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TESE

Whey Drink de Uva Processado por Dioxido de Carbono Supercrítico: Parâmetros de Qualidade e Sensoriais

GABRIELA VIEIRA DO AMARAL

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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GABRIELA VIEIRA DO AMARAL

Sob a Orientação do Professor Doutor
Adriano Gomes da Cruz

e Co-orientação da Professora Doutora
Maria Angela A. Meireles

Tese submetida como requisito parcial para obtenção do grau de **Doutor em Ciências**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Tecnologia de Alimentos.

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____
Adriano Gomes da Cruz, Dr., IFRJ
(Orientador)

____
Rodrigo Nunes Cavalcanti, Dr., UNICAMP

____
Erick Almeida Esmerino, Dr., UFF

____
Maria Ivone Martins Jacintho Barbosa, Dra., UFRRJ

____
Simone Pereira Mathias, Dra., UFRRJ

Dedico este estudo aos grandes amores da minha vida... Meu marido, pais, irmães e sobrinha.

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RESUMO

AMARAL, Gabriela Vieira do. **Whey drink de uva processado por dióxido de carbono supercrítico: parâmetros de qualidade e sensoriais.** 2017. 65p. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2017.

A tecnologia emergente de dióxido de carbono supercrítico (DCSC) vem sendo estudada como agente pasteurização a frio, no entanto, são poucos os estudos disponíveis a cerca da sua eficiência em derivados lácteos. Neste estudo, foram investigados os efeitos do processamento do DCSC por diferentes pressões 14, 16 e 18 MPa (35 ± 2 °C / 10 min) no whey drink de uva, bebida a base de soro de leite e suco de uva, em comparação à pasteurização convencional (tratamento térmico a 72 °C / 15 s). Foram realizadas análises físico-quimicas de pH, acidez titulável, sólidos solúveis totais, compostos fenólicos, antocianinas, atividade antioxidante, atividade inibidora da enzima conversora de angiotensina (ECA) e compostos voláteis. Também foramam alisados a cor, o tamanho de partícula, reologia, estabilidade física, assim como a qualidade microbiológica e analise sensorial das bebidas. Os resultados deste estudo evidenciaram a ausência de diferenças entre os tratamentos nas análises de pH, acidez titulável, sólidos solúveis, antocianinas totais e atividade de DPPH (p> 0,05). Foi observada uma relação direta entre pressão DCSC e atividade inibitória ACE, com 34,63, 38,75 e 44,31% (14, 16 e 18 MPa, respectivamente). Poucas diferenças foram encontratdas no perfil dos compostos voláteis. O processamento das bebidas por DCSC resultou em um produto com menor diâmetro de partícula, menor índice de consistência e uma redução no caráter pseudoplástico em comparação com a bebida tratada pelo processo convencional. Não foi observado efeito de CO_2 de alta pressão nos atributos sensoriais da bebida para os níveis estudados. Os consumidores não encontraram diferenças entre as bebidas tratadas com CO_2 e as bebidas tratadas termicamente. Os resultados confirmam o processamento do DCSC como uma tecnologia promissora para o tratamento não térmico de whey drink de uva disponibilizado uma bebida promotora de saúde e bem-estar.

Palavras-chave: tecnologia emergente, soro de leite, suco de uva.

ABSTRACT

AMARAL, Gabriela Vieira do. **Whey-grape drink processed by supercritical carbon dioxide: quality and sensory parameters.** 2017. 65p. Thesis (Doctor Science in Food Science and Technology). Institute of Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ. 2017.

Emerging supercritical carbon dioxide (SCCD) technology has been studied as a cold pasteurizing agent, however, few studies are available on its efficiency in dairy products. In this study, the effects of SCCD processing by different pressures 14, 16 and 18 MPa (35 ± 2 °C / 10 min) on whey drink, whey drink and grape juice were investigated in comparison To conventional pasteurization (heat treatment at 72 °C / 15 s). Physicochemical analyzes of pH, titratable acidity, total soluble solids, phenolic compounds, anthocyanins, antioxidant activity, angiotensin converting enzyme (ACE) inhibitory activity and volatile compounds were performed. The color, particle size, rheology, physical stability, as well as microbiological quality and sensory analysis of beverages were also smoothed. The results of this study evidenced the absence of differences between treatments in pH, titratable acidity, soluble solids, total anthocyanins and DPPH activity ($p > 0.05$). A direct relationship between SCCD pressure and ACE inhibitory activity was observed, with 34.63, 38.75 and 44.31% (14, 16 and 18 MPa, respectively). Few differences were found in the volatile compounds profile. The beverage processing by SCCD resulted in a product with lower particle diameter, lower consistency index and a reduction in pseudoplastic character compared to the beverage treated by the conventional process. No effect of high pressure CO_2 on the sensorial attributes of the drink was observed for the studied levels. Consumers found no difference between CO_2 treated beverages and heat-treated beverages. The results confirm the processing of SCCD as a promising technology for the non-thermal treatment of grape whey drink made available a health and wellness promoter beverage.

Key Word: Emerging technology, whey, grape juice.

SUMÁRIO

INTRODUÇÃO GERAL

A procura por hábitos saudáveis vem estimulando o consumo de alimentos promotores de saúde, sensorialidade, conveniência, com alto padrão de qualidade, além de operar processos sustentáveis e limpos. Esta tendência estimula a indústria de alimentos na elaboração de novos produtos, que tragam além da praticidade, os aspectos nutraceuticos.

O whey drink de uva pode ser uma maneira refrescante de atender estas necessidades dos consumidores. O whey drink de uva é uma bebida elaborada a base de soro de leite e suco de uva. A proteína do soro de leite é reconhecida pelo seu valor nutricional, como fonte de aminoácidos essenciais, peptídeos bioativos, antioxidantes e imunomopotenciadores. Diversos estudos vêm comprovando são os benefícios à saúde, tais como a eliminação de radicais livres, ação anti-inflamatória, antitumoral, hipotensora, antiobesidade, antidiabética, ação sobre a biossíntese muscular, osteoproteção e radioproteção.

Em paralelo, o suco de uva é apreciado em todo o mundo, não só devido ao seu singular sabor, mas também por ser rico em vitaminas, minerais, fibras, compostos fenólicos bioativos, como antocianinas e proantocianidinas, que são polifenóis antioxidantes e anti-inflamatórios.

É sabido que os tratamentos térmicos convencionais promovem a degradação de nutrientes e compostos bioativos, além de afetar o sabor dos alimentos devido ao processo de cozimento. Por isso, técnicas de tratamentos não térmicos vêm sendo estudadas e aprimoradas visando a produção de alimentos com características sensoriais mais próximas às do produto fresco.

O dióxido de carbono supercrítico (DCSC) é uma tecnologia limpa, reconhecida como environmentally friendly, além do baixo custo de operação, que vem ganhando destaque no tratamento não térmico de alimentos, uma vez que não são expostos aos efeitos nocivos do calor.

Diversos estudos comprovaram a eficiência no processamento por DCSC com a inativação microbiana e enzimática, e ainda, relataram a preservação das propriedades nutricionais e sensoriais de sucos naturais e leite. A tecnologia supercrítica também emerge como alternativa ao processamento a alta pressão hidrostática, que pode atingir até 10.000 bar, enquanto que os tratamentos com DCSC são próximos de 100 bar.

O processamento de alimentos com DCSC utiliza o dióxido de carbono em estado supercrítico (condições críticas de pressão, 7.38 MPa e temperatura, 31.1°C) como agente pasteurizador a frio.

Neste contexto, é importante ressaltar que não foram encontrados na literatura relatos do emprego do processo de DCSC sobre as características físico-químicas de bebidas lácteas do tipo whey-drink.

O processamento do whey drink de uva utilizando tecnologias emergentes não-térmicas é uma interessante alternativa para a disponibilização de alimentos seguros para o consumo, com características nutricionais e sensoriais mais próximas às do produto fresco.

OBJETIVOS

O presente trabalho teve como objetivo geral reunir as principais considerações da tecnologia do DCSC em lácteos e ainda investigar os efeitos desta tecnologia sobre os parâmetros de qualidade e sensoriais do whey drink de uva.

Os **objetivos específicos** foram:

- Investigar os efeitos do processamento do DCSC por diferentes pressões 14, 16 e 18 MPa (35 ± 2 °C / 10 min) no whey drink de uva, bebida a base de soro de leite e suco de uva;
- Em comparação à pasteurização convencional (tratamento térmico a 72 °C / 15s);
- Realizar análises físico-quimicas de pH, acidez titulável, sólidos solúveis totais, compostos fenólicos, antocianinas, atividade antioxidante, atividade inibidora da enzima conversora de angiotensina (ECA) e compostos voláteis; e
- Realizar análises a cor, o tamanho de partícula, reologia, estabilidade física, assim como a qualidade microbiológica e analise sensorial das bebidas.

Esta tese está disposta em formato de capítos (quatro capítulos), os quais são referentes aos artigos publicados. Cada capítulo foi apresentado seguindo as normas das respectivas revistas.

CAPÍTULO I

DIÓXIDO DE CARBONO SUPERCRÍTICO COMO TECNOLOGIA NÃO TÉRMICA NO PROCESSAMENTO DE LEITE E DERIVADOS

Artigo Técnico publicado no site *Milkpoint*

Este artigo técnico foi publicado no site <https://www.milkpoint.com.br/> e está disponível na página do mesmo. O texto apresenta um caráter informativo, como uma nota de jornal e suas referencias estão dispostas ao fim do texto. O tema aborada a tecnóloga emergente do dióxido de carbono supercrítico como agente de pasteurização de leite e derivados, fazendo uso de baixas temperaturas para o tratamento. Possibilitando assim, a manutenção de propriedades nutricionais e sensoriais que são alteradas com a utilização de altas temperaturas, como nos tratamentos convencionais. Portanto, segue abaixo o artigo.

A tecnologia do dióxido de carbono supercrítico (CO_2 – SC) é uma tecnologia usada para diferentes fins e vem ganhando destaque no processamento de leites e derivados. Apesar de ter sido proposta na década de 50, como uma alternativa ao tratamento térmico, esta tecnologia ainda se encontra em fase de pesquisa e aprimoramento.

O termo "supercrítico" refere-se ao estado semilíquido de uma substância quando levada acima de um valor limite de temperatura e pressão, caracterizados como o ponto crítico, que é uma característica única e específica para cada substância. No caso do CO_2, o ponto crítico está em 31,04 °C e 73,8 bar e, para o estado subcrítico, a temperatura ou pressão são abaixo desses valores.

O uso do CO_2 tem várias vantagens, incluindo o fato de ser um gás inerte, disponível em alto grau de pureza, baixo custo, não corrosivo, não inflamável, não explosivo, possuir facilidade de remoção do produto após o uso, e ainda - não há restrições para sua eliminação no meio ambiente.

Além disso, o CO_2 tem uma temperatura e pressão críticas baixas, o que pode ajudar a prevenir a degradação térmica dos componentes dos alimentos, conferindo assim um menor custo de investimento.

Os mecanismos de inativação microbiana que ocorrem com o uso da tecnologia do CO_2- SC atuam de forma complexa e inter-relacionada. Estes principais mecanismos consistem na sua solubilização na fase externa do meio reacional, modificação da membrana celular, redução do pH intracelular, inativação de enzimas, transtorno do equilíbrio intracelular de eletrólitos, remoção dos componentes vitais das células e membranas celulares.

O sucesso da inativação microbiana no leite vai depender dos parâmetros do processo, como a pressão, a temperatura e o tempo de exposição; assim como, o tipo de micro-organismo, a carga microbiana inicial e ainda o teor de gordura e proteína. Estes podem parecer aumentar a resistência bacteriana, atrapalhando a penetração do CO_2 na célula.

Estudos revelam a inativação de micro-organismos patogênicos de interesse na cadeia do leite e derivados, como a *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Brochothrix thermosphacta*, e *Escherichia coli* em leites com diferentes teores de gordura, com um desempenho superior quando temos o leite desnatado.

Reduções nas contagens de micro-organismos deteriorantes, como *Pseudomonas fluorescens* em leite cru foram superiores a pasteurização convencional (72 °C/15 s). Em adição, estudos mostram a redução de bactérias aeróbias em leite cru e leite esterilizado com sistema de microbolhas de CO_2 (MBCO2), demostrando uma melhor eficiência.

A aplicação de CO_2-SC durante todo o processo do tratamento térmico do leite pode reduzir de maneira considerável o tempo requerido para a esterilização ou pasteurização comumente utilizada na indústria láctea, de tal forma a minimizar a degradação térmica de substâncias sensíveis, com destaque para as vitaminas. Contudo, em virtude do número reduzido de estudos, esse efeito sobre a degradação de vitaminas em leite ainda é desconhecido.

Com relação ao processamento de queijos, a estocagem do leite com refrigeração e posterior aplicação do CO_2-SC não provocou efeitos prejudiciais nas propriedades químicas (compostos voláteis e ácidos orgânicos), sendo observado um impacto positivo nos parâmetros tecnológicos intrínsecos dos produtos como: redução do tempo de coagulação e firmeza da coalhada.

O CO_2-SC pode ainda afetar as proteínas do leite devido a sua acidificação e propriedades de solvatação, pois o ácido carbônico formado se liga com os íons cálcio podendo instabilizar internamente as micelas de caseína. Isso pode ser considerado um obstáculo no processamento do leite fluido sendo, contudo, um ponto positivo durante o processamento de derivados como queijos e leites fermentados.

