectar due to its nutritional properties(VIEIRA et al., 2014).

Phytonutrients, also known as phytochemicals, are the nutrients often concentrated in the skins of many fruits and vegetables, and are responsible for their color, hue, scent, and flavor. Plant-based foods are extremely beneficial to consume, and phytonutrients are available in many foods such as fruits and vegetables. The best-known phytonutrients are the carotenoids, flavonoids, polyphenols, indoles, lignans and isoflavone (LIANA MARIA et al., 2014).

The growing consumer demand for safe processed foods that require minimum preparation time has led the food industry to increase its output of products that provide the nutrients and bioactive compounds to consumers are minimally altered during processing and storage (ESTEVE; FRIGOLA, 2008).

Non thermal food preservation technologies can be defined as those in which temperature is not the main factor in the inactivation of microorganisms and enzymes. The purpose of using these technologies is to inactivate the activity of the microorganisms present in the food and of certain enzymes of interest without impacting the nutritional and sensory characteristics that are normally affected during heat treatment (BARBA; ESTEVE; FRÍGOLA, 2012).

High-pressure treatment of fruit, vegetable and fresh herb homogenate products offer nearly fresh products regarding the sensorial and nutritional quality of the original raw materials thus representing relatively stable and safe source of nutrients, vitamins, minerals and health effective components (HOUŠKA et al., 2022). High-pressure treatment is based on two principles: the Le Chatelier principle, which proposes that pressure favors all structural reactions and changes that involve a decrease in volume; and the isostatic principle, which proposes that the distribution of pressure is proportional in all parts of a foodstuff irrespective of its shape and size. In the industry, the installations operate discontinuously and can attain pressures of up to 800 MPa, although pressures exceeding 400 MPa are not normally used for foods because they can bring about a reversible or irreversible disruption of inter- and intramolecular bonds (BARBA; ESTEVE; FRÍGOLA, 2012). The applied pressure can vary between 100 and 900 MPa; however, juices are generally subjected in the range of 400 to 600 MPa, with low retention time and lower temperatures compared to heat treatments (KOUTCHMA et al., 2016a).

The increasing demand for fresh-like juice has resulted in a growing interest in novel non-thermal processes such as high hydrostatic pressure (HHP). It was proved that HHP could inactivate pathogenic microorganisms and enzymes without significantly destroying thermo-sensitive nutrients, texture, color, and flavor of foods (LIU et al., 2013).

A study concluded that high pressure process offers opportunities for food manufacturers to develop new foodstuff with extended shelf life, maintains the organoleptic properties and nutritional values. These processing characteristics cannot be achieved using thermal pasteurization technology, and thus the emerging High Pressure Processing technology better meets the consumer demand for safe, wholesome, new foods containing fewer additives (HUANG et al., 2017).

The objective of this study was to investigate the effect of high hydrostatic pressure treatment on the physicochemical characteristics, antioxidant activity and carotenoids content of watermelon or guava beverages containing.

2. MATERIALS AND METHODS

2.1 RAW MATERIALS

Pink Guava (*Psidium guajava* L.) and red pulp watermelon (*Citrullus lanatus*) fruits were purchased at the local market in Rio de Janeiro/RJ, stored and kept under refrigeration until processing.

The apple juice concentrate, pasteurized and frozen, was supplied by a company specialized in concentrated juices, stored and kept under freezing (-18ºC) until the moment of use.

Frozen coconut water was purchased at the local market in Rio de Janeiro/RJ, stored and kept under freezing (-18ºC) until the moment of use.

2.2 PROCESSING OF GUAVA AND WATERMELON PULP

The fruits were sanitized by immersion in 200 ppm sodium hypochlorite for 20 minutes. After sanitization, the guavas were cut, and injured parts were removed and separated. The watermelon was cut, its rinds and seeds were removed and separated.

The pulps were obtained by grinding in Thermomix ™ (Vorwerk) – max. 3L - until complete pulp formation (without pieces), after which they were subjected to a sieve to separate the seeds. The pulps obtained were stored in sanitized recipients and kept under refrigeration until the use.

2.3 BEVERAGE FORMULATION

The formulation of fruit beverages with coconut water was defined according to a previous study (Chapter II). The formula chosen for application in this study was the one identified in the response surface methodology as the optimized formula. According to the results obtained, it was concluded that the optimized formulas of watermelon with coconut water and guava with coconut water contain 40g of fruit, 30g of coconut water and 30g of apple juice per 100 ml of beverage in their composition.

The ingredients were homogenized (5 min.) under constant stirring and the resulting beverage was kept under refrigeration at 4 ± 2°C until the moment of high hydrostatic pressure process.

2.4 HIGH HYDROSTATIC PRESSURE PROCESS

The processing of fruit beverages with coconut water by HHP was carried out in a Stansted Fluid Power equipment (S-FL-850-09-W, England) with a capacity of 250 ml and a maximum nominal operating pressure of 900MPa, as shown. in figure 1. The mixture of water and ethanol (30/70 v/v) was used as a pressurization medium.

The samples were previously packaged in polyethylene bags (50 mL), which were vacuum sealed.

Figure 1. High Hydrostatic Pressure Equipment

Pressure (MPa) and Time (min.) parameters were controlled using a Central Composite Design (CCD), that is, a factorial scheme of 2^2 treatments, including 4 factorial trials and 3 repetitions at the central point, totaling 7 trials(BARROS NETO; SCARMINIO; BRUNS, 2001). Table 1 shows the experimental design matrix with the coded and uncoded of the factors, with the real values of the independent variables defined according to the restrictions of the equipment and previous evaluations of other studies.

Table 1. Experimental design matrix for high hydrostatic pressure technology uncoded and coded for pressure(X_1) and time (X_2)

Samples	Uncoded and coded values	
	Pressure (X_1) MPa	**Time (X_2) Min.**
1	300 (0)	8 (0)
2	200 (-1)	4 (-1)
3	200 (-1)	12 (+1)
4	300 (0)	8 (0)
5	400 (+1)	4 (-1)
6	400 (+1)	12 (+1)
7	300 (0)	8 (0)

After processing, the samples were immediately stored under freezing (-18ºC) until the analysis was performed. The tests were carried out at Pilot Plant II of Embrapa Agroindústria de Alimentos, Rio de Janeiro.

The samples were coded according to the experimental design, being the samples of guava beverage with coconut water (G1 to G7) and watermelon beverage with coconut water (M1 to M7).

2.5 PHYSICOCHEMICAL CHARACTERIZATION

The pH, titratable acidity (TA) and total soluble solids (TSS) content of the different processing of fruit beverages with coconut water were determined. The pH and TA were determined using an automatic titrator equipment - model 916 Ti-Touch - (Metrohm, Switzerland), calibrated with pH 4.0, 7.0 and 10 buffer solutions. TA was determined by titration with hydroxide of sodium (NaOH) 0.1 N, using 1g of sample. The results were expressed as percentage of citric acid. The TSS content was determined at room temperature using a BS digital refractometer, model RFM340 and the results were expressed in °Brix. The physicochemical analyzes of pH, TA and TSS were performed according to AOAC (2010).

2.6 COLOR MEASUREMENTS

The instrumental color analysis of the different treatments of fruit beverages with coconut water was performed using a CR 400 colorimeter (Konica Minolta, Tóquio, Japão) with CIELAB scales. The parameters measured were lightness (L*) on the scale from 0 (black) to 100 (white), (a*) on the green scale (-80 to 0) to red (0 to +100) and (b*) on the scale from blue (-100 to 0) to yellow (0 to +70). Tests for each sample were conducted in triplicate and the values were averaged. These values were then used to calculate hue degree (h^0=arctangent [b*/a*]), chroma ($C=[(a^{*2}+b^{*2})^{1/2}]$), which is the intensity or color saturation, and ΔE, total differences of color. The total color difference (ΔE) was calculated using the following formula.

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

2.7 ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS

2.7.1 Determination of total phenolic compounds

The analysis of total phenolic compounds was performed according to the Folin-Ciocalteau spectrophotometric method (SINGLETON, V. L.; ORTHOFER, 1999). The determination was performed using aliquots of the obtained extracts, which were later pipetted and added with the Folin-Ciocalteau reagent. The mixture was left to stand for 3 to 8 minutes. Then 4% sodium carbonate was added and kept for 2 hours, protected from light. After this period, the reading was performed with absorbance of 750nm using spectrophotometer (SpectraMax i3X, Molecular Devices). The results were compared with the standard gallic acid curve and expressed in mg of gallic acid (GAE) per 100g of sample.

2.7.2 DPPH radical scavenging activity Assay

The measurement of DPPH radical scavenging activity was performed according to an adaptation of the methodology described by BRAND-WILIAMS, W.; CUVELIER, M.E.; BERSET, (1995). To evaluate the antioxidant activity, samples at three concentrations in triplicate were added for reaction with the stable radical DPPH in a methanol solution. In the radical form, DPPH has a characteristic absorption at 515 nm, which disappears after reduction by hydrogen stripped from an antioxidant compound. The radical reduction of DPPH was measured by reading the absorbance at 515 nm at 30 minutes of reaction using spectrophotometer (SpectraMax i3X, Molecular Devices). Trolox (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) standard was used. Antioxidant activity was expressed in μmol of trolox per gram of sample.

2.7.3 TEAC Assay

The ABTS method (2,2'-azino-bis 3-ethylbenzothiazolin 6-sulfonic acid) was adapted from the method described by RUFINO, MARIA S M, (2007). The ABTS•+ radical is formed by a chemical reaction with potassium persulfate in a stoichiometric ratio of 1:0.5. Once formed, the ABTS•+ radical is diluted in ethanol until obtaining an absorbance measurement of 0.70 (± 0.02) at a wavelength of 734 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). Three different volumes of beverage samples, in triplicate, reacted with the ABTS•+ radical for 6 min. A standard curve with Trolox solutions and the antioxidant activity were expressed in TEAC, Trolox equivalent antioxidant capacity (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) in μmol of trolox per gram of sample.

2.7.4 FRAP Assay

The FRAP assay was done according to ABREU et al. (2019).The FRAP reagent was prepared using 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. 2.7 ml of FRAP reagent at 37ºC was added with 90 µl of sample extract and 270 µl of distilled water. After 30 min in warm bath, the readings of samples were taken at 595 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). The ferrous sulfate was used to the standard curve and the results were expressed µM ferrous sulfate per gram of sample.

2.8 DETERMINATION OF CAROTENOIDS

The determination of total carotenoids, β-carotene and lycopene was performed by HPLC, according to PACHECO et al. (2014) with some adaptations. In the extraction of carotenoids, about 5g of each sample was used and the extraction method was carried out according to the procedure described by RODRIGUEZ-AMAYA, (2001).Profiles of the carotenoids were determined in an acetone extract by HPLC using a Waters TM HPLC system, controlled by the Empower software program with the column oven at 33 °C and photodiode array detector (PDA). Carotenoids were identified based on their retention times and UV/Vis absorption spectra, compared to the retention times of the carotenoid standards. To calculate the concentration of carotenoids in the samples, the standard curve was used and the results obtained were expressed in µg/100 g of sample.

2.9 STATISTICAL ANALYSIS

The physicochemical characterization, antioxidant capacity and carotenoids were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at a 5% probability level and p-values lower than 0.05 were considered significant. GraphPad Prism 5.0 was used. Results were expressed as mean ± standard deviation

The experimental design and analysis of variance (ANOVA) with 95% reliability were performed using Statistica® v.14. P values less than 0.05 were considered significant (TIBCO SOFTWARE INC., 2020). Results were expressed as mean ± standard deviation

3. RESULTS AND DISCUSSIONS

3.1 PHYSICOCHEMICAL CHARACTERIZATION OF FRUIT PULP AND FRUIT BEVEVERAGES WITH COCONUT WATER

The physicochemical analyzes of the fruits after pulping, coconut water and concentrated apple juice, used in the preparation of fruit beverages with coconut water can be observed in table 2.

Table 2. Physicochemical characterization of fruits used in the beverages

Parameters	Watermelon	Guava	Coconut water	Apple juice
TSS (ºBrix)	10.12±0.005[a]	11.01±0.153[b]	5.04±0.003[c]	70.21±0.008[d]
TA% citric acid	0.36±0.015[a]	0.72±0.081[b]	0.34±0.028[a]	1.18±0.005[c]
pH	5.87±0.005[a]	3.71±0.002[b]	5.32±0.005[c]	3.84±0.005[d]

Different letters in same line indicate that the values are statistically significant ($p<0.05$) by Tukey test

A significant difference ($p<0.05$) between them was observed in the TSS and pH parameters of the fruit samples, indicating that the intrinsic characteristics of each fruit impact the result presented. And in the case of apple juice, the impact is higher because the juice is in its concentrated form and during the beverage preparation process, it was diluted to the original concentration. In the case of watermelon (0.36±0.015%) and coconut water (0.34±0.028%) in the acidity parameter, no significant difference ($p>0.05$) was observed.