Finalmente, com relação aos aspectos sensoriais, são escassos os estudos do leite tratado com SC-CO_2. Porém, há relatos de maior aceitação do leite processado por esta nova tecnologia quando comparado ao leite pasteurizado-HTST por consumidores.

De forma geral, estudos adicionais ainda devem ser realizados a fim de avaliar todos os efeitos sobre da tecnologia de dióxido de carbono supercrítico sobre os parâmetros de qualidade do leite e produtos lácteos. No entanto, os bons resultados nos diversos estudos já disponíveis demonstram que a técnica é uma promissora alternativa ao tratamento térmico convencional. Dessa forma, o potencial desta tecnologia ainda está longe de ser esgotado.

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CAPÍTULO II

DAIRY PROCESSING USING SUPERCRITICAL CARBON DIOXIDE TECHNOLOGY: THEORETICAL FUNDAMENTALS, QUALITY AND SAFETY ASPECTS

Artigo de Revisão publicado no periódico *Trends in Food Science & Technology*

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RESUMO

Antecedentes: Os processamentos de alimentos não térmicos são configurados como uma alternativa interessante para a indústria de alimentos devido ao aumento da retenção de nutrientes e mudanças sensoriais mínimas nos produtos processados.
Âmbito e abordagem: o objetivo desta revisão é abordar o potencial da tecnologia de dióxido de carbono supercrítico, enfatizando o processamento de leite e lácteos, incluindo os aspectos históricos, as principais vantagens, os mecanismos de inativação microbiana, bem como os efeitos em alguns parâmetros de qualidade dos produtos lácteos.
Principais conclusões e conclusões: o uso de tecnologia supercrítica de dióxido de carbono (SC-CO2) apresenta grande potencial de aplicação no processamento de lácteos, uma vez que é efetivo reduzir a carga microbiana quando comparado ao processo de pasteurização, obtendo-se assim um produto com maior prateleira e melhores propriedades sensoriais com mudanças mínimas e às vezes positivas nos parâmetros de qualidade intrínseca.

ABSTRACT

Background: Non-thermal food processing is configured as an interesting alternative for the food industry due to the increased nutrient retention and minimal sensory changes in processed products.
Scope and approach: The aim of this review is to address the potential of supercritical carbon dioxide technology, emphasizing milk and dairy processing, including the historical aspects, main advantages, microbial inactivation mechanisms, as well as effects in some quality parameters of dairy products.
Key findings and conclusions: The use of supercritical carbon dioxide technology (SC-CO2) presents great potential application in dairy processing, since it is effective to reduce microbial load when compared to the pasteurization process, thus obtaining a product with greater shelf life and better organoleptic properties with minimal and sometimes positive changes in the intrinsic quality parameters.

1 INTRODUCTION

Milk and dairy products have high nutritional value, being consumed worldwide, and considered as healthy choices by consumers, associated with quality of life (North America Milk Market, 2016; FAO, 2016). In fact, it is estimated that the global consumption of dairy products will increase by around 36% by 2024 (Tetra Pak, 2016).

In many countries, raw milk is required to undergo thermal processing so that the milk is safe to consume (Clayes et al., 2013; Yoon, Lee, & Choi, 2016). Thermal treatments are the most common means of ensuring food safety and shelf-life stability of milk and dairy products (Gulsun, 2015). However, it is known that the high temperatures of conventional heat treatments lead to changes in nutritional (for example, degradation of vitamins) and organoleptic characteristics (aroma, flavor and texture) of the processed products (Barba, Zhu, Koubaa, Sant'Ana, & Orlen, 2016).

Recent interest in the consumption of raw milk and raw milk products has led to the consideration of alternative dairy processing technologies that will not compromise milk quality and safety (McAuley, Singh, Haro-Maza, Williams, & Buckow, 2016). Currently, many studies have addressed the emerging non-thermal technologies in food processes to minimize the deleterious effects of thermal conventional process, like, pulsed electric field (Odriozola- Serrano, Aguilo-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2013), ohmic heating (Jaeger et al., 2016), ultraviolet light (Guneser & Yuceer, 2012), pulsed-light technology (Abida, Rayees, & Masoodi, 2014; Miller, Sauer, & Moraru, 2012), ultrasound (Chandrapala & Leong, 2014), cold plasma (Mir, Shah, & Mir, 2016), high hydrostatic pressure (Yang et al., 2012) and ultra-high pressure homogenization (Valsasina et al., 2015).

Supercritical carbon dioxide technology (SC-CO_2 technology) utilizes pressure in combination with carbon dioxide to destroy microorganisms without affecting the nutritional content, organoleptic attributes, being a promising alternative for pasteurization of bioactive compounds in food and medicine (Jimenez-Sanchez, Lozano-Sanchez, & Fernandez-Gutierrez, 2017) in which compounds would be destroyed by conventional thermal processes (Vigano, Machado, & Martínez, 2015). However, the high cost of the equipment and operation of supercritical systems can be an obstacle to the application of supercritical processes on an industrial scale (Ceni et al., 2016).

Jermann, Koutchma, Margas, Leadley, and Ros-Polski (2015) developed a study involving researchers and CEOs (Chief Executive Officer) of North America, about the trends of utilizing an emerging technology in food process and reported that supercritical carbon dioxide technology has a great commercial potential for the next ten years. Regarding dairy foods processing, SC-CO_2 technology would aim to control the rawmaterial and minimize the use of heat treatment to inactivate the bioactive compounds. Also, it would improve economic and technical efficiency focus on value-added processing of milk and dairy products (Maubois, 2011).

Consumers crave for food with better nutritional quality, coupled with food safety and use of green technology (Barba et al., 2016). Therefore, this review aims to discuss the use of SC-CO2 technology as an innovative method of dairy products processing, exploring the historical perspectives on technology, main advantages, limitations, microbial inactivation mechanisms, and relevance of milk matrix issues will be assessed.

2 THEORETICAL AND FUNDAMENTALS ASPECTS

The term "supercritical" refers to a substance in a noncondensing and single-phase fluid when brought above its critical temperature (Tc) and critical pressure (Pc). Beyond this point, there is a supercritical region where the substance shows some typical physicochemical properties of gases or liquids, such as high density, intermediate diffusivity and low viscosity and surface tension (Table 1) (Cavalcanti & Meireles, 2012).

Table 1. Physical properties of carbon dioxide according to the state of aggregation*.

Property	**Gas 1 atm, 15 - 30°C**	**Supercritical Fluid**		**Liquid 15 - 30°C**
		T_c, P_c	**T_c, $4P_c$**	
Diffusivity, D ($cm^2.s^{-1}$)	0.1 – 0.4	0.7×10^{-3}	0.2×10^{-3}	$(0.2\text{-}2) \times 10^{-5}$
Viscosity, η ($g.cm^{-1} \times s^{-1}$)	$(1 – 3) \times 10^{-4}$	$(1 – 3) \times 10^{-4}$	$(3 – 9)$ X 10^{-4}	$(0.2 – 3) \times 10^{-2}$
Density, ρ ($g.cm^{-3}$)	$(0.6 – 2) \times 10^{-4}$	0.2 – 0.5	0.4 – 0.9	0.6 – 1.6

*Source: Tzia & Liadakis, 2003.

The application of supercritical fluid is directly related to its physicochemical properties. The high-density values combined with the pressure dependent solvent power provides high solubility and selectivity to the supercritical fluid. In addition, low viscosity values and intermediate values of diffusivity combined with the absence of surface tension of these fluids allow its rapid penetration into the cells and particles of the sample matrix extracting their interior material (Osorio-Tobon, Silva, & Meireles, 2016; Silva & Meireles, 2014). These characteristics facilitate process of extraction and inactivation of vegetative cells.

In some cases, similar effects to the supercritical state can be reached at temperatures near to its critical, the liquid state of a substance, with P > Pc and T < Tc characterizing the subcritical state (Ceni et al., 2016). Subcritical fluids exhibit physicochemical properties similar to supercritical fluids and have been suggesting as a practical advantage, once relatively high densities can be found at moderate pressures guaranteeing similar solvent extracting power. For example, subcritical CO_2 density at 20 °C is 0.818 g cm^3 at 7.5 MPa, while for supercritical CO_2 similar density (0.816 g cm^3) only will be achieved at 32 °C and 13.5 MPa (NIST, 2005). This indicates use of subcritical CO_2 is highly advantageous in respect to investment cost and thermal degradation once similar solvent power is achieved at more moderated conditions of temperature and pressure.

Various substances in the supercritical state could be used in the food industry, such as ethylene, water, ammonia. However, most of the studies using supercritical fluid technology use carbon dioxide (CO_2) as supercritical fluid aiming extraction and processing (Zabot, Moraes, Carvalho, & Meireles, 2015). Carbon dioxide is considered a chemically inert, non-corrosive, non-flammable, non-toxic, cheap, readily available, and GRAS (generally recognized as safe) solvent (Khosravi-Darani, 2010). Also, it can be recirculated into the system making this technology safe and environmentally friendly, due to the fact CO_2 can be easily removed from food matrix by pressure reduction, to obtaining a solvent-free product. Furthermore, its low critical temperature (31.04 °C) allows application at near room temperature, which prevents degradation of thermosensitive and volatile compounds, minimizing changes in the physicochemical, sensorial and nutritional characteristics of the food, thus obtaining high quality products. Besides, its moderate critical pressure (7.38 MPa) provides minor energetic and investment costs when compared with other supercritical substances, for example supercritical water (373,95 °C and 22.064 MPa) (Cavalcanti & Meireles, 2012; Cavalcanti, Albuquerque, & Meireles, 2016; Vigano et al., 2015).

Indeed, SC-CO_2 technology has many applications in food processing, with relevance in the fractionation, extraction, microencapsulation, pasteurization, sterilization, and chromatograph techniques, among others (Kulkarni, Kar, & Singhal, 2017; Moraes, Zabot, & Meireles, 2015; Osorio-Tobon et al., 2016; Santos & Meireles, 2013; Silva & Meireles, 2014).

3 DAIRY PROCESSING BY SC-CO2TECHNOLOGY

3.1 General Aspects

Generally, fluids in their supercritical state possess various advantages, including higher diffusion coefficient and lower viscosity, absence of surface tension, allowing rapid penetration into the pores of heterogeneous matrices, and simple control of temperature and pressure, which directly affects the solubility of the fluid (Sanli, Bozbag, & Erkey, 2012).

The main difference between high hydrostatic pressure (HHP) and supercritical carbon dioxide technology (SC-CO_2) processes applied in microbial and enzymatic inactivation processes is that SC-CO_2 technology can be as efficient as HHP but at lower pressures, i.e., much lower processing pressure is employed with supercritical carbon dioxide. Indeed, CO2 pressure applied for preservation purposes is in the range of 10 - 20 MPa, which is almost two orders of magnitude lower than HHP pressure (50- 1000 MPa). This reduces the cost of investment and facilitates the handling of the pressurized CO_2 system. Furthermore, use of SCCO_2 improves mass transfer due to higher diffusivity resulting in shorter processing time with consequent preservation of essential food elements, which means minimal degradation of nutritional and organoleptic properties (Ceni et al., 2016).

Overall, the motivation in using SC-CO_2 technology is related to multidimensional plane: the lightening environmental regulations on conventional solvent residues; concern over the use of chemical solvents in food manufacturing, increased demand for higher quality products which traditional processing techniques cannot meet and increased cost of energy (Khosravi-Darani, 2010).

Besides its use as microbial and enzymatic inactivation agent, others applications of sub- and supercritical CO_2 technology in the dairy processing are: (1) carbonation of milk, aiming lowering of the liquid through the production of carbonic acid, bicarbonates and hydronium ions; and (2) use of supercritical fluid for fractionation and extraction of target compounds, such as fat and cholesterol (Paul, Jayakumar, & Mishra, 2016; Prado, Khan, Saldaña, & Temelli, 2012; Sanchez-Macías, Laubscher, Castro, Argüello, & Jimenez-Flores, 2013; Truong, Palmer, Bansal, & Bhandari, 2017), resulting solvent-free residues and higher quality products due to minimized risk of thermal oxidation (Macías, Castro, Argüello, & Jimenez Flores, 2014).

3.2 Carbon Dioxide Solubility in Dairy Products

CO_2 solubility in dairy products influences the quality factors of these products. Supercritical CO_2 plays a special role on the retention and extraction of natural lipophilic compounds present in food matrix. In general, variations in pressure and temperature directly affect CO_2 solubility in dairy products. However, the CO_2 pressure has a greater influence on solubility when compared to the temperature (Vigano et al., 2015).

Current lines of research are focusing on the use of CO_2 in modified atmosphere packaging because it is one of the most important gases influencing the quality of most semi-hard cheeses. Recently, Acerbi, Guillard, Guillaume, and Gontard (2016) studied the effects of selected composition and ripening conditions on the CO_2 solubility in semi-hard cheese. The authors observed that the CO_2 solubility decreases linearly with increasing temperature considering a range 2 - 25 °C. In addition, they showed that the CO_2 solubility significantly decreases with increasing salt content from 0 to 2.7 g/100 g. Jakobsen, Jensen, and Risbo (2009) evaluated the influence of the fat content (10 - 45 g/100 g fat on dry basis) on the CO_2 solubility coefficients for semi-hard cheese aiming to understand the CO_2 effects on the aqueous and fat phase. They verified that both phases are of equal importance in terms of carbon dioxide solubility. Furthermore, the CO_2 solubility in the fat phase increased with increasing temperature.

The CO_2 solubility behavior is similar to whey protein solutions, since the pH significantly reduced with an increase in CO_2 pressure. When the protein concentration increases, the pH rises because of the strong buffering properties. Moreover, small changes in temperature have little influence on the pH and saturation solubility of CO2 (Yver, Bonnaillie, Yee, McAloon, & Tomasula, 2012; Bonnaillie & Tomasula, 2015).

3.3 Operational Systems

Another advantageous aspect of using supercritical CO_2 technology instead of HHP is that supercritical systems can be operated in any operational mode such as batch, semicontinuous and continuous, while HHP can basically be processed in batch. This makes SC-CO_2 technology more adaptable to any production chain enabling a wide range of applications. Furthermore, semicontinuous and continuous systems allowa better contact between the sample and CO_2 turning microbial and enzymatic inactivation more effective due to more rapid saturation with CO_2, reducing operational time and manufacturing costs.

The batch system consists of a CO_2 pump, a pressure regulator, and an exhaust system to release the pressure after the process. Temperature control is required to establish and control the heating or cooling temperature, which may be a water bath (Moraes et al., 2015), oven, or autoclave (Di Giacomo, Taglieri, & Carozza, 2009). Di Giacomo et al. (2009) used a batch system with a coupled stirring apparatus to evaluate the organoleptic changes in treated milk during storage.

The semicontinuous system allows a continuous flow of CO_2 through the pressure vessel. In semicontinuous processing, several vessels are connected in series; while some are under constant pressure, others are being pressurized, unloaded, reducing the time between the processes and allowing energy recovery (Porto, Decorti, & Tubaro, 2010). Kobayashi, Odake, Akuzawa, & Miura (2016) studied two-stage system with CO_2 microbubbles (twostage $MBCO_2$) to evaluate the efficacy in inactivating E. coli and aerobic bacteria in raw and commercial milk.

Finally, the continuous system, CO_2 and the sample is pumped and mixed before passing through the high pressure pump, which raises the pressure to the desired value for the process.Werner and Hotchkiss (2006) studied single pressurized system to determine the potential of pressurized CO_2 on bacteria reduction in fluid raw milk. The high-pressure CO_2 pilot plant developed by Praxair was used to treat milk at different temperatures (15, 30, 35, and 40 °C), pressures (10.3, 13.8, 17.2, 20.7, 24.1, and 48.3 MPa), and CO_2 concentrations (0.66 and 132 g/kg). In this system, milk was mixed with CO_2 in the first jacketed section. The CO_2 regulated by a microneedle valve and meter was injected into the milk flow. Then, milk passes through a pressure relief area under controlled temperature to avoid freezing and the degassed milk was then collected.

3.4 Non-Thermal Pasteurization Mechanisms

All food processing procedures aims to inactivate foodborne pathogens, spoilage microorganisms and enzymes present in the food that can lead to undesirable changes in flavor, texture, or color or represent any risk to public health (Hu, Zhou, Xu, Zhang, & Liao, 2013). The use of supercritical CO_2 technology to inactivate microorganisms is a novel method and well reported in the literature. Many terms have been adopted by several authors (Marszałek, Skąpska, Wozniak, & Sokołowska, 2015; Ceni et al., 2016), such as dense phase carbon dioxide (DPCD), liquid carbon dioxide (LCD), and high pressure carbon dioxide (HPCD). Indeed, few reviews related to microbial and enzymatic inactivation by supercritical CO_2 technology in food systems are reported in the literature (Hu et al., 2013).

Microorganisms and enzymes can be inactivated by CO_2 in two basically different ways, according to operational temperature and pressure conditions: while at moderate values of pressure and temperature, CO_2 can be used for carbonation, which the main effects are displacement of the oxygen and lowering of the pH in the medium, inhibiting enzymatic reactions and microbial growth, at high pressures and low-to-moderate temperature, CO_2 initiates deleterious activities into vegetative cells, spores and enzymes, providing partially or totally inactivation, which enables its use for pasteurization or sterilization of a product to prevent long-term poilage, respectively (Bonnaillie & Tomasula, 2015).

Table 2 shows the microbial and enzymatic inactivation mechanisms of supercritical carbon dioxide. Among them, CO_2 solubilization in the external phase of the reaction medium, cell membrane modification, reduction of intracellular pH, enzyme inactivation, disorder of intracellular electrolyte balance, removing the vital components of cells and membranes (Perrut, 2012). However, the exact mechanism has not been fully understood yet, being influenced by different parameters during the trial, including CO_2 pressure, temperature, time, substrate, type of microorganism, type of system used in processing (continuous or in batch), and operating parameters.

The efficiency of the microbial and enzymatic inactivation by sub- and supercritical CO_2 can be affected by several factors, including the operating pressure, temperature, time, initial pH, CO_2 state (high pressure liquid/gaseous CO_2 or sub-/supercritical CO_2), the food ingredients and processing media, HPCD processing system, combined treatments, the processing cycle, and storage time (Hu et al., 2013).

Overall, microbial and enzymatic inactivation rates have increased performance due to the increase of the main process parameters involved in the technology, such as pressure, temperature, and exposure time, besides the influence of the initial cell number and food matrix. Strong synergism between the effect of temperature and pressure was observed in the inactivation of various microorganisms such as *E. coli* (Kobayashi, 2007).

Spilimbergo, Mantoan, Quaranta, and Dealla Mea (2009) investigated the real-time monitoring of cellular membrane change during pasteurization by supercritical CO_2, and found a correlation between cell death and CO_2 permeability, which in turn can be considered as the key factor for microbial inactivation.

Previous researches have shown the effects of pressurized CO_2 on inactivation mechanism of vegetative cells. It is a consensus that Gram-negative bacteria are more sensitive to pressure and acidic conditions than the Gram-positive bacteria (Cappelletti, Ferrentino, & Spilimbergo, 2014; Porębska, Sokołowska, Skąpska, & Rzoska, 2017). Even though the spore inactivation mechanism is not well understood, some studies have shown the important role of CO2 in spore inactivation, mainly because of the decrease in the heat tolerance, which achieves a more efficient sterilization when followed by heat treatment (Casas, Valverde, Marín-Iniesta, & Calvo, 2012; Park, Choi, Kim, & Kim, 2013).

Table 2. Non-thermal pasteurization mechanisms of supercritical carbon dioxide* (continua).

Factors	Mechanism
Solubilization	CO_2 in aqueous solution forms carbonic acid (H_2CO_3), which dissociates into bicarbonate (HCO^{3-}), carbonate (CO_3^{2-}) and hydrogen (H^+) ions, thus reducing the interstitial pH of the bacterial cell, and reducing microbial resistance to inactivation due to higher energy consumption for maintaining intracellular homeostasis.
Structural changes in the cell membrane	The high affinity between CO_2 and plasma membrane provides CO_2 accumulation within the internal lipophilic layer, affecting its permeability, with structural and functional disorders to the microorganism.
Reduction in intracelular pH	The increased permeability of the membrane allows penetration of the pressurized CO_2 across the bacterial cell membrane with accumulation within the microbial cel.
Enzyme inactivation	The reduction in intracellular pH can cause inhibition and/or inactivation of essential enzymes for regulating the metabolic processes of the organism, since the catalytic activity of enzymes is particularly sensitive to pH change. The immediate consequence is the loss of biological controls, damaging the intermediary metabolism and cellular function.
Direct inhibitory effect of CO_2 and HCO^{3-}	The concentration of carbonic acid and bicarbonate ion appears to be crucial for the regulation of enzyme activity, reflected in the microbial metabolism due to changes in the carboxylation and decarboxylation of the different reactions.
Disorder in intracellular electrolyte balance	Lethal damage to the biological system of the microbial cells can occur with pressure and additional carbon dioxide accumulation in the cytoplasm. This can convert the HCO^{3-} to CO_2, which could intracellular precipitate inorganic electrolytes from the cells and cellular membranes.
Removal of the vital components and cell	CO_2 accumulated in the interior of the microbial cell can "extract" vital components from the cell membranes of microorganisms such as phospholipids and hydrophobic compounds, altering the structure and / or balance of nutrients.

*Adapted from Perrut, 2012.

Recently, the efficacy of use SC-CO_2 technology in combination with other method was evaluated. The technology when associated to application of peracetic acid (PAA) to control microbial growth in mozzarella cheese (Sikin, Walkling-Ribeiro, & Rizvi, 2016) proportionated interesting findings. The maximum reduction of *Geobacillus stearothermophilus* spores - 1.4 CFU/g - was obtained after treatment with SC-CO2/PAA 100 ppm, where it achieved about 3.8 log reduction during 21-day storage while for vegetative microorganisms above 4.6 log cycles inactivation was obtained. These findings seem relevant for the cheese industry when considering the consumption and popularity of mozzarella cheese worldwide.

4 QUALITY PARAMETERS OF DAIRY FOODS AFFECTED BY SC-CO2 TECHNOLOGY

4.1 Microbiological Aspects

Milk is a low acid food, thus allowing the development of a range of microorganisms, including important pathogens, such as Salmonella, Eschericia coli, Listeria monocytogenes, and *Coxiella burnetti*, among others. In addition to low acidity, microbial inactivation in milk is very difficult due to the baroprotector effect (Cappato et al., 2017). The use of supercritical carbon dioxide technology has also aroused interest in the dairy area. In 1987, in Germany, a patent (DE3734025 A1) proposed the use of supercritical CO_2 to increase the shelf life of dairy products.

Table 3 summarized the main current studies on the use of supercritical CO_2 for microbial inactivation in milk and dairy products. Although the results are directly related to the operating parameters (process time, pressure and temperature), it is clear the potential of the process for obtaining a processed product with no risk to public health. Most of the studies related a distinct inactivation phases for a constant temperature, which the first phase (Shoulder region) was characterized by a slow inactivation rate, suggesting the time that CO_2 entered the cells. CO_2 penetration is more intense when higher pressures are applied, probably due to the increase in the solubilization rate of the gas. After reaching critical CO_2 levels within the cell, the vital components were extracted, which changed their biological equilibrium and showed a second phase, characterized by the rapid death of microorganisms. Besides the effect of the pressure, temperature has a great importance on the inactivation rate. The increase in temperature and pressure causes a raise in the inactivation ratio and reducing shoulder region. The research's suggest that higher temperatures stimulate the diffusivity of CO_2, which increases the fluidity of cell membrane making CO_2 penetration smoother (Ceni et al., 2016).

Spore-formers are important contaminants in the dairy products, for it affect the food quality and safety, besides it facilitates the spoilage of the products with a decrease in their commercial shelf life (Oliveira et al., 2016). Thus, during cheese processing, it is usual to submit raw milk to pasteurization (72-75 °C, 15 s), but this temperature profile is not sufficient for the inactivation of some important microorganisms, like *Bacillus cereus* and *Geobacillus stearothermophilus* (Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz, 2013).

Table 3. Microbial inactivation by supercritical CO_2 in milk.

Product	**Microorganism**	**Pressure (MPa)**	**Temperature (°C)**	**Time (min)[a]**	**System**	**Reduction (log)**	**Shoulder (min)**	**Reference**
Raw skim milk	Native psychrotrophs	20.7	35	10	Continuous	5.36	NI	Werner & Hotchkiss (2006)
Raw skim milk	*Pseudomonas fluorescens*	20.7	35	10	Continuous	5.02	NI	
Raw milk	Aerobic bacteria	25	50	70	Continuous	4.96	NI	Hongnei, Zhong, Liao, & Hu (2014)
Raw milk	Coliforms	25	40	50	Continuous	2 *	NI	
Raw milk	Yeast and molds	25	40	50	Continuous	3 *	NI	
Raw milk	Aerobic bacteria	4	45	1	$MBCO_2$	3	NI	Kobayashi, Odake, Miura, & Akuzawa (2016)
UHT milk	*Escherichia coli*	4	50	5	$MBCO_2$	4.8	NI	
Whole milk	*Escherichia coli*	8	70	1	Continuous	0.09	NI	Ceni et al. (2016)

NI: Not informed; MB: microbubbles; D: total decimal reduction.
[a] Total inactivation time (Shoulder + straight-line).

In spite of this, few studies cover the effects of supercritical CO_2 on spores inactivation in milk. Werner and Hotchkiss (2006) tested the viability of *Bacillus cereus* spores at temperatures of 15, 30, 35, and 40 °C; pressures of 10.3, 24.1, and 48.3 MPa, and CO_2 concentrations of 0, 3, 66, and 132 g/kg in the aged raw milk. The fresh milk was inoculated with 101 and 106 spores/mL, and tested for spore viability after treatment at 40 °C, 48.3 MPa, and 132 g/kg of CO_2. None of the treatments resulted in a significant reduction in the number of spores.

All the studies have shown that SC-CO_2 technology can provide microbial inactivation in milk, despite several factors can influence the microbial inactivation rate, such as milk fat content, age of bacteria, equipment type, and processing parameters.

Therefore, knowledge of the main parameters involved and the microbial inactivation mechanism are important factors for the development and the implementation of SC-CO_2 technology in the manufacture of dairy foods with higher quality to ensure the microbiological safety for consumers.

Therefore, knowledge of the main parameters involved and the microbial inactivation mechanism are important factors for the development and the implementation of SC-CO_2 technology in the manufacture of dairy foods with higher quality to ensure the microbiological safety for consumers.