MA et al. (2019) observed in their study on coconut water processing, a pH of 5.54±0.01, TSS 5.20±0.17ºBrix and TA of 0.075+0.002%, the values are similar to our study, except by TA. The difference may be associated with maturation time and cultivation process. Other study on effect of process in watermelon juice revealed there was no obvious change in titratable acid of thermal process watermelon juice (0.362 mg/L) during the storage at 4 ℃ (LIU et al., 2021). A study on the characterization of different guava varieties identified in red guava a pH of 3.6±0.0, TSS of 8.6±0.6 ºBrix and a titratable acidity of 0.79±0.03% ROJAS-BARQUERA, (2009). The results of TA and pH are similar our study, the difference in TSS can be attributed to ripeness degree and crop conditions.

The results of the physicochemical analysis of the watermelon beverages with coconut water and the guava beverages with coconut water subjected to pressurization through high hydrostatic pressure can be seen in table 3.

For the samples of watermelon beverage with coconut water, a significant difference was observed in the pH parameter ($p<0.05$) between the M1 sample (4.46±0.012) and the control sample (4.34±0.006) - without pressurization. In the case of the TSS results, sample M6 (8.45±0.005ºBrix) presented the lowest average compared to the other samples, with the sample subjected to greater pressure for the longest time 400MPa/12 minutes. By evaluating the acidity parameter, the control sample showed a significant difference ($p<0.05$) when compared to the other samples, indicating that after processing there was a stabilization of acidity.

A study on the effect of high pressure and thermal process on the quality of strawberry juice revealed that at different pressures (400/500 and 600 MPa/ 3 min.) there was no significant difference in pH which remained stable at 3.30±0.01. The same was observed for the TSS parameter, which ranged from 8.1 to 8.3 ºBrix and showed no significant difference between the different pressures (AABY et al., 2018).

Table 3. Physicochemical results for watermelon beverage with coconut water and guava beverage with coconut water after HHP process

Samples	Watermelon and coconut water beverage			Guava and coconut water beverage		
	Physicochemical parameters			Physicochemical parameters		
	pH	**TSS ºBrix**	**TA (% citric acid)**	**pH**	**TSS ºBrix**	**TA (% citric acid)**
1	4.46+0.012[a]	8.80+0.036[a]	0.25+0.007[a]	3.96+0.009[a]	8.71+0.045[a]	0.35+0.008[a]
2	4.40+0.011[ba]	8.73+0.016[b]	0.24+0.002[ab]	3.92+0.011[b]	8.82+0.019[b]	0.39+0.017[ab]
3	4.38+0.006[bc]	8.74+0.021[b]	0.29+0.00[a]	3.89+0.006[c]	8.80+0.022[cb]	0.37+0.002[a]
4	4.39+0.008[bc]	8.73+0.005[b]	0.28+0.018[ab]	3.88+0.006[dc]	8.66+0.005[da]	0.38+0.007[ab]
5	4.39+0.019[bc]	8.72+0.008[b]	0.28+0.002[ab]	3.86+0.005[de]	8.73+0.008[acd]	0.35+0.039[a]
6	4.38+0.009[bc]	8.45+0.005[c]	0.28+0.010[ab]	3.85+0.006[e]	8.79+0.041[abc]	0.40+0.008[ab]
7	4.37+0.006[bc]	8.72+0.005[b]	0.29+0.012[a]	3.85+0.006[e]	8.72+0.005[acd]	0.38+0.011[ab]
Control	4.34+0.028[c]	8.77+0.009[ab]	0.41+0.027[c]	3.88+0.004[cde]	9.04+0.005[e]	0.43+0.002[b]

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

In guava beverages with coconut water, when evaluating the pH results, the sample G1 (3.96±0.009) had the highest average compared to the other samples, followed by G2 (3.92±0.011), the other samples did not show significant differences ($p< 0.05$). In the case of TSS, the control sample (9.04±0.005ºBrix) showed a significant difference ($p>0.05$) compared to the other samples. Evaluating the TA results, extremes can be observed between sample G1 (0.35±0.008%) and G5 (0.35±0.039%) and control sample (0.43±0.002), indicating that there was difference after pressurization.

JAYACHANDRAN; CHAKRABORTY; RAO, (2015) identified in their study on effect of high-pressure processing in litchi with coconut water and lemon beverage that the different pressures conditions did not affect the physicochemical parameters. The TA kept the same results (0.32%) in 600 and 500 MPa, for TSS no significant difference 13.6ºBrix (600 MPa) and 13.5ºBrix (500 MPa), the same for pH 4.16 and 4.12, for 600 and 500 MPa, respectively.

The effect of HPP on mango pulp study concluded that the pH, TA and TSS ranged from 3.76 to 4.02, 0.49 to 0.56% and 17.39 to 17.83ºBrix, respectively within 100 to 600 MPa for 1s to 20 min. TSS increased slightly, however not significantly, after HPP treatments ($p>0.05$). On the other hand, pH decreased, and TA increased significantly after pressurization ($p<0.05$), where the pressure level had a significant effect ($p<0.05$) and no effect of dwell time was observed ($p>0.05$). HPP has been known to change the pH of a system since compression of foods may shift the pH of the food as a function of imposed pressure (KAUSHIK et al., 2014).

3.2 EFFECT OF HHP ON COLOR OF FRUIT BEVEVERAGES WITH COCONUT WATER

The color results of watermelon beverages with coconut after pressurization, can be observed in table 4.

The significant differences in the values of the color parameters in the samples of watermelon beverages with coconut water can be explained by considering the watermelon pulp decantation. This might be attributed to aggregation of pectin with other cell wall materials present during the beverage preparation, process and filling. Amount of pulp may have been unevenly distributed impacting the results. A similar effect was observed in the study on high pressure process in litchi, coconut water and lemon beverage in which there was a visible particle precipitation or layer separation for the samples treated at ≥500 MPa and 70 °C (JAYACHANDRAN; CHAKRABORTY; RAO, 2015).

This difference was not observed in the study conducted by (LIU et al., 2013), which concluded in the samples of watermelon juice submitted to high pressure that after HHP treatment, L*-value increased but the value did not change with increase in pressure and treatment time; a*- and b*-value had no significant difference compared to the control ($p<0.05$).

The parameter a* is related to redness of the watermelon juice, which is its main beverage color. The highest value was observed in the M6 (7.84±0.91) – 400MPa/12 min. A study revealed the color a* of the watermelon juice subjected to the high-pressure treatment at 600 MPa was similar to that of the control, whilst that subjected to the high-pressure treatment at 300 and 900 MPa was statistically different to that of the control. The high-pressure treatment at 600 MPa was effective to keep the color of the treated watermelon juice as the control (ZHANG, 2011).

Table 4. Color results for watermelon with coconut beverages after HHP process

	Watermelon and coconut water beverage					
	Color					
Samples	**L***	**a***	**b***	**Chroma**	**Hue angle**	**ΔE**
M1	36.12±3.12[a]	7.11±1.09[a]	8.68±4.40[a]	11.58±2.89[a]	47.94±16.87[a]	12.51
M2	37.48±2.68[a]	6.87±0.93[a]	8.69±3.92[a]	11.35±1.90[a]	49.40±16.40[a]	11.38
M3	42.98±9.70[a]	5.19±3.49[a]	12.62±3.84[a]	14.11±2.69[a]	66.52±19.40[a]	3.64
M4	42.12±4.67[a]	4.56±1.92[a]	14.54±0.65[a]	15.32±0.38[a]	72.62±7.49[a]	3.21
M5	44.52±5.47[a]	4.60±2.33[a]	15.10±1.69[a]	15.85±2.22[a]	73.72±6.95[a]	0.21
M6	35.58±2.50[a]	7.84±0.91[a]	13.30±1.20[a]	15.48±0.58[a]	59.34±5.18[a]	7.70
M7	40.63±2.64[a]	6.00±1.00[a]	15.64±0.90[a]	16.78±0.50[a]	68.96±4.31[a]	2.15
Mcontrol	44.61±3.31[a]	5.96±1.52[a]	13.86±1.51[a]	15.15±1.25[a]	66.67±6.63[a]	0

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

According to CSERHALMI et al. (2006) a study, depending on the value of ΔE, the color difference between the treated and untreated samples can be estimated such as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0) values >12 for a very great difference.

The smallest color difference (ΔE) was observed in sample M5 (0.21) that can be as not noticeable difference, which was subjected to higher pressure (400 MPa), on the other hand, sample M6 (7.70), which was subjected to highest pressure and time (400MPa/12 min.), obtained a higher color difference compared to the M5 sample. This result reinforces the observation that the decanting of watermelon pulp in the beverage can impact the color result of the product, in addition to the increase in lightless.

Other study observed in litchi based mixed fruit beverage the ΔE* was significantly higher in samples treated between 400 and 600 MPa (60 and 70 °C). At 600 MPa/ 30 °C/20 min, the maximum value of ΔE* was 1.55, while at 600 MPa/ 70 °C/20 min the corresponding value was 7.39 (JAYACHANDRAN; CHAKRABORTY; RAO, 2015).

The color results of guava beverages with coconut after pressurization, can be observed in table 5.

The lowest value of parameter L* was observed in sample G6 (41.11) which was subjected to highest pressure and time (400MPa/12 min.).

Beverage luminosity (L*) decreased when the treatment time was longer, while an increase in L* was observed when pressure was higher, although when pressure was higher than 9 min a decrease in L* value was observed when pressure was 600 MPa (BARBA; ESTEVE; FRIGOLA, 2013).

Table 5. Color results for guava with coconut beverages after HHP process

	Guava and coconut water beverage					
	Color					
Samples	**L***	**a***	**b***	**Chroma**	**Hue angle**	**ΔE**
G1	41.92±1.55[a]	10.98±0.57[a]	6.69±1.64[a]	12.92±0.16[a]	31.13±7.08[a]	0.77
G2	41.94±0.27[a]	10.60±0.30[a]	6.45±1.89[a]	12.47±0.63[a]	30.88±6.53[a]	0.17
G3	42.00±0.56[a]	9.72±0.51[a]	4.44±1.44[a]	10.72±0.81[a]	24.15±4.04[a]	2.66
G4	42.96±0.77[a]	10.15±0.60[a]	6.73±0.29[a]	12.17±0.65[a]	33.57±0.70[a]	1.01
G5	42.61±0.42[a]	11.09±0.26[a]	8.24±1.03[a]	13.83±1.06[a]	36.52±2.80[a]	3.12
G6	41.11±0.14[a]	10.15±0.26[a]	6.13±0.47[a]	11.86±1.26[a]	31.12±1.11[a]	1.43
G7	43.16±0.63[a]	10.06±0.49[a]	7.98±0.37[a]	12.84±0.78[a]	38.44±1.94[a]	2.38
GControl	43.00±0.88[a]	10.91±0.67[a]	4.92±1.51[a]	12.01±1.06[a]	24.02±6.03[a]	0

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

In relation to the parameter a* that indicates the redness of the samples, it can be observed there are no significant difference ($p>0.05$) among the samples. Regarding the green/yellow hue (b*) and saturation (Chroma), no significant difference was observed between the samples.

BARBA; ESTEVE; FRIGOLA, (2013), verified in their study with no significant changes observed in a* values (0.36±0.04) after different HPP treatments. L* values significantly ($p<0.05$) decreased (31.32±0.10 to 32.09±0.20) in relation to the unprocessed blueberry juice (32.66±0.23). The multifactor analysis of variance showed the existence of interaction ($p<0.01$) between pressure and time of treatment, when applied pressure was 200 MPa.

The lowest color difference (ΔE*) was observed in the G2 (0.17) sample with lower pressure (200 MPa) and the highest difference in the G5 (3.12) sample with the highest pressure

(400 MPa), when compared with the control sample. A similar observation was verified by KAUSHIK et al. (2014), ΔE* values increased with increasing pressurization and varied from 1.21 to 1.92 within 100 to 600 MPa pressures whereas pressure range 100–300 MPa led to small differences in color for mango pulp after single pulse, and pressures of 400–600 MPa produced distinct color differences.

3.3 EFFECT OF HHP PROCESS ON TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF FRUIT BEVEVERAGES WITH COCONUT WATER.

The figure 2 shows the effect of the HHP process on the quantification of total phenolic compounds in fruit beverages with coconut water. In figure 2A it was observed that the pressure and time in the process did not influence the amount of total phenolic compounds in the watermelon beverage with coconut water, that is, there is no significant difference ($p>0.05$) between the samples. Figure 2B revealed that the G4 (267.62 ±9.30 mg GAE/100g) sample showed a significant difference when compared to the other samples. Since the process parameters for producing G4 samples are the same of the samples G1 and G7, new analysis must be performed to check these differences.