4.2 Physicochemical Parameters

The effect of treatment on the sensory and nutritional characteristics of the food product is an important aspect for evaluation of an emerging technology for food processing and conservation (Cruz, Faria, Saad, Bolini, San' Ana, & Cristianini, 2010). Table 4 shows the main studies which report the effect of SC-CO_2 technology on the physicochemical, sensory and/or nutritional properties of milk.

The application of supercritical carbon dioxide technology can considerably reduce the time required for heat treatment (pasteurization or sterilization) of food products, while minimizing thermal degradation of thermolabile compounds, especially vitamins (Spilimbergo, Komes, Vojvodic, Levaj, & Ferrentino, 2013). However, its effect on the degradation of vitamins in milk is still unknown which reinforces the need of additional studies. Indeed, with respect of vitamins degradation, the acidified milk showed better preservation to retinol, β-carotene, and α-tocopherol, when compared to heat treated milk (Ruas-Madiedo, Bada-Gancedo, Fernandez-Garcia, Llano, & Reyes-Gavilan, 1996).

Besides the effect on vitamins, supercritical CO_2 can affect milk protein due to acidification and solvation properties, as the carbonic acid formed binds with calcium ions, which may internally destabilize the casein micelles (Dalgleish & Corredig, 2012). Although casein precipitation may be an obstacle in the treatment of milk by supercritical CO2, the treatment can contribute to the manufacture of dairy products, including cheese.

Studies have stated that supercritical CO_2 can be used for casein precipitation and fractionation of α and β-lacto albumin (Yver et al, 2012; Bonnaille & Tomasula, 2012). The precipitated casein has industrial applications, such as an ingredient for enrichment of nutrition formulas. According to Yver et al. (2012), supercritical carbon dioxide technology is an effective method for precipitation and fractionation of protein concentrate solutions

Whey proteins are commonly used in the dairy industry as an ingredient due to its functionalities, nutritional value and physical form of the industrial formulations (Krolczyk, Dawidziuk, Janiszewska-Turak, & Sołowiej, 2016).

Table 4. Effects of supercritical CO_2 technology in physico-chemical, sensory and quality parameters of milk.

Parameters	Assay	Main Findings	Reference
Sensory Analysis	Sensory evaluation of skimmed milk processed by supercritical CO_2 (15 MPa, 35-38 °C for 15 min) as compared to the HTST pasteurized milk, along the refrigerated storage using a panel of trained assessors.	Greater acceptance of milk processed by supercritical CO_2 was observed when compared to HTST pasteurized milk under the same storage conditions. However, higher pressures of CO_2 resulted in higher extraction of aromatic compounds from milk during depressurization, causing undesirable changes in the sensory characteristics.	Di Giacomo, Taglieri, & Carozza (2009)
Lipolytic and Proteolytic activity	Lipolysis assessed by the free fatty acids (FFA) content, and evaluation of proteolysis from casein nitrogen in whole milk processed by supercritical CO_2 at different pressures (7-62 MPa) and temperatures (15-40 °C) for 30 min., as compared to non-treated milk, and subsequently stored longer than 4 days at 25 °C.	The higher FFA content (5.2 meq / kg after 3 days at 25 °C) was obtained in two treatments: 62 MPa at 15 °C and 7 MPa at 40 °C. However, the FFA level obtained during the treatment at 62 MPa at 40 °C was lower (1.5 meq / kg), suggesting that the increase in temperature was not sufficient to inactivate lipolytic enzymes. Additionally, there shall be a minimum combination of pressure and temperature to inhibit lipolysis in milk treated by supercritical CO_2. In general, bacterial lipases were inactivated at lower temperatures in the process by supercritical CO_2 when compared to the thermal processing. All treatments with supercritical CO_2 had a lower reduction of proteolytic activity, with lower proteolytic rate.	Tisi (2004)
Physicochemical characteristics of milk	pH, Protein, Fat, Acidity, lactose, calcium, iron, potassium and manganese in whole milk processed by supercritical CO2 at different pressures (80-180 bar), temperature (30-70 °C) and time (10-30 min.) as compared to untreated milk.	The authors found no difference in the results, demonstrating the processing by supercritical CO_2 did not result in changes in physicochemical characteristics of milk. However, greater retention of the indicators assessed was not mentioned by the authors.	Ceni et al. (2016)
Proteolysis	Casein resistance to proteases (papain and thermolysin) and free amino acid content in whole milk processed by two stage MBCO2 as compared to untreated milk, using different parameters in the mixing vessel (1-2MPa at 10 ° C) and the heating coil (4 MPa, 35-50 °C for 1-10 min.).	The treatment induced changes in casein resistance to proteases. In the mixing vessel, casein was decomposed easier by thermolysin (17% increase). In the heating coil, the hydrolysis by thermolysin reduced by 90% (45 °C), but increased with increasing temperature (50 °C), while the hydrolysis of papain decreased to 82%. The treatment promoted less formation of free amino acids except for phosphoethanolamine (PEA) and valine (Val), with the largest reduction rate observed for lysine (LYS), induced by temperature increase.	Kobayashi, Odake, Miura, & Akuzawa (2016)

MB: microbubbles.

In addition, the consumption of whey beverages is increasing around the word and several varieties supplemented with fruits, probiotic bacteria and prebiotic ingredients are available on the market (Castro et al., 2013; Siqueira, Machado, & Stamford, 2013). Although it is a consensus that whey proteins are more resistant to the effects of high pressure CO_2 than caseins (Macías et al., 2014), SC-CO_2 technology is able to change the structure and conformation of whey proteins offering a new or different functionality for this component. Previous study reported the improvement of gelling properties after dissolution in water and powder form after SC-CO_2 technology (40 °C / 10 MPa or 65 °C / 30 MPa during 1 h) with positive impact on the rheology aspects due the change of composition, the surface hydrophobicity, which resulted an increase of the gel strength (Zhong & Jin, 2008).

Milk fat mainly presents in small globules in which triacylglycerol (TAG) predominates while other lipids classes as diacylglycerol and cholesterol can be also found (Pereira, 2014). These globules are surrounded by the milk fat globule membrane (MFGM), which presents interesting composition in which highlights the presence of phospholipids, sphingolipids, and glycolipids, all linked to several biological processes (Yao et al., 2016). The SCCO_2 technology when associated with the microfiltration, being convenient to concentrate MFGM components using as raw material butter milk, whey, and whey cream that are considered byproducts for the dairy processors. In a final perspective, it was able to give a unique opportunity to develop a functional product with differentiated composition (Spence, Jimenez-Flores, Qian, & Goddik, 2009), which can be used as ingredient in several food formulations. In this moment, it is prudent to comment the enormous diversity in the operational parameters used to manufacture butter and cheese for dairy industry worldwide, which reflects on the whey and buttermilk composition. Therefore, it is compulsory to perform previous experiments to find the optimal conditions to be used in SC-CO_2 technology.

The effects in supercritical carbon dioxide technology on milk enzymes are not well studied, although it is of great importance, since milk contains over 50 different types of native enzymes, with proteases and lipases as major endogenous enzymes responsible for changes in milk quality. Lipases or lipoproteins are the most important, due to the cleavage of ester linkage, which can lead to free fatty acids formation that is related to spicy aroma and ultimately causes rancidity in milk (Campbell & Drake, 2013). Proteolysis in milk is mainly induced by the action of plasmin, an endogenous enzyme normally associated with the casein fraction while plasminogen, which is also present in milk, can be readily converted to plasmin and activated (Stoeckel et al., 2016).

However, storage time, heat treatment, and pH are the most important factors affecting the plasmin system in milk and dairy products (Burbrink & Hayes, 2006). Milk for human consumption is normally pasteurized (72 °C, 15 s) and these parameters can inactivate plasminogen activator inhibitor, but not plasmin, besides it improves the conversion of plasminogen to plasmin, thus contributing to proteolysis. Indeed, plasmin is extremely heat-stable, and its activity causes severe sensory defects (Stoeckel et al., 2016). So far, there are no studies on the inactivation of plasmin by supercritical carbon dioxide technology. Another important milk endogenous enzyme is the alkaline phosphatase, which is used as an indicator of the effectiveness in milk pasteurization. Ceni et al. (2016) studied the continuous inactivation of alkaline phosphatase in milk using supercritical carbon dioxide, utilizing pressures from 8 to 18 MPa, temperature of 30, 50 and 70 °C, and CO_2 to milk mass ratio of 0.05 and 0.45 wt % during 30 min. Thus, considering initial alkaline phosphatase activity of 5.5 $U.mL^{-1}$, it was observed an increase in the enzymatic activity in the first assay (21.8%) at 0.05 wt%, 30 °C and 8

MPa, and also in the third (9.0%) and forth (23.6%) assays at 30 °C and 18 MPa using CO_2 and milk mass ratio of 0.05 and 0.45 wt%, respectively. Similar findings were reported past research (Liu, Chen, & Wang, 2013). The best inactivation rate of alkaline phosphatase (98.2%) was observed for CO_2 and milk mass ratio of 0,45 % wt at 70 °C and 8 MPa during 30 min, which revealed potential application of continuous supercritical carbon dioxide process for inactivation of alkaline phosphatase in milk (Ceni et al., 2016). The processing of low-fat dairy foods with healthier lipid profile has gaining attention from the dairy industry (Ferrão et al., 2016). For the cheese industry, the main obstacle is to manufacture low fat cheeses with similar characteristics compared to full-fat ones, as these present a compact matrix, abnormal instrumental texture, color characteristics and absence of the typical flavor (Macías et al., 2014), besides different development of proteolysis and lipolysis presents a decrease in the performance in sensory tests. The adoption of the SC-CO_2 technology seems to be a promising option to manufacturing low-fat cheeses, as showed in previous reports for cheddar and Parmesan grated cheeses (Yee, Khalil, & Jimenez- Flores, 2007; Yee, Walker, Khalil, & Jimenez-Flores, 2008) and Majorero (an artisanal goat cheese) and Gouda-type goat cheese (Sanchez-Macías et al., 2013). The effect was clearly dependent on the cheese matrix, which was clearly noticed the need of optimization in the processing parameters - pressure, temperature and carbon dioxide mass flow, which are directly related to the efficiency of lipid removal.

Regarding the development of dairy products with a healthier lipid profile, Chitra, Deh, and Mishra (2015) relate an important contribution and incentive to use the SC-CO_2 technology. After performing a two-factor design (temperature and pressure ranging from 40 to 80 °C, 15 - 25 MPa, respectively), they were able to determine the optimal operational conditions to remove cholesterol from whole milk powder: 68 ºC, 20.7 MPa, 40 min static time and 2 h dynamic time at flow rate of 6 L min^{-1} of CO_2. Those parameters guaranteed the removal of 55.8% in cholesterol content, while kept unaltered the free fatty acids, the lightness values and the solubility index. Due the deleterious role of cholesterol as risk factor for cardiovascular diseases, this finding emphasizes in a positive manner to potential of using supercritical carbon dioxide technology for the dairy processors.

Overall, the supercritical dioxide carbon technology has proven to be an interesting option for maintaining the sensory and nutritional quality of the dairy product and inactivating important enzymes for the dairy industry. Additional studies are needed to elucidate the effects of supercritical CO_2 on the main intrinsic indicators parameters involved in dairy foods processing and quality, to contribute in this way for its complete application for the dairy industry. In special, research about covering the effect of SC-CO_2 technology to decrease milk protein allergenicity would very useful and can contribute to a more disseminated adoption by dairy processing lines.

5 PERSPECTIVES

Supercritical dioxide carbon technology is an innovative and promising technique for treatment of milk and dairy products and has the potential of improving dairy products formulation affecting in a positive way the nutritional value, while providing at the same time the safety of final products.

However, additional studies on the mechanisms of inactivation of pathogenic and spoilage microorganisms are needed, as well as monitoring of commercial shelf life of processed dairy products. Additionally, it is extremely important to perform optimization design of the parameters involved taking in account each dairy food processing. Finally, in an economic perspective, feasibility studies of the process in order to enable the implementation of this technology at industrial scale are also welcome and compulsory.

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CAPÍTULO III

WHEY-GRAPE JUICE DRINK PROCESSED BY SUPERCRITICAL CARBON DIOXIDE TECHNOLOGY: PHYSICO-CHEMICAL CHARACTERISTICS, BIOACTIVE COMPOUNDS AND VOLATILE PROFILE

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RESUMO

O efeito da tecnologia de dióxido de carbono supercrítico (SCCD, 140, 160 e 180 bar a 35 ± 2 °C durante 10 min) em características de bebidas de suco de uva foi investigado. Caracterização físico-química (pH, acidez titulável, sólidos solúveis totais), compostos bioativos (compostos fenólicos, antocianinas, DPPH e atividade ACE) e os compostos voláteis foram realizados. A ausência de diferenças foi encontrada entre tratamentos para pH, acidez titulável, sólidos solúveis, antocianinas totais e atividade de DPPH ($p > 0,05$). Foi observada uma relação direta entre pressão SCCD e atividade inibitória ACE, com 34,63, 38,75 e 44,31% (140, 160 e 180 bar, respectivamente). Atende aos compostos voláteis, observou-se poucas diferenças, exceto pela presença de cetonas. Os resultados confirmam o processamento do SCCD como uma potencial tecnologia promissora para o tratamento térmico convencional.

Palavras-chave: tecnologia de carbono com dióxido supercrítico, bebida de soro de uva, compostos bioativos, compostos voláteis.

ABSTRACT

The effect of supercritical carbon dioxide technology (SCCD, 140, 160, and 180 bar at 35 ± 2 °C for 10 min) on whey-grape juice drink characteristics was investigated. Physicochemical characterization (pH, titratable acidity, total soluble solids), bioactive compounds (phenolic compunds, anthocyanins , DPPH and ACE activity) and the volatile compounds were performed. Absence of differences were found among treatments for pH, titratable acidity, soluble solids, total anthocyanins and DPPH activity ($p>0.05$). A direct relationship between SCCD pressure and ACE inhibitory activity was observed, with 34.63, 38.75, and 44.31% (140, 160, and 180 bar, respectively). Regards the volatile compounds, it was noted few differences except by the presence of ketones. The findings confirm the SCCD processing as a potential promising technology to the conventional thermal treatment.

Key-words: supercritical dioxide carbon technology, whey-grape juice drink, bioactive compounds, volatile compounds.

1 INTRODUCTION

The lifestyle of consumers around the world has changed. Large and complex economic, social, cultural and political movements have led to a strong tendency to change the consolidated consumption habits (Brazil Food Trends 2020, 2010). The search for healthier life practices has led the industrial sector to develop health-promoting products with convenience and high quality, besides adopting sustainable and clean processes.

In this sense, the consumption of whey-grape juice drink is an alternative for obtaining a range of nutrients that promote health, along with the new tendencies of the global market. The nutritional value of whey and its high bioavailability have motivated its incorporation as an ingredient in food products due to the nutritional appeal and functional properties. In addition, grape juice is appreciated all over the world due to its unique taste, besides being a source of phenolic compounds that exert health benefits when consumed regularly (Granato, 2015).

Conventional heat treatments promote the degradation of nutrients and bioactive compounds, as well as affect the taste of food due to the cooking process (Marszałek, Skąpska, Woźniak & Sokołowska, 2015). Therefore, non-thermal treatments have been studied aiming at the production of foods with sensory characteristics closer to those of the fresh product.

Supercritical carbon dioxide (SCCD) is a clean technology, recognized as environmentally friendly, which has been gaining prominence in the non-thermal treatment of foods, due to the absence of the harmful effects of heat, thus keeping the physicochemical, nutritional, and sensory characteristics of the fresh product (Ramírez-Rodrigues et al., 2012, Ceni et al., 2016, Marszałek, Skąpska, Woźniak, Sokołowska, 2015). Carbon dioxide (CO_2) has critical properties at low temperatures (31.1 ° C) and moderate pressure (73.8 bar) (Chen et al., 2010). Therefore, SCCD processing for pasteurization can occur under temperature conditions lower than those in the conventional heat treatments, characterizing this technology as a non-thermal pasteurizing process of food products. Supercritical technology has also emerged as an alternative to hydrostatic high-pressure processing, which can reach up to 10,000 bar, while SCCD treatments are close to 100 bar (Ceni et al., 2016). Other terms adopted for the technology are dense phase carbon dioxide (DPCD), and liquid carbon dioxide (LCD) and/or high-pressure carbon dioxide (HPCD) (Damar&Balaban, 2006; Garcia-Gonzalez et al., 2007; Marszałek, Skąpska, Woźniak, &Sokołowska, 2015).

Several studies have demonstrated the efficiency of SCCD processing in the preservation of juices such as orange, melon, hibiscus, kiwi, pear, and strawberry (Yuk, Sampedro, Fan & Geveke, 2014; Ramírez-Rodrigues et al. However, there is no report in the literature about the effect of the SCCD on the physicochemical characteristics of whey-based beverages. Therefore, the purpose of this study was to investigate the effects of SCCD treatment (140, 160, and 180 bar at 35 ± 2 ° C, for 10 min) compared to the conventional heat treatment by HTST (72 ° C for 15 s) on the physicochemical characteristics of whey-grape juice drink in relation to pH, titratable acidity, total soluble solids, bioactive compounds, angiotensin converting enzyme (ACE), and volatile compounds.

2 MATERIAL AND METHODS

2.2 Whey-Grape Juice Drink Processing

The following ingredients were used for the preparation of whey-grape juice drink: 50% (v/v) of whole red grape juice (Mitto ®, Brazil, natural juice without preservatives, sugar or sodium) and 50% (v/v) whey powder (Alibra®, Brazil) reconstituted in distilled water, according to the manufacturer's instructions. About 0.108 g xanthan gum (Satiaxane™, Cargill, Spain) and 7.53 g sucrose (Union®, Brazil) were added per 100 g of sample. After mixing the ingredients, the beverage was homogenized in a rotor-stator type homogenizer (Walita®, Brazil) for 5 min. Then, the beverage was subjected to conventional thermal processing (HTST pasteurization) and non-thermal treatment using SCCD technology. The HTST was applied at 72 ° C for 15 s in a temperature-controlled water bath (Marconi®, MA126 / BO, Piracicaba, Brazil). The temperature of the sample was monitored using a digital thermometer (Digital Thermometer Hanna®, Check temp, HI98501, Nusfalau, Romania), and the samples were cooled to 10 ° C immediately after treatment.

For supercritical dioxide carbon technology, a homemade equipment was used assembled by the research group in LASEFI (Laboratory of Supercritical Technology: Extraction, Fractionation, and Identification of Vegetal Extracts) – Department of Food Engineering (DEA)/School of Food Engineering (FEA), University of Campinas (UNICAMP)/Brazil. Figure 1 shows the flow diagram of the experimental apparatus. A volume of 400 mL of whey-grape juice drink was manually introduced into the 316 stainless steel reactor, fitted with a flange closure system, with a maximum capacity of 630 mL. Carbon dioxide (99.9% CO_2, Gama Gases Especiais Ltda., São Bernardo do Campo, Brazil) cooled at -6 ° C in a thermostatic bath was pumped by a pneumatic pump to the high-pressure reactor, promoting the homogenization of the sample until reaching the operating pressure.

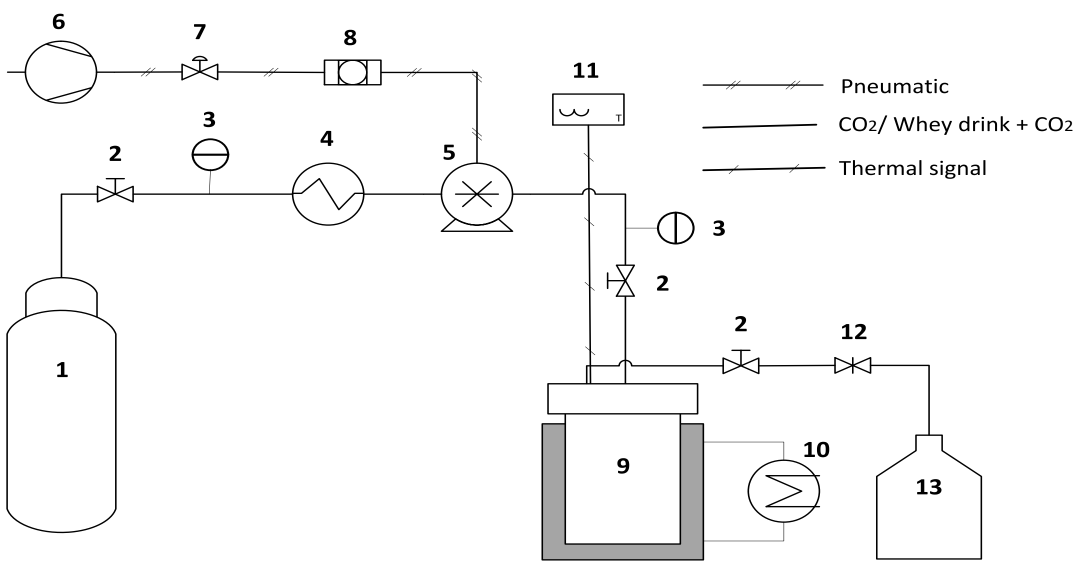

Figure 1. Diagram of the homemade equipment for the supercritical dioxide carbon technology. 1 – CO_2 Cylinder; 2 – Control valve; 3 – Manometer; 4 – Cooling bath; 5 – Pump (Booster); 6 – Air compressor; 7 – Control valve (air flow); 8 – Air filter; 9 – Batch reactor; 10 – Heating bath; 11 – Temperature indicator; 12– Micrometer valve; 13 – Grape whey drink + CO_2.

The 10-minute processing time was counted from the temperature stabilization at 35 ± 2 ° C, monitored by means of a digital thermocouple. At the end of each treatment, the equipment was depressurized and the sample collected and immediately cooled. All procedures of hygiene and asepsis were carefully carried out during the experiments, considering the microbiological safety and quality aspects.

The samples were subjected to SCCD at 140, 160, and 180 bar and 35 ± 2 ° C for 10 min, as described in the next section. All experiments were performed in duplicate. Samples were collected immediately after the process and frozen (-18 ° C) for further analysis, except the pH and soluble solids determinations, which were performed immediately after the processing.

2.2 Physicochemical Characterization

The pH was measured using a pH meter (AKSO, AK103, São Leopoldo / RS, BR) calibrated with pH 7 and pH 4 buffer solutions, according to AOAC (2000). The total soluble solids were measured with a digital refractometer (Atago®, PAL-1, Tokyo, Japan). The titratable acidity was determined using a bench titrator (PHS-3B, Phtek, China) by titration with 0.01 M NaOH, according to AOAC (2000) and the results expressed as g tartaric acid/L. The color of the samples was a limiting factor for the use of the phenolphthalein indicator, thus a calibrated potentiometer with automatic temperature adjustment was used to identify the turning point. All analyses were performed in triplicate at 25 °C.

2.3 Bioactive Compounds

Total phenolic compounds (TPC) were determined using the Folin-Ciocalteau reagent (Singleton & Rossi, 1965), as described by Sabokbar & Faramarz (2016). The absorbance was measured at 760 nm in a spectrophotometer (Biospectro, SP-220), and the results were calculated using a calibration curve. All determinations were performed in triplicate and the results expressed as Gallic acid equivalents per liter of sample (GAE g / L).

Total anthocyanins were determined by the differential pH spectrophotometric method, according to Klopotek, Otto & Böhm (2005), in triplicate. Absorbance values were measured at 515 and 700 nm using pH 1.0 and 4.5 buffer solutions. The results were expressed as mg malvidin-3-glucoside / 100 mL.

The antioxidant capacity of the beverages was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reducing power, according to the method of Brand-Williams et al. (1995), as described by Sabokbar & Faramarz (2016). (Biospectro, SP-220) with absorbance readings at 510 nm. A DPPH solution (0.06 mM) was prepared by mixing 2.4 mg DPPH and methanol in a 100 mL volumetric flask. To obtain the extracts, 5 mL sample were mixed with 25 mL distilled water at 100 ° C, remaining for 60 min and then centrifuged (12,000 RPM) for 15 min, and the supernatant was collected. Then, in dark environment, 0.1 mL extract aliquots were transferred to tubes containing 3.9 mL of 0.06 mM DPPH. The blank consisted of a mixture containing 0.1 mL control solution (distilled water at 100 ° C) and 3.9 mL DPPH. Methyl alcohol was used as a blank for calibration of the spectrophotometer.

The ACE inhibitory activity was determined in the whey-grape juice filtrates, as described by Ramc, Ran & Shah (2010), using the methodology reported by Cushman & Cheung (1971).

2.4 Volatile Compounds

The volatile compounds were extracted by solid phase microextraction (SPME) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS, Varian 3800 gas chromatograph directly interfaced with Varian 2000 ion trap mass spectrometer, VarianSpa, Milan, Italy). The methodology was performed as described by Felicio at al. (2016). SPME extractions were made using 50/30 µm DVB/Car/PDMS fiber (divinylbenzene/carboxy dimethyl siloxane) (Supelco, Bellefonte, PA, USA) and 40 mL flasks fitted with mininert valves (Supelco). For that, 8 g sample were dissolved in 12 mL saturated NaCl solution and the mixture was maintained at 40 ° C. The equilibration time lasted 20 min and the extraction time 30 min. The sample was stirred continuously during extraction at 750 rpm using a magnetic stirrer. After extraction, SPME fiber was introduced into the GC-MS splitless injector and maintained at 260 ° C for 3 min for thermal desorption of the analytes. The volatile compounds were identified by comparing their experimental spectra with those from the National Institute of Standards & Technology (NIST/EPA/NIH Mass Spectra Library, version 1.7, USA), using the linear retention indices (LRI).

2.5 Statistical Analysis

The results were presented as means ± standard deviation, and analyzed by ANOVA followed by Tukey's test (p-value $\leq$ 0.05) using the software Statistica 10 (StatSoft®, Tulsa, OK, EUA).

2 RESULTS AND DISCUSSION

3.1 Physicochemical Characterization

The whey-grape juice drinks presented similar pH, soluble solids, and titratable acidity, without significant differences among the CO_2-treated and pasteurized samples and untreated beverages (Table 1), as also observed in other studies. Grape juices processed by a continuous SCCD system (34.5 MPa/30 ° C/6.25 min) and thermal pasteurization (HTST 75 ° C/15 s) presented no changes in pH, soluble solids, and titratable acidity (Pozo-Insfran, Balaban & Talcott, 2006a; Pozo-Insfran, Balaban & Talcott, 2006b). Similar results were also reported by Yuk et al. (2014) and Fabroni et al. (2010) in SCCD-treated orange juice (7.6 MPa/40 ° C for 20 min, 130 - 230 bar/36 ° C, respectively), and by Spilimbergo & Ciola (2010) in kiwi and pear juices (10 MPa/35 ° C) and strawberry juice (30 and 60 MPa/45 ° C/30 min) (Marszałek, Skąpska, Woźniak, Sokołowska, 2015).