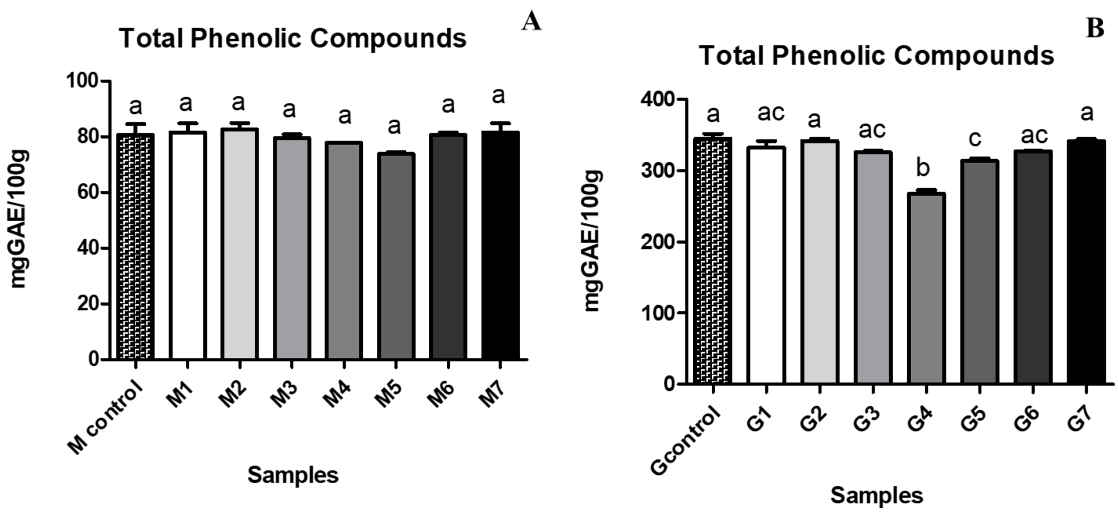

Figure 2. Effect of pressure and time for total phenolic compounds (Mean±SD) in fruit beverage with coconut water. (**A**) watermelon beverage with coconut water and (**B**) guava beverage with coconut water. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

A similar result was obtained on the effect of high-pressure processing on a litchi based mixed fruit beverage, in which the authors observed no impact of pressure on phenolic content in the initial of the fresh sample (untreated = 33.6 mg GAE/100 mL). At 200, 300 and 400 MPa, the TP (total phenols) content of the samples were well retained as much as in the untreated samples (JAYACHANDRAN; CHAKRABORTY; RAO, 2015). FERNANDEZ et al. (2018), on optimization study of high pressure processing in mixed fruit (orange juice, apples, carrots, beet leaves and beet stems) revealed that the total phenols content of the high pressure treated samples ranged between 62.07 ± 0.91 and 68.33 ± 1.35 mg GAE 100 mL^{-1}. This increase was proven to be not statistically significant ($p > 0.05$) in relation to untreated samples. BARBA et al., (2014) observed that the phenols appeared to be relatively resistant to HPP, no significant decrease was found for any of the pressure/time combination applied in a study on high pressure processed fruit extract (Papaya, mango and Orange).

The figure 3 shows the effect of the HHP process on antioxidant capacity considering different methods in watermelon beverage with coconut water. Figure 3A indicates in DPPH method that sample M1 (8.19±0.39 µmol trolox/g) showed a significant difference with sample M4(9.46±0.41 µmol trolox/g) and not with sample M7 (11.15±0.00 µmol trolox/g). The 3 samples were submitted to the same process parameters (300MPa/8 minutes), new analysis must be performed to check these differences.

LI; PADILLA-ZAKOUR, (2021) evaluated in their study on High Pressure Processing vs. Thermal Pasteurization of Whole Concord Grape Puree that there was no significant difference ($p>0.05$) between the results of the control sample and the sample submitted to the high-pressure process (600MPa/3 min.), considering the DPPH method. In the TEAC method the observation was different, there was an increased from control sample to HPP sample 34.7±0.6, 38.2±0.1 µmol trolox/g, respectively for the same condition.

The same results can be observed in TEAC method figure 3B, which obtained the highest mean of antioxidant capacity in the M4 (8.69±0.86 µmol trolox/g) and M7(8.68±0.00 µmol trolox/g) samples, but different from M1(5.33±1.12 µmol trolox/g). CHANG et al., (2017) identified in their study that the HPP significantly enhanced the TEAC value of grape juice from 10.64 mmol L^{-1} Trolox in the control group to 11.75 and 13.34 mmol L^{-1} Trolox in the HPP-300MPa and HPP-600MPa, respectively.

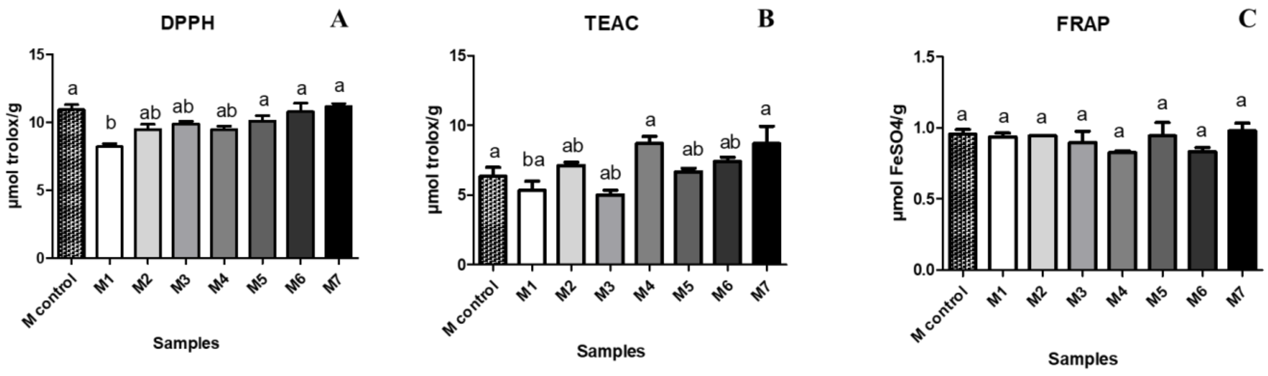

Figure 3. Effect of pressure and time for antioxidant capacity (Mean±SD) in watermelon beverage with coconut water. (**A**) DPPH method (**B**) TEAC method and (**C**) FRAP method. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

A study verified the effect of HHP in antioxidant capacity considering the DPPH method of fresh smoothies (whole apple, apple juice from concentrate, strawberry, banana and orange) was similar to HHP-450 MPa samples. There were differences between the two high pressure treatments were observed. HHP-450 MPa samples had higher TEAC, than HHP-600 MPa samples ($p < 0.001$) (KEENAN et al., 2012).

In figure 3C it was observed that the pressure and time in the process did not influence the antioxidant capacity in the watermelon beverage with coconut water, considering the FRAP method that is, there is no significant difference ($p>0.05$) between the samples.

The figure 4 shows the effect of the HHP process on antioxidant capacity considering different methods in guava beverage with coconut water.

Figure 4A identified that the highest average antioxidant capacity by the DPPH method is in the G6 (47.56±4.59 µmol trolox/g) sample, which comprises the highest pressure and process time (400MPa/12 min.), indicating that high pressure and time impacted positively in the results.

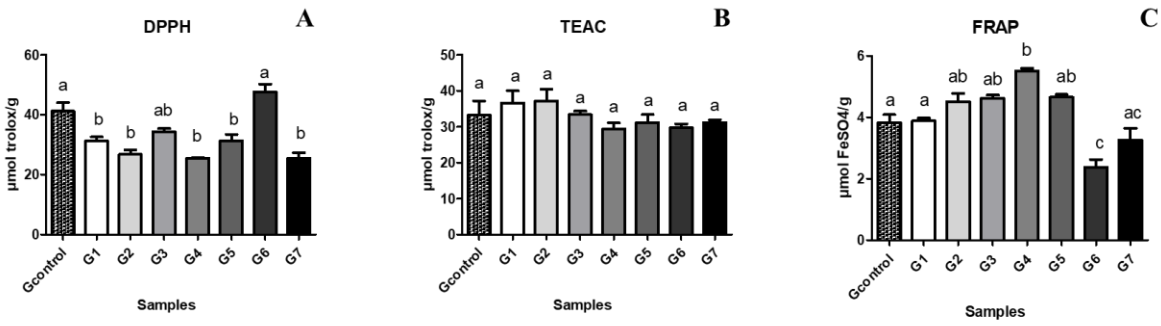

Figure 4. Effect of pressure and time for antioxidant capacity (Mean±SD) in guava beverage with coconut water. (**A**) DPPH method (**B**) TEAC method and (**C**) FRAP method. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

In figure 4B it was observed that the pressure and time in the process did not influence the antioxidant capacity in the guava beverage with coconut water, considering the TEAC method that is, there is no significant difference ($p>0.05$) between the samples.

A study on effects of high-pressure processing in jaboticaba (*Myrciaria cauliflora*) juice demonstrated the HPP-600MPa had 55.4% higher antioxidant capacity in TEAC method, followed by the HPP-400MPa, with 30.9%, and not significantly different for the HPP-200MPa. The results for DPPH indicated that there were three HPP groups without significantly difference among their components (HU; WANG; CHEN, 2020).

Figure 4C identified that the lowest average antioxidant capacity by the FRAP method is in the G6 (2.37±0.44 µmol $FeSO_4$/g) sample, which comprises the highest pressure and process time (400MPa/12 min.), indicating that high pressure and time decreased the antioxidant capacity in this method, contrary to what was observed in Figure 4A related to DPPH method. A study on bee-pollen pastes and bee-pollen-based beverage had a different result compared our study and identified that the bee-pollen increased the FRAP levels significantly ($p < 0.05$) in pressurized food matrix-based beverage samples. The pressure and bee pollen concentration had a significant effect on antioxidant activity in HPP-treated samples, reaching values of 140.30 ± 4.90 µmol Trolox/g with the highest treatment tested (400 MPa, 15 min, 10% (w/v) bee pollen). This can be attributed to the extraction of carotenoids and polyphenols, responsible for antioxidant capacity in bee-pollen (ZULUAGA et al., 2016).

3.4 EFFECT OF HHP PROCESS ON CAROTENOIDS OF FRUIT BEVEVERAGES WITH COCONUT WATER

The table 6 shows the effect of HHP process in carotenoids content for watermelon beverage with coconut water.

A decrease in the of total carotenoids content was observed after application of high hydrostatic pressure. The M2 (951±37 µg/100g) sample was the most impacted, losing about 27% when compared to the unprocessed sample. In the study effect of process in orange juice it was observed a decrease of total carotenoids content compared to sample submitted to high pressure (4000 Bar for 5 min) the decrease was from 1367.2 ± 64.7 to 1309.2 ± 46.7 µg/100 g (ESTEVE; FRIGOLA, 2008).

In the β-carotene analysis, it was observed a higher retention in the samples when compared to the control sample. Only the M2 (326,0±10 µg/100g) sample had a decay. TADAPANENI et al. (2014) observed in their study, the apricot nectars submitted at the 300−500 MPa/5−20 min no effect on total carotenoids and individual carotenes, except for the treatment at 500 MPa/20 min, which increased total carotenoids and β-carotene

The same situation was observed in lycopene results, the M2 (350±23 µg/100g) sample has a decrease of lycopene content compared to the control sample. This shows that the lower pressure (200MPa) and time (4 min.) does not retain of increase the of carotenoids content in the watermelon beverage with coconut water. A similar result was observed by LIU et al. (2013) on the effect of high hydrostatic pressure on overall quality parameters of watermelon juice, in the lycopene solution, pressure under 400MPa did not affect the content of total lycopene, while pressure of 500 and 600MPa caused significant losses compared to the control. Other study on quality parameters of watermelon juice study revealed total lycopene concentration of the treated watermelon juice was statistically similar after the high-pressure treatment at 300 MPa and 600 MPa (ZHANG, 2011).

Table 6. Effect of HHP process in carotenoids content for watermelon beverage with coconut water.

	Carotenoids (µg/100g)		
Sample	Total carotenoids	β-carotene	Lycopene
M1	1169±2^{a}	413±14^{a}	407±8^{a}
M2	951±37^{b}	326±10^{b}	350±23^{ba}
M3	1132±2^{a}	385±1^{a}	501±19^{a}
M4	1060±4^{ab}	374±4^{a}	426±17^{a}
M5	1104±21^{a}	370±10^{a}	483±3^{a}
M6	1147±42^{a}	387±9^{a}	497±15^{a}

M7	1097±48[a]	387±21[a]	476±47[a]
Control	1296±48[c]	364±6[b]	611±45[c]

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

Analysis of variance (ANOVA) was determined according to table 7, p values of pressure and time were observed for the carotenoids parameters in watermelon beverage with coconut water. It was revealed that pressure (MPa) and time (min.) showed no significant difference $p>0.05$. This means that the variation of pressure and time does not impact the results of total carotenoids, β-carotene and lycopene.