However, previous studies have found significantly higher titratable acidity in SCCD-treated hibiscus juice (34.5 MPa / 40 ° C / 6.5 min) (Ramírez-Rodrigues et al., 2012) and coconut water (34.5 MPa/25 ° C/6 min). The results were probably due to the concentration of residual CO_2 in the beverage, which tends to form carbonic acid in aqueous media, altering the acidity of the beverage (Damar et al., 2009). Similar results were observed for pH and titratable acidity in whole milk processed by SCCD in a continuous system at different pressures (80-180 bar), temperatures (30-70 ° C), and process time (10-30 min) when compared to milk not subjected to supercritical technology (Ceni et al., 2016).

3.2 Bioactive Compounds

The HTST and SCCD treatments did not affect the total phenolics, total anthocyanins, and antioxidant capacity of the whey-grape juice beverages, as can be seen in Table 1. Although no significant differences were found among treatments, we can observe a trend of pasteurization treatment in reducing total anthocyanins levels (7.8%) when compared to other treatments.

Recent studies have shown no differences in anthocyanins content in strawberry juice processed at 30 MPa (45 ° C/30 min) when compared to the untreated juice. However, the SCCD-treated juice subjected to 60 MPa (45 ° C/30 min) presented significant low anthocyanins levels (about 3%) when compared to the untreated samples (Marszałek, Skąpska, Woźniak, Sokołowska, 2015).

Some authors have studied grape juice, and found that thermal pasteurization (HTST 75 ° C/15 s) negatively affected the anthocyanins, soluble phenolics, and antioxidants, with reductions of 16% 26%, and 10% when compared to SCCD processing (34.5 MPa/6.25 min/30 °C), which presented no significant changes (Del Pozo-Insfran, Balaban & Talcott, 2006a). Furthermore, a shelf-life study of these grape juices showed that the SCCD-treated samples maintained the highest anthocyanins content and antioxidant capacity when compared to the thermally pasteurized samples, after 10 weeks of storage (Del Pozo-Insfran, Balaban&Talcott, 2006a).

In another study, under similar treatment conditions, Del Pozo-Insfran, Balaban & Talcott (2006$_b$) evaluated the resistance of phytochemical compounds in natural grape juice, ascorbic acid-fortified juice, and juice with the addition of thyme polyphenolic co-factors. The thermal pasteurization reduced the contents of anthocyanins, ascorbic acid, soluble phenolics, and the antioxidant capacity, while no significant changes were observed for the SCCD-treated samples. This trend was maintained over the storage period, and the results showed that increasing the CO_2 level from 8 to 16% in the SCCD process was fundamental in reducing the phytochemical and antioxidant degradation of juices. The authors stated that the absence of dissolved oxygen led to the retention of phytochemicals during storage (Del Pozo-Insfran, Balaban & Talcott, 2006$_b$) since there was a reduction in the oxidation rate of these compounds. It is worth emphasizing that the whey-grape juice drink is not exclusively made from grape since it contains whey in the formulation. Therefore, anthocyanins levels were lower when compared to natural grape juice. It is also worth mentioning the need for final treatment in the elaboration of the whey drink, as is usually done in the processing units. In addition, no differences were observed in the anthocyanins levels of strawberry juice processed at 30 MPa (45 ° C/30 min) when compared to the untreated juice, which decreased slightly when processed at 60 MPa (45 ° C/30 min) with significant differences (about 3%) from the raw samples (Marszałek, Skąpska, Woźniak, Sokołowska, 2015).

A small reduction of anthocyanins levels was observed in heat-treated (75 ° C/15 s) and SCCD-treated hibiscus juice (30.6 MPa/40 ° C/6.8 min). The CO_2-treated juice was similar to the pasteurized juice, with significant differences from the control, with a loss of about 3%. Similar behavior was observed for the phenolics levels, with no significant differences in the antioxidant capacity (Ramírez-Rodrigues et al., 2012). Fabroni, Amenta, Timpanaro & Rapisarda (2010) observed that thermal pasteurization (88-91 ° C/30 s) was more detrimental to the antioxidant compounds of orange juice than the SCCD treatment (230 and 130 bar/36 ± 1 ° C/15 min) since it retained more compounds at levels similar to the untreated juice. In orange juice, the antioxidant behavior was similar to that observed in the present study, and the different CO_2

concentration (3.91 or 1.96 L/h CO_2) did not affect the retention of antioxidants (Fabroni, Amenta, Timpanaro&Rapisarda, 2010). In addition, the authors observed that lower operating pressures contribute to the retention of antioxidant compounds, especially ascorbic acid, probably due to the displacement of the dissolved oxygen in the liquid matrix (Fabroni, Amenta, Timpanaro&Rapisarda, 2010).

Previous studies have shown the SCCD treatment as a viable alternative for the preservation of phytochemical compounds of juices due to less formation of inhibitory compounds such as furfural. Phenolics and anthocyanins degradation is presumably due to the formation of carbohydrates and organic acids degradation products during thermal processing, such as furfural and other carbonyl compounds that may form condensation products with anthocyanins and polyphenols (Fabroni, Amenta, Timpanaro & Rapisarda, 2010; Del Pozo-Insfran, Balaban, & Talcott, 2006b). Another benefit of this technology is the absence of dissolved oxygen, which is critical in preventing the degradation of phytochemical antioxidant compounds (Del Pozo-Insfran, Balaban, &Talcott, 2006b). Angiotensin-converting enzyme (ACE) is responsible for the cleavage of two important substrates involved in the regulation of blood pressure, angiotensin, and bradykinin (Rai, Sanjukta & Jeyaram, 2017). Hypertension (blood pressure > 140/90) is one of the risk factors for coronary heart disease and stroke and is one of the leading causes of mortality in developing countries (Lin, Lv, Li, 2012).

The SCCD-treated samples presented relevant ACE inhibitory activity (Table 1), and the sample treated at 180 bar presented the highest potential, followed by those subjected to 160 bar and 140 bar (44.31, 38.75, and 34.63%, respectively). The pasteurized drink had a lower ACE inhibitory activity, 23.89%. Recent studies have been discussed the ACE inhibitory capacity in foods. Several factors can affect the production and activity of compounds with this potential, including the food composition, bacteria, processing conditions, and storage (Rai, Sanjukta&Jeyaram, 2017; Susmita, 2013). Some *in vitro* and *in vivo* studies have shown the relationship between phytochemical compounds, such as flavonoids, and ACE activity (Kwon, Vattem & Shetty, 2006; Actis-Gorettaa, Ottaviani, Keenb & Fraga, 2003). In contrast, other studies have not observed this correlation in several flavonoid-rich fruit extracts, suggesting that non-phenolic compounds may also be responsible for the ACE inhibitory activity (Susmita, 2013; Apostolidis, Kwon & Shetty, 2007). Also, it is believed that the phenolic profile, that is, the qualitative composition of phenolics is more important than the total content estimated in the analysis.

Milk and dairy products are provided with functional peptides, derived from caseins, with ACE inhibitory activities (Rai, Sanjukta & Jeyaram, 2017). ACE inhibitor peptides are found in ripened cheeses, even at short ripening periods, and may be due to proteolysis during ripening (Smacchi & GobbettI, 1998; Addeo, et al. 1992). Our results showed that the SCCD treatment of the whey-grape juice drinks was an important factor in the ACE inhibitory activity, probably exposing bioactive peptides or interacting with components of grape juice. Further studies are needed to better understand the effect of SCCD on the beverage components, assessing their profile and bioavailability in the human body.

3.3 Volatile Compounds

Thirty-one peaks (Table 2) were identified in the beverages of this study, of which 2-heptanone, eucalyptol, 1-hexanol, 1-octanol, α-terpineol, phenyl ethyl alcohol, and 2,5-dimethyl-Benzaldehyde have previously been identified in grape juices (Burdock, 2001; Kalua&Boss, 2010; Lee,Lin,Cha, Luo& Chen, 2016). The volatile organic compounds in grape juice, which are quite varied, are responsible for the aroma, and originate in the grape itself or during the fermentation process, which comprises hundreds of compounds from different chemical groups (Lee, Lin, Cha, Luo & Chen, 2016). In grapes, the free volatiles can contribute directly to the aromas whereas the attached glycosidic forms are odorless and considered flavor precursors (Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande &Simal-Gándara, 2013; Verzera et al., 2008). The compounds 2-heptanone and 4-hydroxydihydro-2(3h)-furanone can be found in some fruits, such as grapes, pear, papaya, and strawberry, but also in dairy products such as butter and cheese (Burdock, 2001). In addition, 2-nonanone, 2-octanone, 2-undecanone, 8-Nonen-2-one have already been identified in milk and dairy products such as butter, yogurt, and ripened cheeses (McNaught & Wilkinson 1997; Burdock, 2001; Lamsen & Zhong, 2011).

Compounds responsible for the taste and aroma of whey can vary due to many factors, including the source of whey, storage, and processing conditions (Carunchia Whetstine et al., 2005). It is worth mentioning that the distinct behavior of volatile compounds may be due to several factors, including the different extraction methods (Lamsen&Zhong, 2011).

Table 1. pH, soluble solids, titratable acidity, total phenolic compounds, anthocyanin (total, momeric and polymeric), DPPH and ACE activity of whey-grape juice drink*.

Analysis		**Control**	**HTST**	**140 bar**	**160 bar**	**180 bar**
pH		3.6 ± 0.01[a]	3.6 ± 0.01[a]	3.6 ± 0.01[a]	3.6 ± 0.01[a]	3.6 ± 0.01[a]
Soluble Solids		15.7 ± 0.1[a]	15.8 ± 0.1[a]	15.8 ± 0.1[a]	15.8 ± 0.1[a]	15.8 ± 0.1[a]
Titratable Acidity		0.44 ± 0.02[a]	0.44 ± 0.01[a]	0.44 ± 0.02[a]	0.45 ± 0.01[a]	0.45 ± 0.01[a]
Compounds Phenolic		1.8 ± 0.01[a]	1.8 ± 0.03[a]	1.8 ± 0.01[a]	1.8 ± 0.04[a]	1.7 ± 0.03[a]
	Total	7.1 ± 0.09[a]	6.5 ± 0.37[a]	6.9 ± 0.43[a]	7.2 ± 0.15[a]	6.7 ± 0.31[a]
Anthocyanins	**Monomeric**	2.0 ± 0.23[b]	1.5 ± 0.18[b]	3.2 ± 0.34[a]	3.5 ± 0.22[a]	3.11 ± 0.67[a]
	Polymeric	5.1 ± 0.31[a]	5.0 ± 0.31[a]	3.7 ± 0.51[b]	3.7 ± 0.32[b]	3.6 ± 0.54[b]
DPPH		27.3 ± 3.06[a]	31.3 ± 1.26[a]	29.4 ± 3.85[a]	28.6 ± 1.29[a]	25.7 ± 3.09[a]
ACE		19.2 ± 0.16[e]	23.8 ± 0.67[d]	34.6 ± 1.12[c]	38.7± 1.05[b]	44.3 ± 1.21[a]

*Results are presented as the mean ± standard deviation. Control (without processing), HTST (72 °C/15 s) and SCCD technology (14,16,18 MPa). pH is adimensional. Soluble solids are expressed in °Brix. Titratable acidity is expressed in g tartaric acid/L. Total phenolic compounds is expressed in Gallic acid equivalents per liter of sample. Anthocyanins (total, momeric and polymeric) are expressed in mg malvidin-3-glucoside/100 mL. ACE (angiotensin-converting enzyme activity) is expressed in %. DPPH (1,1-diphenyl-2-picrylhydrazyl radical activity) is expressed in %. abcdeDifferent letters in the same column denote difference according the Tukey test ($p < 0.05$

Table 2. Volatile compounds of whey grape juice drink*

Compounds	LRI	Control	HSTS	140 bar	160 bar	180 bar
Ketones						
2-Heptanone	1161	--------	X	X	X	X
2-Octanone	1268	--------	X	X	--------	--------
2-Nonanone	1376	--------	X	X	X	X
8-Nonen-2-one	1432	--------	X	X	--------	--------
2-Undecanone	1589	--------	X	--------	--------	--------
Isophorone	1592	X	X	X	X	X
Dihydroxyacetone	2066	--------	--------	X	--------	--------
Acids						
2-Butenoic acid, ethyl ester, (Z)-	1140	X	X	X	X	X
Acetic acid	1438	X	X	X	X	X
Butanoic acid, 3-hydroxy-, ethyl ester	1506					
Butanoic acid	1616	X	X	X	--------	X
Hexanoic acid	1829	X	--------	X	X	X
Octanoic acid	2040	X	--------	X	X	X
n-Decanoic acid	2250	--------	--------	X	--------	--------
Alcohols						
3-Methyl-1-Butanol	1197	X	X	X	X	X
1,4-Cineol	1152	x	--------	--------	--------	--------
Eucalyptol	1187	X	--------	--------	--------	--------
1-Hexanol	1338	X	X	X	X	X
3-Hexen-1-ol	1370	X	--------	--------	--------	--------
(E)-2-Hexen-1-ol	1391	X	X	X	X	X
2-Ethyl-1-Hexanol	1475	X	--------	--------	--------	--------
1-Octanol	1543	--------	X	--------	--------	
2-Furanmethanol	1643	--------	--------	X	--------	--------
α-Terpineol	1687	X	X	X	X	X
Phenylethyl Alcohol	1895	X	X	X	X	X
1,2-Ethanediol, 1-(2-furanyl)-	2540	X	--------	--------	--------	--------
Others						
2-Propen-1-amine	1195	X	X	--------	X	X
Acetoin	1293	X	X	--------	X	--------
Hydroxyacetone	1295	--------	--------	X	--------	--------
Furfural	1453	X	X	X	X	X
Phenol, 2-nitro-	1805	X	X	X	X	X
2,5-Dimethyl-Benzaldehyde	1808	X	X	--------	X	--------
Dihydroxyacetone	2066	--------	--------	X	--------	--------
5-Hydroxymethylfurfural	2474	--------	--------	X	--------	--------
Dihydro-4-hydroxy-2(3H)-Furanone	2574	--------	--------	X	--------	--------

* LRI/= Linear Retention Index on CP-Wax 52 CB according to the Van der Dool and Kratz equation. Organized by families. x= presence, ----- = absence Control (without processing), HSTS (72 °C/15s) and SCCD technology (140,160,180 bar).

All the whey-grape juice drinks of the present study, the alcohols constituted a volatiles fraction. The absence of eucalyptol and 2-ethyl-1-hexanol was observed in all treatments, in addition to the compounds 2-propen-1-amine, acetoin, and 2,5-dimethyl-benzaldehyde in the sample subjected to 140 bar. Severe heat treatments can cause the loss of volatile compounds, altering the sensory or functional characteristics of the product (Fellows, 2006). The absence of certain compounds during the treatments indicates degradation with the formation of other compounds. Furthermore, in the SCCD processing, probably CO_2 dissolved the volatiles that was entrained during the depressurization. However, to minimize possible losses of volatiles during the SCCD process, these compounds should be recovered at the exit system and added back to the beverage (Ramírez-Rodrigues et al., 2012). In addition, the presence of 1-octanol in the drink treated by HTST, and 2-furanmethanol in the drink treated at 140 bar was also observed. The compound 2-furanmethanol has been found in wines and is related to bitterness (Flamini, 2008). The compound (E)-2-hexen-1-ol present in all samples may be due to the damages to fruit, from the enzymatic reactions of linolenic and linoleic acids from grape (Bamforth & Ward, 2014). The moderate heat treatment may induce the hydrolysis of some compounds resulting in free forms (Coelho, Rocha, Barros, Delgadillo, Coimbra, 2007), suggesting an enrichment of aromas (Lee, Lin, Cha, Luo & Chen, 2016). The presence of three other compounds including dihydroxyacetone, 5-hydroxymethylfurfural, and 4-hydroxydihydro-2 (3H)-furanone was also observed in the samples subjected to 140 bar.

SSCD technology contributed to the presence of ketones, since the compounds isophorane, 2-heptanone, and 2-nonanone were detected in all treatments. In addition, 2-undecanone and dihydroxyacetone were also identified in the HTST-treated drink and drink treated at 140 bar, respectively, while 2-octanone, 8-nonen-2-one were found in both beverages. The compound 2-nonanone is found in dairy products and alcoholic beverages, such as wine (Burdock, 2001). In this context, 3-methyl-1-butanol is the main higher alcohol in alcoholic beverages, and is present in cider, mead, beer, wine, and beverages with varying degrees, being obtained from starch fermentation (Kostrubsky et al., 2015). Isofuran can also be found in wines, as a result of the degradation of certain carotenoids, known for its peppermint-like aroma (Panighel et al., 2014). Dihydroxyacetone may also appear in the vinification process and can affect the sensory quality of the sweet wine, besides participating in glycolysis and being an intermediary product of the metabolism of fructose (Boulton et al., 1999).

Regarding the organic acids, the presence of n-decanoic acid was detected in the treatment at 140 bar, unlike the other treatments. Still, the absence of two compounds was observed for the HTST treatment, including hexanoic and octanoic acids. Butanoic acid was not observed in the treatment at 160 bar. The compounds n-decanoic acid, hexanoic acid, and butanoic acid are members of the fatty acids series found in oils and animal fats and are related to rancid, cheesy, waxy, and sour odor (Atkins & Paula, 2006). Some studies have shown the higher efficiency of SCCD treatments when compared to conventional pasteurization treatments. A considerable loss (88%) of volatiles was observed in HTST-treated hibiscus juice, with a slight decrease (21%) in alcohols and ketones in the beverage processed by SCCD (34.5 MPa /40°C/6.5 min) (Ramírez-Rodrigues et al., 2012). Similar results were observed for melon juice, which evidences the effects of SCCD (35 MPa/55°C/60 min) on the higher volatiles retention when compared to the conventional heat treatments (Chen et al., 2010).

4 CONCLUSION

The treatment of whey-grape juice drink with supercritical technology, using carbon dioxide as a high-pressure pasteurizing agent did not change the physicochemical properties of the beverages such as pH, soluble solids, titratable acidity, and bioactive compounds (phenolics and anthocyanins) when compared to the conventional heat treatment HTST. However, the SCCD treatment altered the volatiles profile of the beverage, although this alteration was not enough to effectively modify the characteristics of the final product. The main physicochemical response observed in the SCCD treatment was in relation to the angiotensin converting enzyme inhibitory activity since a positive linear effect was observed on the inhibition effectiveness when increasing the CO_2 pressure, that is, the higher the pressure used, the higher the inhibition levels. Therefore, the results of the present study confirm that the pasteurization using CO_2 supercritical technology may be a viable alternative for processing of whey-grape juice drink, since the physicochemical characteristics of the beverage remained intact, with positive effects on the angiotensin converting enzyme inhibitory activity.

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CAPÍTULO IV

SUPERCRITICAL CO_2 PASTEURIZATION OF WHEY-GRAPE JUICE DRINK: PHYSICAL AND SENSORY PROPERTIES

Artigo submetido ao Periódico *Journal of Supercritical Fluids*

RESUMO

O uso da tecnologia supercrítica como processo de pasteurização a frio da bebida de suco de uva e soro de uva foi investigado neste estudo. Os efeitos do dióxido de carbono supercrítico em 14, 16 e 18 MPa (35 ± 2 °C / 10 min) nas propriedades físicas e sensoriais da bebida, quando comparados à pasteurização convencional (tratamento térmico a 72 °C / 15 s) Foram avaliados. O processamento de CO_2 de alta pressão da bebida de suco de soro de soro de leite resultou em um produto com menor diâmetro de partícula, menor índice de consistência e uma redução no caráter pseudoplástico em comparação com a bebida tratada pelo processo convencional. Não foi observado efeito de CO_2 de alta pressão nos atributos sensoriais da bebida para os níveis estudados. Os consumidores não encontraram diferenças entre as bebidas tratadas com CO_2 e as bebidas tratadas termicamente. Nossas descobertas sugerem o uso da tecnologia supercrítica com dióxido de carbono como uma alternativa efetiva para a produção e disponibilidade de uma bebida promotora de saúde e bem-estar.
Palavras-chave: Processamento não térmico; Fluido supercrítico; bebida láctea.

ABSTRACT

The use of supercritical technology as a non-thermal pasteurization process of the whey-grape juice drink was investigated in this study. The effects of supercritical carbon dioxide at 14, 16, and 18 MPa (35 ± 2°C/10 min) on the physical and sensory properties of the beverage, when compared to conventional pasteurization (heat treatment at 72°C/15 s) were evaluated. High-pressure CO_2 processing of whey-grape juice drink resulted in a product with lower particle diameter, lower consistency index, and a reduction in pseudoplastic character when compared to the beverage treated by the conventional process. No effect of high-pressure CO_2 was observed on the sensory attributes of the beverage for the levels studied. Consumers did not find differences between the CO2-treated and heat-treated beverages. Our findings suggest the use of supercritical technology with carbon dioxide as an effective alternative for the production and availability of a health and wellness promoting beverage.
Keywords: Non-thermal process; supercritical fluid; whey - drink.

1 INTRODUCTION

Whey-grape juice drink is a beverage made from whey and grape juice, being considered as a refreshing way to consume health promoting compounds. Whey protein is a recognized source of essential amino acids, bioactive peptides, antioxidants and immunopotentiators, which confer a number of functions in human body, such as elimination of free radicals, anti-inflammatory, antitumor, immunostimulatory, hypotensive, homeostatic action, antiobesity and antidiabetic properties, action on muscle biosynthesis, osteoprotection and radioprotection (Patel, 2015; Sinha, Radha, Prakash, & Kaul, 2007). In parallel, grape juice is appreciated worldwide and is rich in vitamins, minerals, fibers, and bioactive phenolic compounds such as anthocyanins and proanthocyanidins, which are antioxidant and anti-inflammatory polyphenols (Perestrelo, Lu, Santos et al., 2012; Ruggiero,. Vitalini, Burlini, et al., 2013).

The processing of whey-grape juice drink using emerging non-thermal technologies is an interesting alternative for the availability of safe foods for consumption, with nutritional and sensory characteristics closer to those of the product in natura. In contrast, the conventional heat treatments can lead to the degradation of thermosensitive nutrients, besides affecting the taste of the food due to the cooking process (Cappelletti, Ferrentino, Endrizzi, et al., 2015; Spilimbergo, Komes, Vojvodic, et al., 2013).

Among the emerging non-thermal technologies, supercritical technology, especially using supercritical carbon dioxide technology (SCCD) as a pasteurizing agent, has gained prominence in recent years for being a clean and environmentally friendly technology, in addition to presenting relatively low operating costs (Ferrentino & Spilimbergo, 2017). Food processing with SCCD results in a cold pasteurization, once the critical CO_2 pressure and temperature conditions are 7.38 MPa and 31.1°C, respectively (Brunner, 2005; Gasperi, Aprea, Biasioli, et al., 2009). Several studies have reported microbial and enzymatic inactivation using supercritical technology (Perrut, 2012). Other authors have also reported preservation of nutritional and sensory properties of natural juices subjected to SCCD. The most recent publications evaluated the effects of the supercritical CO_2 on mulberry juice (Zou, Lin, Bi, et al., 2016), strawberry juice (Marszałek, Skąpska, Woźniak, et al, 2015), coconut water (Cappelletti, Ferrentino, Endrizzi, et al., 2015), among others. In addition, studies on milk pasteurization using SCCD have been reported (Hongmei, Zhong, Liao, et al., 2014; Kobayashi, Odake, Miura, et al., 2016; Amaral, Silva, Cavalcanti, et al., 2017). However, few studies have evaluated the effects of SCCD processing on the physicochemical and sensory properties of the products.

The physical properties of a product are fundamental in the evaluation of its quality, being decisive in consumers' acceptance. Thus, the purpose of this study was to evaluate the effects of supercritical technology, as a non-thermal pasteurization process on the quality of whey-grape juice drink, with emphasis on physical and sensory properties.

2. MATERIAL AND METHODS

2.1 Whey-Grape Juice Drink Processing

The following ingredients were used for the preparation of whey-grape juice drink: 50% (v/v) of whole red grape juice (Mitto®, Brazil, natural juice without preservatives, sugar or sodium) and 50% (v/v) whey powder (Alibra®, Brazil) reconstituted in distilled water, according to the manufacturer's instructions. About 0.108 g xanthan gum (Satiaxane™, Cargill, Spain) and 7.53 g sucrose (Union®, Brazil) were added per 100 g of sample. After mixing the ingredients, the beverage was homogenized in a rotor-stator type homogenizer (Walita®, Brazil) for 5 min.

The whey-grape juice drink was subjected to conventional thermal processing known as high-temperture shor-time (HTST) at 72°C for 15 s in a temperature-controlled water bath (Marconi®, MA126 / BO, Piracicaba, Brazil). The temperature of the sample was monitored using a digital thermometer (Digital Thermometer Hanna®, Check temp, HI98501, Nusfalau, Romania). The experiment was performed in duplicate. The samples were also subjected to SCCD technology at 14, 16, and 18 MPa and 35 ± 2°C for 10 min. A homemade equipment was used assembled by the LASEFI (Laboratory of Supercritical Technology: Extraction, Fractionation, and Identification of Vegetal Extracts) research group – Department of Food Engineering (DEA)/School of Food Engineering (FEA), University of Campinas (UNICAMP)/Brazil. Figure 1 shows the flow diagram of the experimental apparatus.