Table 7. p- value of pressure and time for carotenoids in high pressure processed watermelon beverage with coconut water

	Carotenoids		
Constituents	**Total carotenoids**	**β-carotene**	**Lycopene**
(1) Pressure (MPa) (L)	0.2686	0.3571	0.2126
Pressure (MPa) (Q)	0.6100	0.2426	0.5196
(2) Time (min.) (L)	0.1805	0.1901	0.1487
1L by 2L	0.3389	0.4004	0.1952

Another way to visualize the impact of constituents on the acceptance of fruit beverages with coconut water is through the standardized estimated effect of the constituents pressure and time as well in the linear and quadratic terms and their interactions.

In the Pareto chart of standardized effect, it can be observed which factors and interactions were statistically or not significant and the most relevant in order of importance. The bars that go beyond the region to the right of the vertical where $p=0.05$ is indicated (5% of significance) represent the parameters that have a significant effect on the evaluated response. Considering that none of the factors had a significant difference in this study, the chart shows which were the most important constituents for each parameter evaluated**.**

The figure 5 shows Pareto chart of standardized effect of pressure and time for carotenoids in watermelon beverage with coconut water. The results revealed that the most important constituent for total carotenoids, β-carotene and lycopene results was the time in its linear mode.

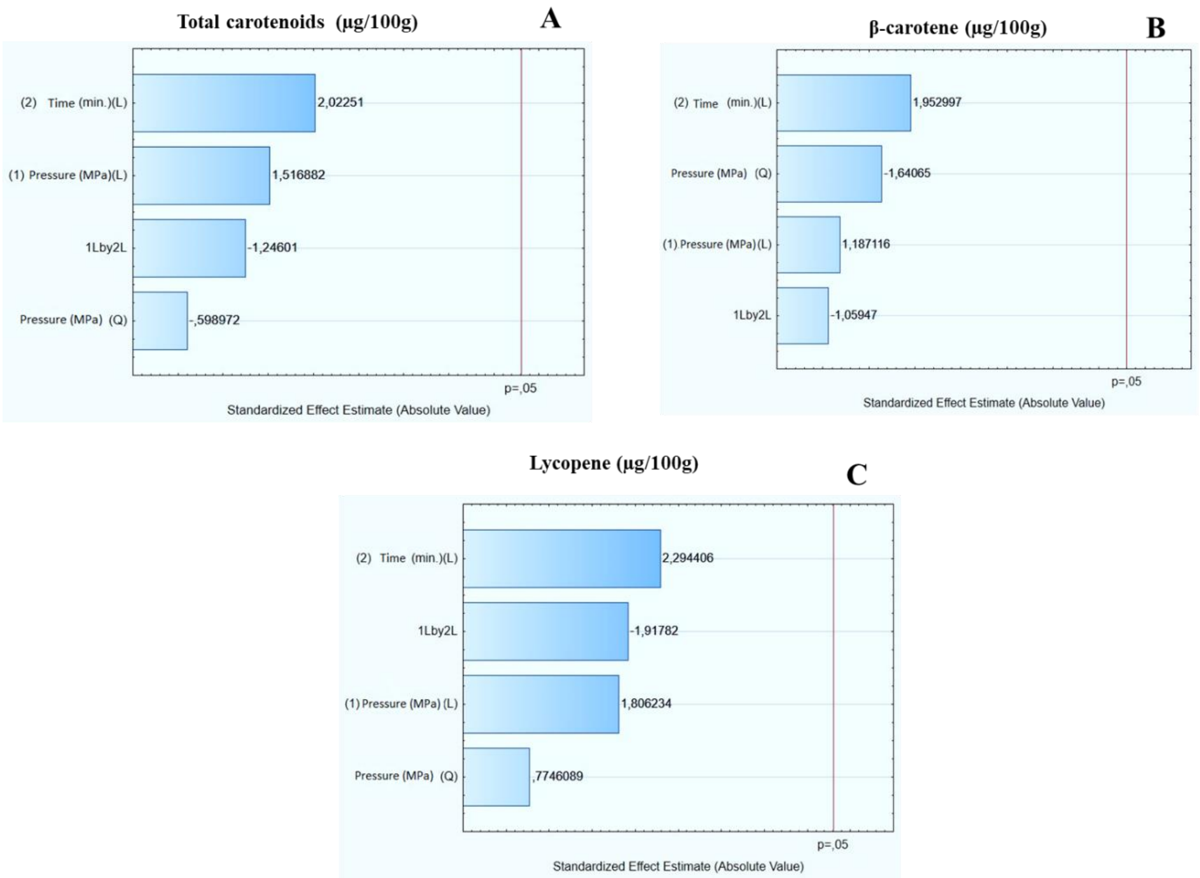

Figure 5. Pareto chart of standardized effect of pressure and time for carotenoids in watermelon beverage with coconut water. (**A**) Total carotenoids (**B**) β-carotene and (**C**) Lycopene

Table 8 shows the effect of HHP process in carotenoids content for guava beverage with coconut water.

An increase in the of total carotenoids content was observed after application of high hydrostatic pressure. The G6 (2277±91µg/100g) sample was the most impacted, increasing about 26% when compared to the unprocessed sample. A study observed in orange juice an increase of 45% in total carotenoids submitted to 400MPa/40ºC/1 min (KOUTCHMA et al., 2016a).

Table 8. Effect of HHP process in carotenoids content for watermelon beverage with coconut water.

	Carotenoids (µg/100g)		
Sample	Total carotenoids	β-carotene	Lycopene
G1	1939±78^{ac}	101.0±4.2^{a}	1364±68^{a}
G2	1852±44^{ac}	106.5±2.1^{a}	1317±88^{a}
G3	2225±30^{b}	109.0±0.0^{a}	1465±15^{a}
G4	2121±40^{abc}	110.5±3.5^{a}	1422±74^{a}

G5	2083 ± 59^{d}	117.5 ± 12^{a}	1400 ± 69^{a}
G6	2277 ± 91^{eb}	142.0 ± 1.4^{b}	1486 ± 30^{a}
G7	1872 ± 115^{a}	98.5 ± 4.9^{a}	1246 ± 86^{a}
Control	1800 ± 83^{f}	101.5 ± 7.8^{a}	1251 ± 64^{a}

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

In the β-carotene analysis, it was observed a higher retention in the samples when compared to the control sample. Only the G7 (98.5±4.9 µg/100g) sample had a decay. The same situation was observed in lycopene results, in which the G6 (1486±30µg/100g) sample has a higher increase of lycopene content compared to the control sample. This shows that the higher pressure (400MPa) and time (12 min.) retain or increase the carotenoids content in the guava beverage with coconut water. A review on implications for bioactive compounds considering the high-pressure process observed the significant increase (approximately 56%) in concentrations of total extractable carotenoids as a result of HPP (600MPa/3 min) in avocado paste and higher increases for individual carotenoids were observed, for example β-carotene (107%) (TADAPANENI et al., 2014).

Analysis of variance (ANOVA) were performed, and p-values can be seen in table 9 for the carotenoids' parameters for guava beverage with coconut water. The pressure constituents in its linear and quadratic form, considering the β-carotene ($p=0.0738$) and ($p=0.086$) were the ones that obtained the p-value closest to the significant difference $p<0.05$.

Table 9. p- value of pressure and time for carotenoids in high pressure processed guava beverage with coconut water

	Carotenoids		
Constituents	**Total carotenoids**	**β-carotene**	**Lycopene**
(1) Pressure (MPa) (L)	0.3871	0.0738	0.6214
Pressure (MPa) (Q)	0.3120	0.0859	0.3983
(2) Time (min.) (L)	0.1589	0.1667	0.3219
1L by 2L	0.5599	0.2244	0.7638

The figure 6 shows Pareto chart of standardized effect of pressure and time for carotenoids in guava beverage with coconut water. The results revealed that the most important variable for total carotenoids (figure 6A), and lycopene (figure 6C) results was the time in its linear mode. For β-carotene (figure 6B) pressure in linear mode was instead the main factor, that is, the pressure had the highest influence on the results of this parameter.

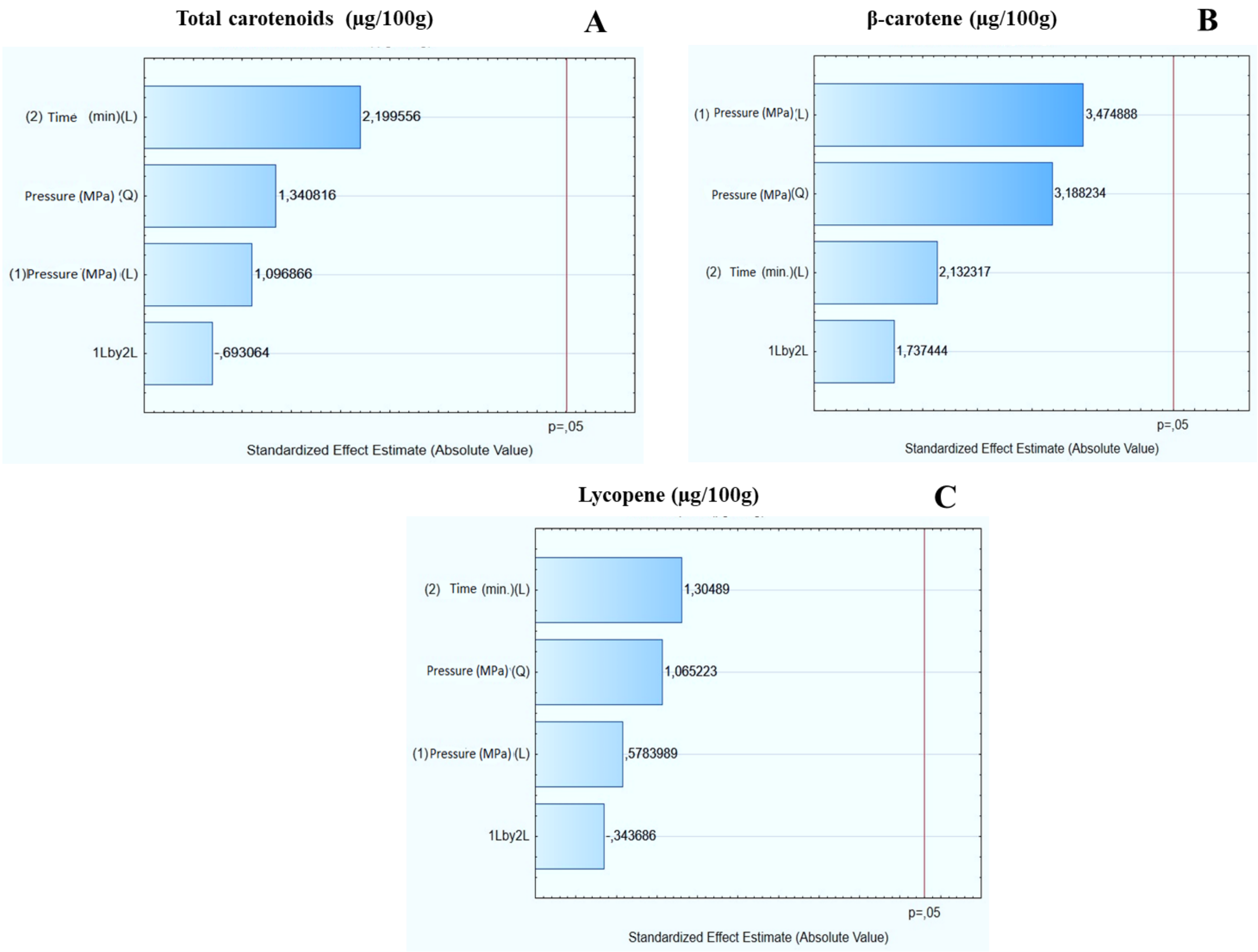

Figure 6. Pareto chart of standardized effect of pressure and time for carotenoids in guava beverage with coconut water. (**A**) Total carotenoids (**B**) β-carotene and (**C**) Lycopene

Researchers have shown that the extractability and potential bioaccessibility of compounds such as flavanols, carotenoids, and lycopene are improved with HPP. However, the biological significance of high pressure processed fruit products, specifically as it relates to the bioavailability of nutrients and polyphenolic compounds, has not been broadly studied (TADAPANENI et al., 2014).

4. CONCLUSION

The results of the present study showed that hydrostatic high-pressure process is a promising technology as a preservation method of the fruit beverage with coconut water. The HPP did not affect pH and total soluble solids, had a slight difference on color, retained the total phenolics compounds and antioxidant capacity and improved the carotenoids content. The results of analysis of variance (ANOVA) and the standardized effect through pareto chart indicate that the variation of pressure (MPa) and time (minutes) does not influence the results of total carotenoids, β-carotene and lycopene. The pressure and time can be defined considering the variables to predict the quality characteristics of fruit beverages with coconut water during the HHP process within the studied domain of process conditions. Future studies could be oriented to evaluate the stability of the different quality attributes during storage, shelf-life estimation and possible scaling-up for evaluating the industrial HHP process application to fruit beverages.

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CAPÍTULO V

COMPARISON OF HEAT TREATMENT AND HIGH HYDROSTATIC PRESSURE ON CHARACTERISTICS OF FRUIT BEVERAGES WITH COCONUT WATER

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ABSTRACT

Fruit juices have become an important product for the healthy food world and alternative technologies to heat treatment have been studied to preserve the nutritional characteristics. The effects of high hydrostatic pressure (HHP) compared to heat treatment (HT) were studied in fruit beverages with coconut water. The impact of the processing technologies on the microbiological and phytochemical profile was assessed by applying two HHP treatments at 200 MPa/4min. and 400 MPa/12 min. and HT 124-126ºC and 104-109ºC for 17s. All treatments ensured microbiological stability. Aside from that, the physicochemical parameters were not significantly different considering the same process conditions. Regarding color parameters, the color difference (ΔE) was higher in HT compared to the HHP. In relation to total phenolic compounds content there was an increase in the samples with guava and a decrease in the samples with watermelon in the HT, the samples submitted to HHP had a retention. In the antioxidant capacity considering only the DPPH, FRAP and TEAC methods, all treatments had a retention of amounts. In the evaluation of carotenoids, samples with HT had a decrease in the total carotenoids content, β-carotene and lycopene, in samples submitted to HHP there was a retention. It can be concluded that HHP is an effective alternative to HT, achieving effective microbial inactivation the beverages by contributing to a better preservation of color and bioactive compounds.

Keywords: Fruit beverage; High hydrostatic pressure; Heat treatment; Carotenoids

1. INTRODUCTION

The health-conscious market's interest in functional foods such as longevity foods, nutritional foods, super foods, pharmaceutical foods, phyto foods and therapeutic foods provide a bright prospect for coconut water to penetrate the beverage industry (HIDALGO, 2017).

The growing consumption of premium and ultra-premium categories of juices has been attributed to the perceived health benefits of reduced calories, reduced sugar, and the "all natural" message based on high contents of enzymes, nutrients, and bioactive constituents. The addition of vegetable juices and fruit-vegetable blends also drives the low-calorie and health-beneficial messages. To achieve these attributes, the premium juice category is minimally processed using cold-pressing or other extraction methods to minimize treatment temperature and exposure to oxygen (KOUTCHMA et al., 2016b).

The academia and the food industry have shown a great deal of interest in exploring alternative food processing technologies that use minimal heat and/or preservatives. Increased consumer demand for fresh products has encouraged research into non-thermal preservation techniques, the use of which results in minimal organoleptic property loss (CHANG et al., 2017).

The technique of heat or thermal treatment is well proven in various food sectors: from bakery and dairy to fruits and vegetables. The process generally involves heating of foods at a temperature between 75 and 90º C or higher with a holding time of 25–30 s. The heating of foods reduces the pathogens. However, extensive research has also concluded nutrient losses, energy wastages, flavor changes and reduction in the food matrix (SRIDHAR et al., 2021).

High-pressure processing (HPP) is a non-thermal processing technology that uses pressure instead of high temperature heating to inactivate microorganisms and enzymes, while also reducing the damage to the nutrients, texture, and appearance (HU; WANG; CHEN, 2020) . In the food industry, treatments of 500–600 MPa have yielded good quality and safety of food products thus 400 and 600 MPa for 3 min were selected for low (HPP1) and high intensity (HPP2) treatments, respectively (WIBOWO et al., 2019) .

A study concluded that, the commercial production of coconut water has applied a heat treatment preservation process, and it eliminates the delicate flavor along with the microbes. From promoting product differentiation perspective, high pressure process treated fresh-like coconut water could be a competitive option. There is no doubt that economic effectiveness of high-pressure process should be considered as well, and microbiological shelf-life stability and sensory properties of this process treated coconut water should be further optimized in future product development (MA et al., 2019).

Phytonutrients are plant-derived substances that play a key role in maintaining human health, especially in disease prevention. These substances based on phytochemicals from fruit and vegetables have recently become increasingly popular due to consumer awareness of their health benefits. This broad group of natural substances includes phenolic compounds and carotenoids, showing considerable antioxidant activity(TREMLOVA et al., 2021).

The objective of this study was to compare the impact of heat treatment and high hydrostatic pressure on the microorganisms, physicochemical properties, color, antioxidant capacity and carotenoids content in fruit beverages with coconut water.

2. MATERIALS AND METHODS

2.1 SAMPLES

The formulation of fruit beverages with coconut water was defined according to a previous study (Chapter II). The formula chosen for application in this study was the one identified in the response surface methodology as the optimized formula. According to the results obtained, it was concluded that the optimized formulas of watermelon with coconut water and guava with coconut water contain 40g of fruit, 30g of coconut water and 30g of apple juice per 100 ml of beverage in their composition.

The samples with the application of technologies were defined according to the results obtained in chapters III and IV. The configuration and denomination are shown in table 1 and 2, considering the parameters and technology applied.

Table 1. Temperature and time parameters of fruit beverages with coconut water uncoded and coded.

Code	Samples	Parameters	
		Temp. (ºC)	Time (sec.)
HT GControl	Guava + Coconut Water	0	0
HT G1	Guava + Coconut Water	124-126	17
HT G2	Guava + Coconut Water	104-106	17
HT MControl	Watermelon + Coconut Water	0	0
HT M1	Watermelon + Coconut Water	124-126	17
HT M2	Watermelon + Coconut Water	107-109	17

Table 2. Pressure and time parameters of fruit beverages with coconut water uncoded and coded.

Code	Sample	Parameters	
		Pressure (MPa)	Time (min.)
HHP GControl	Guava + Coconut Water	0	0
HHP G1	Guava + Coconut Water	200	4
HHP G2	Guava + Coconut Water	400	12
HHP MControl	Watermelon + Coconut Water	0	0
HHP M1	Watermelon + Coconut Water	200	4
HHP M2	Watermelon + Coconut Water	400	12

2.2 HEAT TREATMENT PROCESS

The processing of fruit beverages with coconut water by heat treatment was carried out in HTST - "high temperature, short time" - model HT 122 (OWVE) equipment with a flow capacity of 10 liters/h and a maximum temperature of 165ºC.

The process parameters and the evaluated samples are described in table 1.

After heat treatment, the beverages were filled in 300 ml PET bottles, sealed, capped and the bottles inverted for 3 minutes. After the inversion time, they were immersed in an ice bath. The samples were removed from the ice bath after 20 minutes and stored at 4 ± 2°C until the analysis was performed. The heat treatment were carried out at the Coca-Cola Brasil Pilot Plant, Rio de Janeiro

2.3 HIGH HYDROSTATIC PRESSURE PROCESS

The processing of fruit beverages with coconut water by HHP was carried out in a Stansted Fluid Power equipment (S-FL-850-09-W, England) with a capacity of 250 ml and a maximum nominal operating pressure of 900MPa. The mixture of water and ethanol (30/70 v/v) was used as a pressurization medium. The samples were previously packaged in polyethylene bags (50 mL), which were vacuum sealed.

The process parameters and the evaluated samples are described in table 2.

After processing, the samples were immediately and stored at 4 ± 2°C until the analysis was performed. The tests were carried out at Pilot Plant II of Embrapa Agroindústria de Alimentos, Rio de Janeiro.

2.4 PHYSICOCHEMICAL CHARACTERIZATION

The pH, titratable acidity (TA) and total soluble solids (TSS) content of the different processing of fruit beverages with coconut water were determined. The pH and TA were determined using an automatic titrator equipment - model 916 Ti-Touch - (Metrohm, Switzerland), calibrated with pH 4.0, 7.0 and 10 buffer solutions. TA was determined by titration with hydroxide of sodium (NaOH) 0.1 N, using 1g of sample. The results found were expressed as percentage of citric acid. The TSS content was determined at room temperature using a BS digital refractometer, model RFM340. Results were expressed in °Brix. The physicochemical analyzes of pH, TA and TSS were performed according to AOAC (2010).

2.5 COLOR MEASUREMENTS

The instrumental color analysis of the different treatments of fruit beverages with coconut water was performed using a CR 400 colorimeter (Konica Minolta, Tóquio, Japão) with CIELAB scales. The parameters measured were lightness (L*) on the scale from 0 (black) to 100 (white), (a*) on the green scale (-80 to 0) to red (0 to +100) and (b*) on the scale from blue (-100 to 0) to yellow (0 to +70). Tests for each sample were conducted in triplicate and the values were averaged. These values were then used to calculate hue degree (h0=arctangent [b*/a*]), chroma $[C=(a^{*2}+b^{*2})^{1/2}]$, which is the intensity or color saturation, and ΔE, total differences of color. The total color difference (ΔE) was calculated using the following formula. $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.6 MICROBIOLOGICAL ANALYSIS

The analyzed samples followed the recommendations established in the Brazilian legislation for juices and other beverages submitted to technological processes for microbial reduction, which require refrigeration (BRASIL, 2019). The microorganisms considered were, *Salmonella*/25mL, *Enterobacteriaceae*/mL and Molds and Yeasts/mL. The analyzes were performed according to AOAC (2010) in an external private laboratory and the results were expressed in CFU/mL (molds and yeasts and *Enterobacteriaceae*) and absent for *Salmonella.*

2.7 ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS

2.7.1 Determination of total phenolic compounds

The analysis of total phenolic compounds was performed according to the Folin-Ciocalteau spectrophotometric method (SINGLETON, V. L.; ORTHOFER, 1999).The determination was performed using aliquots of the obtained extracts, which were later pipetted and added with the Folin-Ciocalteau reagent. The mixture was left to stand for 3 to 8 minutes. Then 4% sodium carbonate was added and kept for 2 hours, protected from light. After this period, the reading was performed with absorbance of 750nm using spectrophotometer (SpectraMax i3X, Molecular Devices). The results were compared with the standard gallic acid curve and expressed in mg of gallic acid (GAE) per 100g of sample.

2.7.2 DPPH radical scavenging activity Assay

The measurement of DPPH radical scavenging activity was performed according to an adaptation of the methodology described by BRAND-WILIAMS, W.; CUVELIER, M.E.; BERSET, (1995).To evaluate the antioxidant activity, samples at three concentrations in triplicate were added for reaction with the stable radical DPPH in a methanol solution. In the radical form, DPPH has a characteristic absorption at 515 nm, which disappears after reduction by hydrogen stripped from an antioxidant compound. The radical reduction of DPPH was measured by reading the absorbance at 515 nm at 30 minutes of reaction using spectrophotometer (SpectraMax i3X, Molecular Devices). Trolox (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) standard was used. Antioxidant activity was expressed in µmol of trolox per gram of sample.

2.7.3 TEAC Assay

The TEAC method (2,2'-azino-bis 3-ethylbenzothiazolin 6-sulfonic acid) was adapted from the method described by RUFINO, MARIA S M, (2007). The ABTS•+ radical is formed by a chemical reaction with potassium persulfate in a stoichiometric ratio of 1:0.5. Once formed, the ABTS•+ radical is diluted in ethanol until obtaining an absorbance measurement of 0.70 (± 0.02) at a wavelength of 734 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). Three different volumes of beverage samples, in triplicate, reacted with the ABTS•+ radical for 6 min. A standard curve with Trolox solutions and the antioxidant activity were expressed in TEAC, Trolox equivalent antioxidant capacity (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) in µmol of trolox per gram of sample.

2.7.4 FRAP Assay

The FRAP assay was done according to ABREU et al., (2019). The FRAP reagent was prepared using 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. 2.7 ml of FRAP reagent at 37ºC was added with 90 µl of sample extract and 270 µl of distilled water. After 30 min in warm bath, the readings of samples were taken at 595 nm using a spectrophotometer (SpectraMax i3X, Molecular Devices). The ferrous sulfate was used to the standard curve and the results were expressed µM ferrous sulfate per gram of sample

2.8 DETERMINATION OF CAROTENOIDS

The determination of total carotenoids, β-carotene and lycopene was performed by HPLC, according to PACHECO et al., (2014) with some adaptations. In the extraction of carotenoids, about 5g of each sample was used and the extraction method was carried out according to the procedure described by RODRIGUEZ-AMAYA, (2001). Profiles of the carotenoids were determined in an acetone extract by HPLC using a Waters TM HPLC system, controlled by the Empower software program with the column oven at 33 °C and photodiode array detector (PDA). Carotenoids were identified based on their retention times and UV/Vis absorption spectra, compared to the retention times of the carotenoid standards. To calculate the concentration of carotenoids in the samples, the standard curve was used and the results obtained were expressed in µg/100 g of sample.

2.9 STATISTICAL ANALYSIS

The physicochemical characterization, antioxidant capacity and carotenoids were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at a 5% probability level and p-values lower than 0.05 were considered significant. GraphPad Prism 5.0 was used. Results were expressed as mean ± standard deviation

3. RESULTS AND DISCUSSIONS

3.1 PHYSICOCHEMICAL RESULTS OF FRUIT BEVEVERAGES WITH COCONUT WATER IN DIFFERENT TECHNOLOGIES

According to table 3, it was observed that total soluble solids (TTS ºBrix), titratable acidity (AT% citric acid) and pH do not present a significant difference ($p>0.05$) considering the same technology or heat treatment (HT) or high pressure hydrostatic (HHP) for watermelon beverages with coconut water. Except the HHP M2 (8.45±0.005) sample which is significantly different than the HHP M1 (8.73+0.016) sample in the TSS parameter.

Table 3. Physicochemical results for watermelon beverage with coconut after HT and HHP process

Parameters	HT M1	HT M2	HHP M1	HHP M2
TSS (ºBrix)	8.28±0.012[a]	8.29±0.009[a]	8.73±0.016[b]	8.45±0.005[c]
TA% citric acid	0.38±0.041[a]	0.38±0.016[a]	0.24±0.002[b]	0.28±0.010[b]
pH	4.54±0.009[a]	4.52±0.002[a]	4.40+0.011[b]	4.38±0.009[b]

Different letters in same line indicate that the values are statistically significant ($p<0.05$) by Tukey test

Comparing the technologies, the samples showed a significant difference ($p<0.05$), which can be explained considering that they were made with different fruits and periods.

In the study on effects of high-pressure processing and thermal pasteurization on quality of jaboticaba was observed the pH values for the thermal process and three high pressure processes were the same (3.31) at day 0. No detectable changes were found for the acidity of the thermal process and three high pressure processes from the beginning to the end of storage. The result for soluble solids was 12.97 Brix in the control, and there were no significant between-group effects for the thermal process and three high pressure processes (HU; WANG; CHEN, 2020).

The physicochemical results of the guava beverages with coconut water (table x) showed that there was no significant difference between the samples of the same technology, but when we compared the different technologies, all the analyzes showed a significant difference.

Table 4. Physicochemical results for guava beverage with coconut after HT and HHP process

Parameters	HT G1	HT G2	HHP G1	HHP G2
TSS (ºBrix)	8.19±0.022[a]	8.19±0.019[a]	8.82±0.019[b]	8.79±0.041[b]
TA% citric acid	0.26±0.009[a]	0.28±0.014[a]	0.39±0.017[b]	0.40±0.008[b]
pH	4.23±0.002[a]	4.21±0.011[a]	3.92±0.011[b]	3.85±0.006[c]

Different letters in same line indicate that the values are statistically significant ($p<0.05$) by Tukey test

A study revealed that high pressure process (600MPa/3 min) and thermal process (90ºC/60s) had no significant impact on the soluble solid content of grape juice. In the HPP, the total titratable acidity was maintained in the range 0.76–0.84% during storage, which is like that seen in the HT. No significant pH changes were detected in the HPP- and HT during 20 days of storage (CHANG et al., 2017).

3.2 COLOR RESULTS OF FRUIT BEVEVERAGES WITH COCONUT WATER IN DIFFERENT TECHNOLOGIES

The color results of watermelon beverages with coconut water in different technologies can be observed in table 5.

The a* value, showed red–green, when $a^* > 0$, the sample tended to be red, and when $a^* < 0$, the sample tended to be green. It was observed in the samples with watermelon that the parameter a* there was no significant difference ($p<0.05$) in the samples HHP Mcontrol and HHP M2, indicating that higher pressures can maintain the reddish hue of the beverages. In the HT M2 sample, a negative value (-1.04) was observed, indicating that the sample has a greenish hue compared to the other samples.

The ΔE value is the total color difference between a sample and the control demonstrated that the sample with the highest difference in color was HT M2 (ΔE= 14,42), which indicates that temperature can impact the color and the sample presents a very great difference compared to the control. According to a study on color changes, values of ΔE* between 0 and 0.2 indicate an imperceptible color difference, 0.2–0.5 for a very small difference, 0.5–1.5 for a small difference, 1.5–3.0 for distinct, 3.0–6.0 for very distinct, 6.0–12.0 for great and values >12 for a very great difference (SILVA; SILVA, 1999).

In other study was observed that thermally processed smoothies exhibited the largest degree of color change (ΔE) compared to fresh samples. ΔE values were higher ($p < 0.001$) for thermally processed (ΔE = 5.42±0.69) samples than HHP (ΔE= 2.30±0.50) process (KEENAN et al., 2012).

Table 5. Color results for watermelon with coconut beverages after HT and HHP process

	Watermelon with coconut water beverage					
	Color					
Samples	**L***	**a***	**b***	**Chroma**	**Hue angle**	**ΔE**
HT M1	41.96±5.67^{a}	1.42±1.69^{a}	6.67±1.75^{ac}	7.00±1.47^{a}	76.05±15.51^{a}	3.94
HT M2	54.21±1.38^{a}	-1.04±0.16^{a}	7.36±0.87^{ac}	7.43±0.85^{a}	98.10±1.72^{a}	14.42
HHP M1	37.48±2.68^{b}	6.87±0.93^{b}	8.69±3.92^{ab}	11.35±1.90^{b}	49.40±16.40^{b}	11.38
HHP M2	35.58±2.50^{b}	7.84±0.91^{b}	13.30±1.20^{b}	15.48±0.58^{c}	59.34±5.18^{b}	7.70
HT MControl	32.91±5.49^{b}	8.97±0.75^{b}	4.23±3.13^{c}	10,23+0.92^{b}	24.32±17.06^{c}	0
HHP MControl	44.61±3.31^{a}	5.96±1.50^{b}	13.86±1.5^{b}	15.15±1.25^{c}	66.67±6.63^{b}	0

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

In watermelon samples between the processes, the samples had an increase in hue angle parameters in the HT, indicating that the temperature affects the intensity, the temperature changes the hue to a reddish brown, the presence of apple juice and coconut water can further affect this parameter. LIU et al., (2021) concluded in their study that the degree of browning of HT watermelon juice was particularly obvious. It was well known that there was lycopene in watermelon, and lycopene was sensitive to environmental factors such as oxygen, high temperature, and light, and it was prone to be degradation and isomerization. MA et al., (2019) observed in the study on comparison of coconut by HPP and HT process the color deterioration was attributed the increasing hue angle in HT process to the destabilization of emulsion a protein precipitation.

For color parameters, small differences were found between treatments in L* values at day 0, with a minimum of 37.0 for HHP samples and a maximum of 41.5 for HT samples. HT produced a small increase in hue angle that maintained during the whole storage period and that can be associated with rust-brown tonalities (which agrees with the sensory results) (PICOUET et al., 2016).

The color results of guava beverages with coconut water in different technologies can be observed in table 6.

For guava compared between the processes, the guava samples had a decrease in a* parameter in the HT and kept in the HHP, indicating that the temperature affects the reddish hue of the sample. Evaluating the hue angle of the HT samples, a significant increase was observed when compared to the samples with HHP, indicating that the temperature changes the hue to a reddish brown. Brown pigments can also be generated through nonenzymatic activity, such as Maillard reactions during the beverages heat treatment. Considering ΔE, the smallest difference was obtained in the HHP G1 (0.17), the sample with the lowest pressure and time (200 MPa/4min.) indicate an imperceptible color difference and the smallest impact on color from processing.

Table 6. Color results for guava with coconut beverages after HT and HHP process

	Guava with coconut water beverage					
	Color					
Samples	**L***	**a***	**b***	**Chroma**	**Hue angle**	**ΔE**
HT G1	47.83±1.06[a]	11.28±0.71[a]	10.10±2.11[a]	15.17+1.94[a]	41.51+4.05[a]	15.10
HT G2	49.63±0.69[a]	9.81±0.55[a]	14.69±1.90[b]	17.67+1.88[a]	56.15+1.90[b]	10.18
HHP G1	41.94±0.27[b]	10.60±0.30[a]	6.45±1.89[c]	12.47±0.63[a]	30.88±6.53[c]	0.17
HHP G2	41.11±0.14[b]	10.15±0.26[a]	6.13±0.47[c]	11.86±1.26[a]	31.12±1.11[c]	1.43
HT GControl	40,60±0.31[b]	14,15+0.48[b]	4.27+2.09[c]	14,86+1.12[a]	16.46+6.94[d]	0
HHP GControl	43.00±0.88[b]	10.91±0.67[a]	4.92±1.51[c]	12,01±1.06[a]	24.02±6.03[c]	0

Different letters in same column indicate that the values are statistically significant ($p<0.05$) by Tukey test

In a study on comparison between HPP and HT in whole concord grape puree was identified as a consequence of the increased b* value, the Browning Index (BI) in HT-treated samples was significantly higher than that in the HPP treated and control samples on day 1 and during storage. The color change (ΔE) of both HPP- and HT-treated Concord grape purees remained consistent after a refrigerated storage period of 4 months, suggesting that the unique grape puree matrix containing grape skin and seeds was able to preserve the fresh-like color despite of the possible deleterious enzyme activity in HPP-treated Concord grape puree (LI; PADILLA-ZAKOUR, 2021).

The biggest color difference was observed in the heat-treated sample HTG1 (ΔE= 15.10), comparing with the technologies and the fruits. Indicating that heat treatment significantly impacts the color of beverages. Observed similar results, the samples treated under both conditions exhibited significant color differences (ΔE) in comparison with the untreated beverage ($p<0.05$) in the aftermath of processing. The changes were more prone to thermal treatment, while HHP processing caused minor variations in the drinks' colors (SALAR et al., 2021).

3.3 MICROBIOLOGICAL RESULTS IN DIFFERENT TECHNOLOGIES

The microbiological results of fruit beverages with coconut water in different technologies can be observed in table 7

The results of the microbiological analyzes observed for samples of fruit beverages with coconut water in heat treatment and high hydrostatic pressure technologies are in accordance with Brazilian legislation, that is, the conservation methods used maintained microbiological stability of the samples. The HHP M1 sample is at the limit of the legislation, containing 100 CFU/mL in the analysis of molds and yeasts. Presence of molds and yeasts may be an indication of contamination in the environment.

Table 7. Microbiological results of fruit beverages with coconut water in HT and HPP process, compared with legislation

Samples	Molds and yeasts (CFU)	*Salmonella spp*	*Enterobacteriaceae* (CFU)
HT G1	<10	absent	<10
HT G2	<10	absent	<10
HHP G1	<100	absent	<10
HHP G2	<100	absent	<10
HT M1	<10	absent	<10
HT M2	<10	absent	<10
HHP M1	100	absent	<10
HHP M2	<100	absent	<10
Legislation	100/mL	absent 25 ml	10/mL

A study on use of High-Pressure Processing to improve the safety and quality of raw coconut water observed, the HPP at 593 MPa for 3 min is effective in eliminating inoculated strains of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes*, this condition gave microbiologically stable (<2-logs) product during storage at 4 °C for 120 days with no off odors and similar taste to fresh coconut water. In contrast, non-HPP treated coconut water showed elevated microbial counts, gas production, cloudiness, and off-odor within two weeks of storage at 4 °C (RAGHUBEER et al., 2020).

(IU et al., (2016) exhibited in their study that the HPP and HT processes a significant inactivation effect on natural microorganisms in clear cucumber juices. The counts of the Total Aerobic Bacteria in clear cucumber juices after HPP treatment were significantly reduced from 3.88±0.13 to 0.70±0.10 log cycles, and after HT process to 0.30 ± 0.17 log cycles. The yeast and molds were completely inactivated by HPP and HT processes in this study, and their levels were always below the detection limit during 20 days of refrigerated storage at 4 °C.

In the review on the effect of high-pressure processing on the microbial inactivation in fruit, the criteria for microbial inactivation have been reported. For Red fruits with orange, banana, and lime, 350 MPa/7 min. was used to reduce the count of Aerobic mesophilic bacteria, Psychrotrophic bacteria, Yeasts/Molds and Enterobacteria (DAHER; LE GOURRIEREC; PÉREZ-LAMELA, 2017) . The samples HHP M2 and HHP G2 were processed with 400MPa/12 min., condition adequate for reducing the microbiological counts according to the study above.

MA et al., (2019) in the study of the quality attributes of coconut water in the process of high pressure and heat treatment, observed, the counts of total aerobic bacteria and molds and yeasts in high pressure process groups and molds and yeasts in heat treatment group are undetectable, and the counts of total aerobic bacteria in heat treatment the coconut water is 0.597 ± 0.02 log CFU/ml right after processing.

3.4 ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS IN DIFFERENT TECHNOLOGIES

3.4.1 Total phenolic compounds (TPC) of fruit beverages with coconut water in different technologies

The figure 1 shows the results of TPC of fruit beverages with coconut water in different technologies.

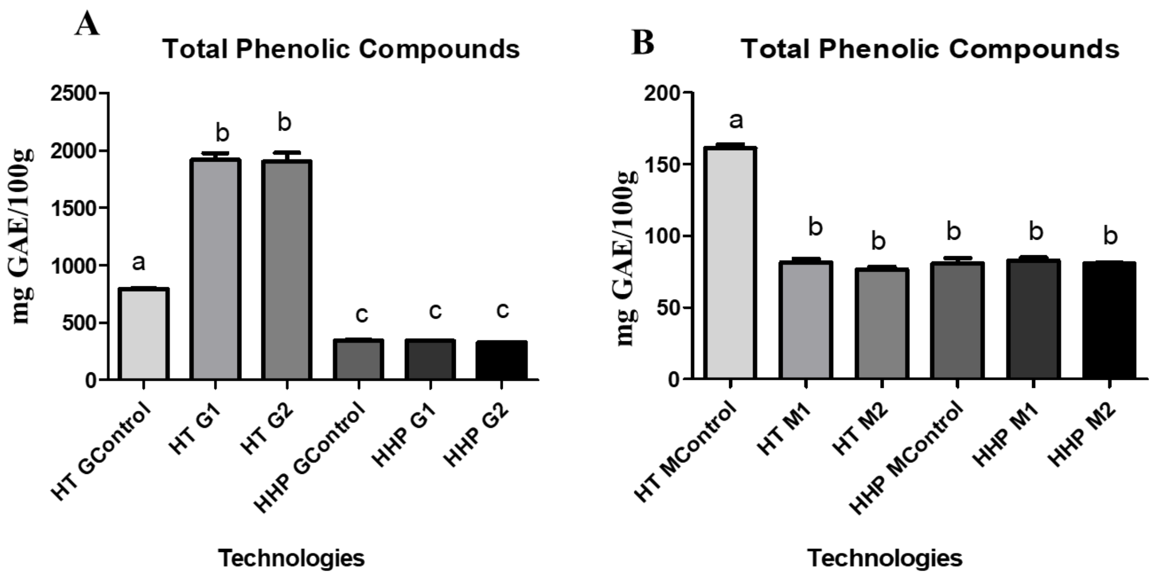

Figure 1. Values of Total Phenolic Compounds (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

In figure 1A, it was shown that in the high-pressure treatment the samples had no significant difference ($p>0.05$) for the TPC. In the case of samples with heat treatment HTG1(1915.06±100.74 mg GAE/100g) and HTG2 (1901.74±130.67 mg GAE/100g), an increase in the TPC content was observed compared to the sample without heat treatment HT Gcontrol (795.95±9.16 mg GAE/100g). Indicating that temperature had a positive impact on the result. LI; PADILLA-ZAKOUR, (2021) observed similar results in their study on whole concord grape puree, after HPP and HT treatment, the TPC value was significantly ($p<0.05$) higher than that of control puree from 3.0 to 3.8 (HPP) and 3.6 (HT) mg/g as GAE.

For the watermelon samples, a significant difference was observed on figure 1B, only for the HT Mcontrol (161.47±4.10 mg GAE/100g) sample, compared to the other samples with HT and HHP processes. The HT M1 (81.53±4.31 mg GAE/100g) and HT M2 (76.74±3.26 mg GAE/100g) samples had a significant reduction after heat treatment.Depending on the composition of the phenolic compounds, an increase or decrease may occur considering the processing conditions.

A study on effect of thermal processing on free and bound phenolic compounds and antioxidant activities of hawthorn conclued that thermal processing decreased the total soluble phenolic contents significantly ($p<0.05$), while increasing the total insoluble-bound phenolic contents. The effect of thermal processing on the phenolic compounds and antioxidant activities depended on the type of phenolic compounds and processing conditions (LI et al., 2020).

3.4.2 Antioxidant capacity results of fruit beverages with coconut water in different technologies

In figure 2A and 2B it was shown that there was no significant difference between the samples without treatment and with treatment in both technologies and for guava and watermelon fruits. This indicates that the treatments did not affect the antioxidant capacity of the beverages, considering the DPPH method.

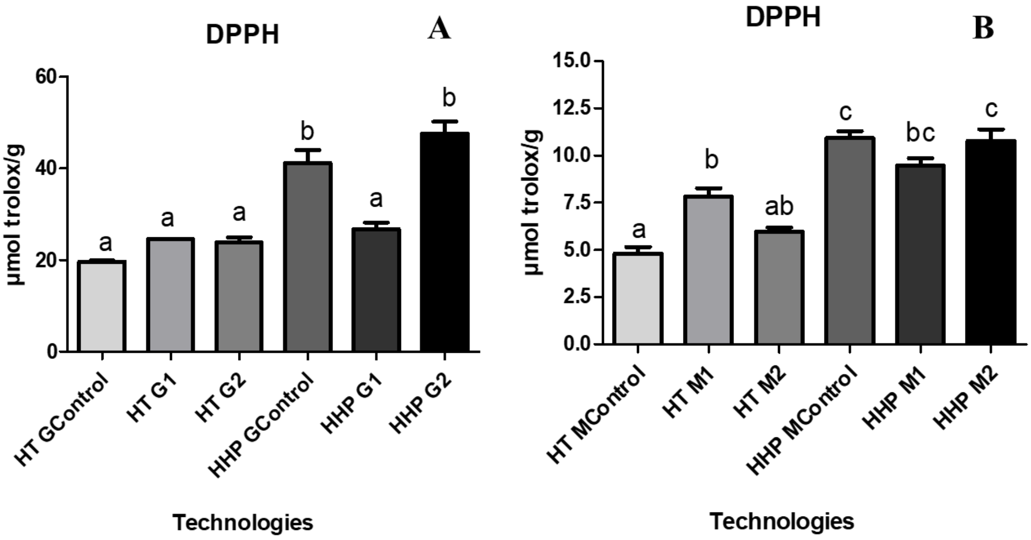

Figure 2. Antioxidant capacity results in the DPPH method (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

A study reported of fresh, thermal (end-point temperature 70ºC > 10 min) and high hydrostatic pressure (HHP -450 MPa/20ºC/5 min and 600 MPa/20 ºC/10 min) processed fruit smoothies indicated the thermal processing induces degradation of bioactive compounds.The same study observed the fresh smoothies was 277.89 mg TE/100g in DPPH method, the levels were similar to HHP-450 samples (KEENAN et al., 2012).

Figure 3A revealed that there was no significant difference ($p>0.05$) between the samples of guava beverage with coconut water in both processes, considering the TEAC method. Figure 3B indicated that there was a significant difference ($p<0.05$) between the HT and HHP processes in the samples of watermelon beverage with coconut water, but not between the samples considering the same process and method

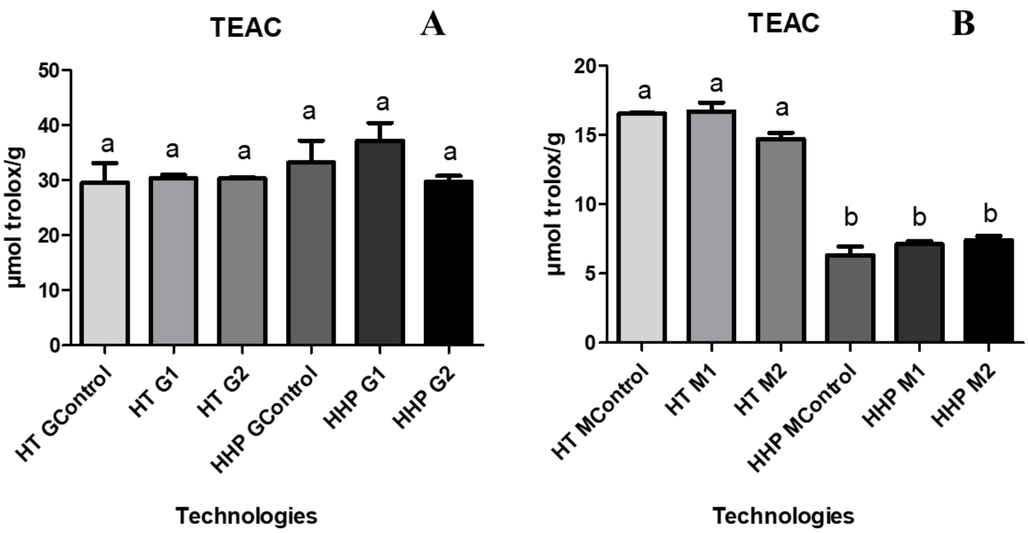

Figure 3. Antioxidant capacity results in the TEAC method (Mean+SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. . Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

No significant differences were found between HPP (600 MPa/3 min) and HT (63ºC/ 3 min) samples for antioxidant activity in ABTS and DPPH methods compared to the control sample in the study of whole concord grape puree (LI; PADILLA-ZAKOUR, 2021).

Figure 4A revealed that there was no significant difference ($p>0.05$) between the samples of guava beverage with coconut water in both processes. Figure 3B indicated that there was a significant difference ($p<0.05$) between the HT and HHP processes in the samples of watermelon beverage with coconut water, but not between the samples considering the same process.

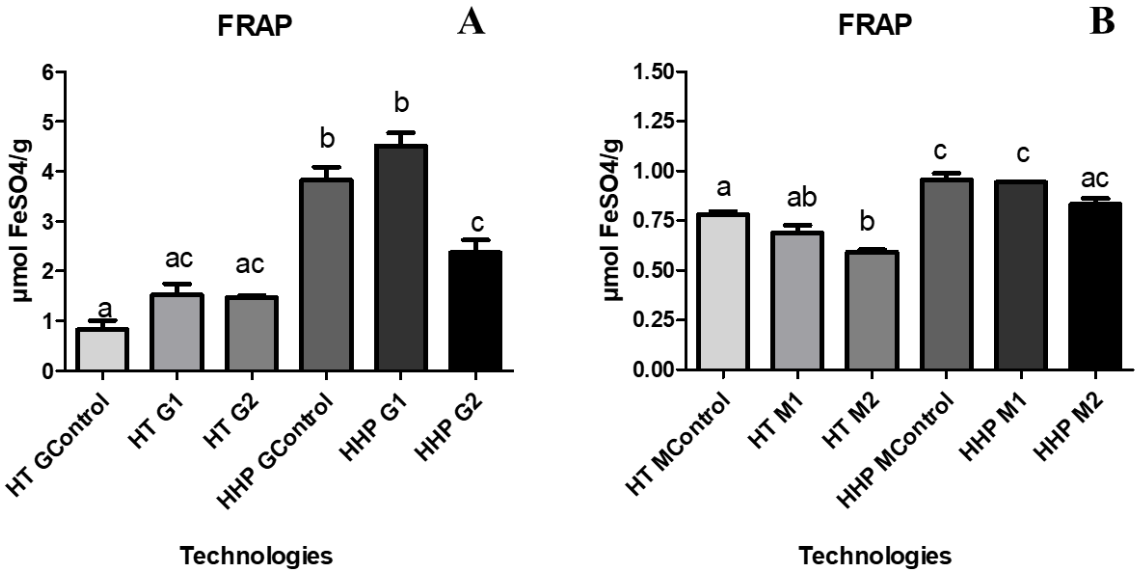

Figure 4. Antioxidant capacity results in the FRAP method (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. . Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

The mixture composed of purple passion fruit (67 %), green passion fruit (17 %) and carrot (17 %) juices treated at 500 MPa and 25 °C for 250 s showed the highest antioxidant capacity expressed as Trolox equivalent (899.63 and 1281.69 mg/L by FRAP and DPPH assay, respectively). The thermal treatments affected the antioxidant capacity in the mixtures, while the high hydrostatic pressure preserved it (MARENGO-OROZCO; TARAZONA-DÍAZ; RODRÍGUEZ, 2020).

In the study on the effect of thermal treatment for tropical juices the results for both the non-thermal and thermal (85ºC/30 s) juices showed high antioxidant activity with similar values ($p<0.05$) measured by ABTS and FRAP, indicating that heat treatment did not impact antioxidant activity in these two methods (WURLITZER et al., 2019).

3.5 CAROTENOIDS RESULTS IN DIFFERENT TECHNOLOGIES

The total carotenoids content in fruit beverages with coconut water are shown in figure 5A and 5B.

The samples submitted to heat treatment had a significant decay when compared to the control sample in both guava and watermelon.

In the high-pressure process, an increase in the of total carotenoids content was observed in the HHP G2 (2277±91 µg/100g) sample when compared to the HHP Gcontrol (1800±83 µg/100g) sample. In the watermelon samples, there was a decrease in the HHP M1 (951±37 µg/100g) when compared to the HHP Mcontrol (1296±48 µg/100g) sample.

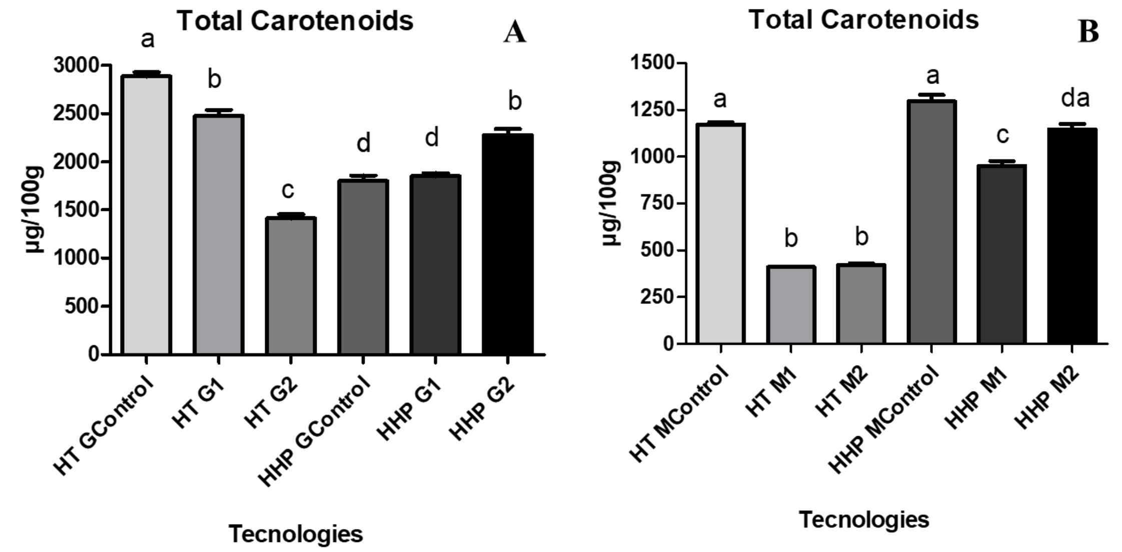

Figure 5. Total carotenoids results (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. . Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

Comparing the two fruits, guava stands out for the higher amount of total carotenoids when compared to watermelon. ZHAO; LV; GU, (2013) in the studies on carotenoids in watermelon flesh identified 46.14±1.74 mg/kg of total carotenoids in watermelon from Zaohua - red cultivar.

Regarding the β-carotene results observed through figures 6A , that the HT G1 (207±9 µg/100g) sample there was significant difference ($p<0.05$) compared to the HT Gcontrol (182±8 µg/100g) sample. In the case of the watermelon (figure 6B), the samples submitted to heat treatment had a decay when compared to the sample without treatment.

In the samples submitted to the HHP process, a stability was observed in guava beverages between the HHP Gcontrol and HHP G1 samples and an increase in the amount of β-carotene when compared to the HHPG2 sample.

In the samples submitted to the HHP process, a stability was observed in guava beverages between the HHP Gcontrol and HHPG1 and an increase in the amount of β-carotene compared to the HHPG2 sample. In the case of watermelon, stability was observed in the HHP Mcontrol and HHPM2 samples when compared to the increase observed in the HHPM1 sample.

In the study on the level of carotenoids in foods, 365 µg/100g of β-carotene was observed in watermelons sold in Brazil and 3550 µg/100g of lycopene in the same sample (DIAS et al., 2021).

Comparing fruits, this is the case where watermelon contains the highest amount of carotenoids compared to the guava, in this case β-carotene.ZHAO; LV; GU, (2013) in the studies on carotenoids in watermelon flesh identified of β-carotene 4.56±0.07mg/kg of in Hongyihao – coral cultivar and 4.42±0.06 mg/kg in Zaohua - red cultivar.

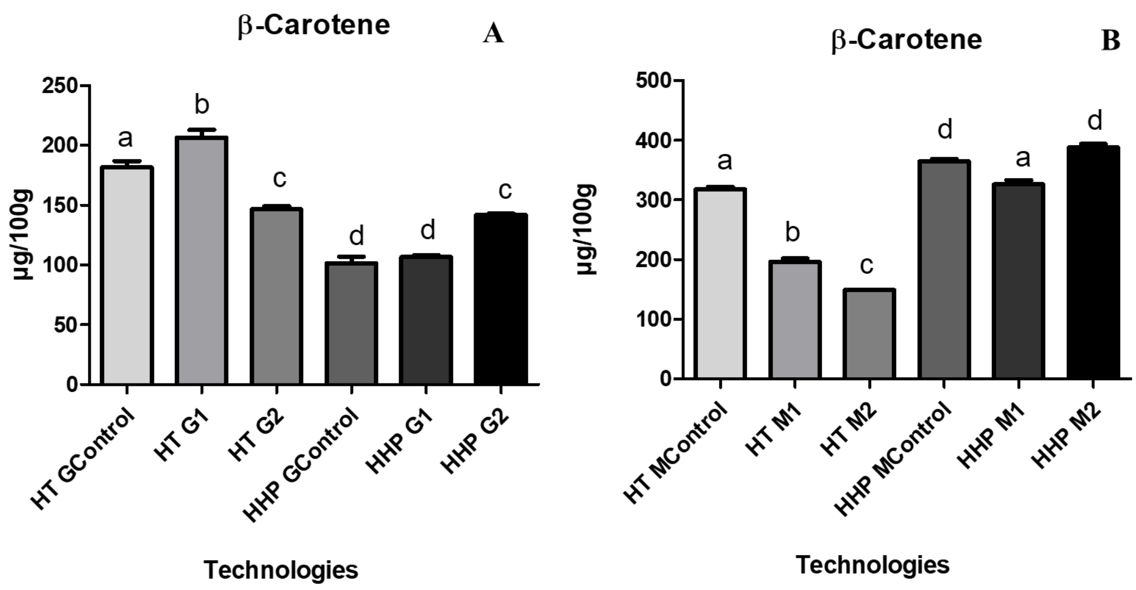

Figure 6. β-carotene results (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. . Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

The quantification of lycopene in the different technologies and for the different fruits can be seen in figure 7A and 7B.

It was observed that the samples submitted to heat treatment had a significant reduction in the amount of lycopene for both guava and watermelon when compared to the control sample.

Regarding the samples submitted to the HHP process, there was no significant difference between the guava beverages samples. There was a decrease in the amount of lycopene in HHP M1 (350±23 µg/100g), compared to the HHP Mcontrol (611±45 µg/100g). The presence of various macromolecules in the juices could play protective role to enhance pressure resistance of lycopene as compared to standard lycopene solution.

YETENAYET; HOSAHALLI, (2015) observed in their study that watermelon juice subjected to high pressure retained more lycopene (99.3% at 400MPa/60min) compared to watermelon juice subjected to heat treatment (95.8% at 90ºC/15 min.).

I was observed in a study that the total lycopene concentration of the treated watermelon juice was statistically similar after the high-pressure treatment at 300 MPa and 600 MPa. The HPP treatment was effective to hold the total lycopene concentration of the treated watermelon juice as the control compared to the thermal and UV-C treatments (ZHANG, 2011).

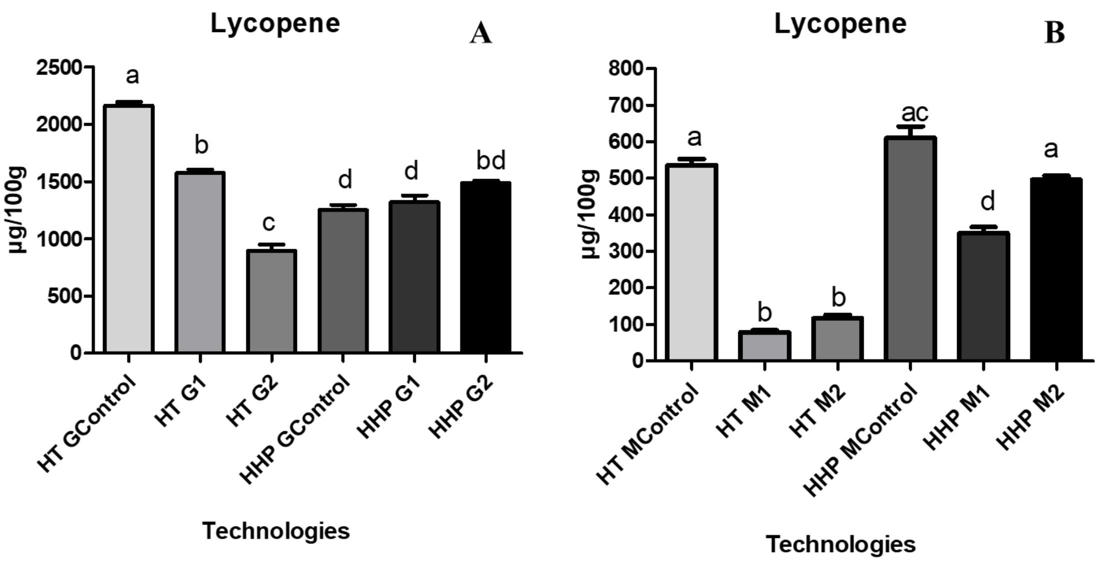

Figure 7. Lycopene results (Mean±SD) of fruit beverages with coconut water. (**A**) Guava beverages with coconut water in HT and HHP process and (**B**) Watermelon beverages with coconut water in HT and HHP process. . Different letters indicate that the values are statistically significant ($p<0.05$) by Tukey test

Comparing guava and watermelon, HT Gcontrol (2161±47 µg/100g) had the highest amount of lycopene compared to HT Mcontrol (536±23 µg/100g).

LIANA MARIA et al., (2014) identified in their study a amonunt different from our study. In watermelon fresh fruit was observed a 4868 µg/100g of lycopene whend comparade to guava fresh fruit 1520 µg/100g.

A study observed that watermelon contained the highest content of lycopene (144.27±0.001 mg/kg) when compared to the other fruits and vegetables as tomato that contain lycopene (104.699±0.000 mg/kg) (SUWANARUANG, 2016).

4. CONCLUSION

The application of HHP and HT in different conditions observed a stability in the physicochemical and microbiological quality in fruit beverages with coconut water. The highest color differences were observed in beverages subjected to heat treatment. The amount of total phenolic compounds had an increase in the guava samples and a decrease in the watermelon samples after HT and a retention in the samples with HHP. In the analysis of the antioxidant capacity, there was a retention in the 3 methods studied both in guava and in watermelons in all treatments. The evaluation of carotenoids concluded that HT impacts by decreasing the amount while HHP retains total carotenoids, β-carotene and lycopene. Due to these results, HHP could be considered an effective alternative to conventional HP in the food industry for the production of high functional quality fruit-based beverages. Other evaluations such as financial impacts and product objectives in the market must be considered to define the best technology to be applied.

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CONSIDERAÇÕES FINAIS

O presente estudo forneceu informações sobre o desenvolvimento e efeitos de diferentes métodos de conservação nas bebidas de frutas com água de coco. Foram desenvolvidas bebidas de goiaba com água e coco e bebidas de melancia com água de coco através de um planejamento fatorial e submetidas a análise sensorial para avaliação da fórmula otimizada. As fórmulas otimizadas foram submetidas a tratamento térmico e alta pressão hidrostática, análises físico-química, microbiológica, análise de cor, compostos fenólicos totais, capacidade antioxidante e quantificação de carotenoides foram performadas e impacto entre as frutas e processos foram avaliados.

As bebidas de frutas com água de coco apresentaram uma boa aceitação sensorial, sendo a bebida com goiaba e água de coco a com maior aceitação comparada a bebida de melancia com água de coco.

A aplicação do tratamento térmico demonstrou através dos resultados avaliados um baixo impacto nas características físico-químicas e nutricionais, podendo ser utilizado como método de conservação para bebidas de frutas.

Alta pressão hidrostática através dos resultados obtidos demonstrou ser uma tecnologia emergente que não afeta as características das bebidas e pode ser considerada uma alternativa eficaz na indústria de bebidas funcionais.

Comparando os processos observamos que a alta pressão hidrostática é uma alternativa eficaz ao tratamento térmico, conseguindo inativação microbiana efetiva das bebidas e contribuindo para uma melhor preservação da cor e dos compostos bioativos.

As bebidas de frutas com água de coco submetidas a alta pressão hidrostática é uma alternativa para produtos com apelo funcional, porém avaliação de impacto financeiro e objetivo do produto no mercado deve ser avaliado para definir a melhor tecnologia a ser aplicada.

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APÊNDICES

APÊNDICE A – Artigo aceito

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APÊNDICE B – Artigo submetido

Gmail **Anderson Teodoro <atteodoro@gmail.com>**

[cmbio] Agradecimento pela submissão

1 mensagem

Roberto Paulo Correia de Araújo <revistacmb@gmail.com> 17 de novembro de 2022 14:29
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