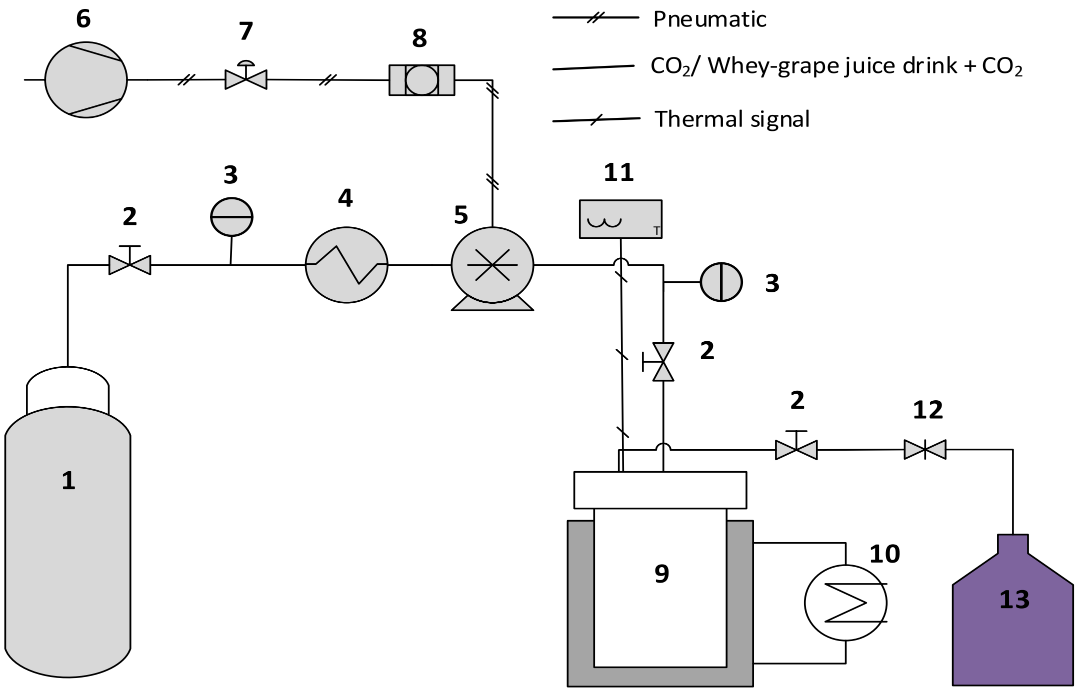

Figure 1. Diagram of the homemade equipment for the supercritical pasteurization. 1 – CO_2 Cylinder; 2 – Control valve; 3 – Manometer; 4 – Cooling bath; 5 – Pump (Booster); 6 – Air compressor; 7 – Control valve (air flow); 8 – Air filter; 9 – Batch reactor; 10 – Heating bath; 11 – Temperature indicator; 12– Micrometer valve; 13 – Whey-grape juice drink + CO_2.

A volume of 400 mL of whey-grape juice drink was manually introduced into the 316 stainless steel reactor, fitted with a flange closure system, with a maximum capacity of 630 mL. Carbon dioxide (99.9% CO2, Gama Gases Especiais Ltda, São Bernardo do Campo, Brazil) cooled at - 6°C in a thermostatic bath was pumped by a pneumatic pump to the high-pressure reactor, promoting the homogenization of the sample until reaching the operating pressure. The 10-minute processing time was counted from the temperature stabilization at 35 ± 2 ° C, monitored by means of a digital thermocouple. The selection of the process conditions in this study was based on the study reported by Gasperi, Aprea, Biasioli, et al. (2009), who found that supercritical CO_2 at 10 MPa and 36°C for 10 min was effective for the pasteurization of apple juice.

At the end of each treatment, the equipment was depressurized and the sample collected. All procedures of hygiene and asepsis were carefully carried out during the experiments, considering the microbiological safety and quality aspects. All experiments were performed in duplicate.

2.2 Microbiological Quality

The enumeration of mesophilic bacteria, thermotolerant coliforms, molds, and yeasts was carried out to evaluate the microbiological quality of the samples. The analyses were performed in triplicate according to the methodology established by the American Public Health Association, APHA (2015). The samples were collected aseptically in a sterile vessel, immediately after processing.

For mesophiles bacterial counts, serial dilutions of 10^{-1}, 10^{-2}, 10^{-3} were plated into Plate Count Agar (PCA, Kasvi), and incubated at 30 °C for 72 h. The thermotolerant coliforms were enumerated by the multiple-tube method. The inoculation was done in Lauryl Tryptose Broth (LST, Kasvi®) with inverted Durham tubes, incubated for 48 hours at 37 °C, and the results were expressed as the most probable number (MPN/mL).

Yeast and mold counts were determined by surface plating on Dichloran Rose Bengal Chloramphenicol (DRBC, Kasvi®) agar, incubated at 25 °C for 5 to 7 days. For both analyses, the viable colonies were counted manually using a colony counter (Bantex Colony Counter 920A, Burlingame, CA) and the values were expressed in Log 10 CFU/g.

2.3 Physical Properties

2.3.1 Color

Color measurements were performed in a Color Quest XE Spectrophotometer (Hunter Associates Laboratory, City, USA) in glass cuvettes with an optical path of 10 mm in diameter. About 10 readings were performed for each sample. The measurement was made in the CIEL a* b* system, using the D65 illuminant and previously calibrated with white reference standards (L = 92.03; a* = - 0.88; b* = 0.63). The parameters determined were: L indicating brightness, ranging from 100 (white) to zero (black), green/blue positive, red negative, and gray neutral.

2.3.2 Particle size distribution

The size distribution and mean particle diameter of the beverages were determined by the laser diffraction technique using the Mastersizer 2000 equipment (Malvern Instruments Ltd, Malvern, UK). The measurements were performed at 25°C immediately after pasteurization. The mean diameter was determined considering the mean diameter of a sphere of similar area, mean surface diameter (D_{32}), Equation 1, and the mean diameter of a sphere of the same volume, Brouckere's diameter (D_{43}), Equation 2. The dispersion index (Span) was determined according to Equation 3. The samples were analyzed in triplicate for each drink, by wet dispersion method, using a refractive index of 1.52.

$$D_{32} = \frac{\sum n_i {d_i}^3}{\sum n_i {d_i}^2} \tag{1}$$

$$D_{43} = \frac{\sum n_i {d_i}^4}{\sum n_i {d_i}^3} \tag{2}$$

$$span = \frac{(d_{90} - d_{10})}{d_{50}} \tag{3}$$

Where: d_i is the mean particle diameter, n_i is the number of particles, and D_{10}, D_{50}, and D_{90} are the particle diameters at 10%, 50%, and 90% cumulative distribution, respectively.

2.3.3 Rheological assays

Flow curves were obtained using a stress-controlled rheometer (AR1500ex, TA Instruments, England) with stainless steel flat plate geometry (4 cm) and a 200 µm gap. The shear rate varied between 0 and 300 s−1, and the flow curves were obtained using an up-down-up steps program. The third flow curve data were fitted to the models for power-law fluids (Eq. 4).

$$\sigma = k(\dot{\gamma})^n \tag{4}$$

Where σ is the shear stress (Pa), is the shear rate (s−1), k is the consistency index (Pa.sn) and n is the flow behavior index (dimensionless).

The parameters k and n were estimated by non-linear regression using the Quasi-Newton method with a convergence criterion of 10-4 using Statistica 8® software. The model was evaluated according to the coefficient of determination (R2) and mean relative percentage deviation modulus (E), as shown in Equation (5). Measurements were made in triplicate at 25°C.

$$E = \frac{100}{N} \sum_{i=1}^{N} \left| \frac{m_i - m_p}{m_i} \right| \tag{5}$$

Where mi is the experimental value, mp is the predicted value, and N is the population of the experimental data.

2.3.4 Physical stability

The physical stability of the beverages was monitored through visual inspection with the optical scanning instrument Turbiscan ASG (Formulaction, l'Union, France). The whey-grape juice drink freshly prepared were placed in flat-bottomed cylindrical glass tubes (140 mm, height; 16 mm, diameter), and the first measure of backscattered light intensity was performed. The tubes were stored at 25°C, and backscattered light was scanned after 24 h. Beverage destabilization was analyzed using backscattering (BS) profiles, with scans at 880 nm.

2.4 Sensory Evaluation

Fifty assessors aged 16-38 years, both gender (57.5% female and 42.5% male), with different instruction levels, consumers of dairy beverage and grape juice, participated in the test. The analyses were performed in individual cabins with controlled temperature (20 °C) and incandescent lighting. The pasteurized beverages (14 MPa, 16 MPa, and 18 MPa) cooled at 7 °C were packed in plastic cups (30 mL) and coded with 3 random digits. All samples were evaluated in a single section, with a balanced order of presentation (Macfie, Bratchell, Greenhoff, et al., 1989). The treated beverages were evaluated using a 9-point hedonic scale (9 = extremely liked; 5 = neither liked nor disliked, and 1 = disgusted extremely), where consumers were asked to score the attributes appearance, aroma, flavor, texture, and overall impression. The purchase intention was evaluated using a 5-point scale (5 = I would certainly buy; 3 = I doubt if I would buy, and 1 = would certainly not buy).

2.5 Statistical Analysis

The results were expressed as the mean ± standard deviation. The effects of treatments were analyzed by analysis of variance (ANOVA) followed by the Tukey's test ($p\text{-value} \leq 0.05$). The intention to purchase was assessed by means of logistic regression, based on the binomial scale (0/1). Statistical analyses were performed using Statistica 10 statistical package (StatSoft®, Tulsa, OK, EUA).

3. RESULTS AND DISCUSSION

3.1 Microbiological Results

Bacterial counts lower than 1 CFU/mL were observed in the microbiological analyses of whey-grape juice drink for all treatments (HTST, 14 MPa, 16 MPa, 18 MPa). The effect of the pasteurization processes on the reduction of the microbiological load of the beverage was not studied, once the control (untreated) drink also presented counts lower than 1 CFU/mL. These findings in the control drink can be due to the use of quality raw materials and good handling practices. Therefore, these results demonstrated that the formulated drink is safe for consumption. As it was not possible to evaluate the effects of the supercritical pasteurization, new studies with the inoculation of contaminating microorganisms may be carried out to evaluate the effect of CO_2 pressure on the reduction of the microbial load of whey-drink type drinks.

Several studies have demonstrated the efficacy of microbial inactivation through SCCD processing in different plant products such as apple juice (Gasperi, Aprea, Biasioli, et al. 2009), coconut water (Cappelletti, Ferrentino, Endrizzi, et al., 2015), hibiscus drink (Ramírez-Rodrigues, Plaza, Azeredo, et al., 2012), strawberry juice (Marszalek, Skapska, Wozniak, et al., 2015), among others. Pozo-Insfran, Balaban, and Talcott (2006) evaluated the effects of supercritical pasteurization using CO_2 on grape juice. The authors inoculated yeast strains (8.1×10^6 CFU/mL) and aerobic microorganisms (1×10^5 CFU/mL) in grape juice and incubated at 21°C for 4 days. A log reduction greater than 5 log was observed in the condition of 34.5 MPa and residence time of 6.25 min at 30 ° C, using 16% of CO_2.

The effectiveness of the pasteurization of whole and skim milk using SCCD was also evaluated by different authors (Hongmei, Zhong, Liao, et al., 2014; Werner & Hotchkiss, 2006). Recently, Ceni, Silva, Valério, et al. (2016) studied a continuous pasteurization system with SCCD and found a microbial inactivation rate of 0.09 min-1 in raw milk processed at 8 MPa and 70°C, with a residence time of 30 min.

3.2 Effects Of The Supercritical Pasteurization On The Physical Properties

3.2.1 Color measurements

The results of the color measurements of the beverages are shown in Table 1. In general, the parameters L and a * had similar behavior. A reduction of these values was observed in the treated samples when compared to the control (untreated), suggesting a decrease in the intensity of the bright red color, which was imperceptible to the naked eye. Similar results were observed for the color parameters of the control and HTST samples, as well as the beverages treated with SCCD at 14 and 16 MPa. The beverage subjected to 18 MPa differed from the others in relation to the parameters L and a*. Regarding the parameter b*, the control sample showed greater intensity in blue color, followed by the SCCD-treated beverage, without significant differences between them, which were different from the HTST-treated sample that exhibited less intensity of blue color.

Products containing high anthocyanins levels, such as grape juice, are susceptible to color deterioration during processing and storage due to the combined effects of anthocyanins degradation and formation of dark pigments (Martynenko & Chen, 2016). However, although a significant difference was observed between treatments, numerically the difference was small. Thus, the effects of the pasteurization processes on the color of the whey-grape juice drink were not visually perceptible when compared to the control beverage (untreated).

Zou, Lin, Bi, et al. (2016) compared three different pasteurization treatments of mulberry juice, naturally rich in anthocyanins. The authors used High Hydrostatic Pressure (HHP) (500 MPa / 5 min), SCCD (15 MPa/55 ° C/10 min), and HTST (110 °C/8.6 s), and observed no significant difference in color of the treated samples when compared to the control.

3.2.2 Particle size distribution

All treatments led to a significant reduction (p-value < 0.05) in the particle size of the beverage when compared to the control. The results of the effect of treatments on size distribution and particle size are shown in Figure 2 and Table 1, respectively.

Although similar peak heights were observed, the CO2-treated samples (14, 16, and 18 MPa, 35°C/10 min) formed a peak at 10 μm in diameter, presenting a bimodal distribution, with the second peak between 100 and 1000 μm. The HTST pasteurized sample and control (untreated) presented a distribution with larger particle sizes, with maximum peak height close to 100 µm. In these two samples, there is a formation of a second discrete peak on the right, evidencing a more pronounced bimodal distribution when compared to the CO_2-treated beverages.

The lower particle size distribution of the SCCD-treated beverages is due to the homogenization effect promoted by high-pressure CO_2. The microstructure of the beverage ingredients, mainly related to the cellular material coming from grape juice, when submitted to high pressures, undergoes a process of rupture due to the tension applied to the product, leading to the formation of smaller structures, with smaller particle diameter. HTST pasteurization reduced about 18% of D_{32}, while a reduction of 62% was observed in the CO_2-treated samples. Similar behavior was observed for D_{43}. Whereas D_{32} is more influenced by the smaller particles, and D_{43} by greater particles, the results evidenced a considerable increase in the number of particles with smaller mean diameter in the whey-grape juice drink processed by supercritical technology (Kubo, Augusto & Cristianini, 2013). The results confirm the homogenization effect of CO_2 pressure on the beverage (Table 1). The *span* expresses the degree of uniformity of the size distribution and the smaller the value, the narrower the distribution, thus implying a more uniform process.

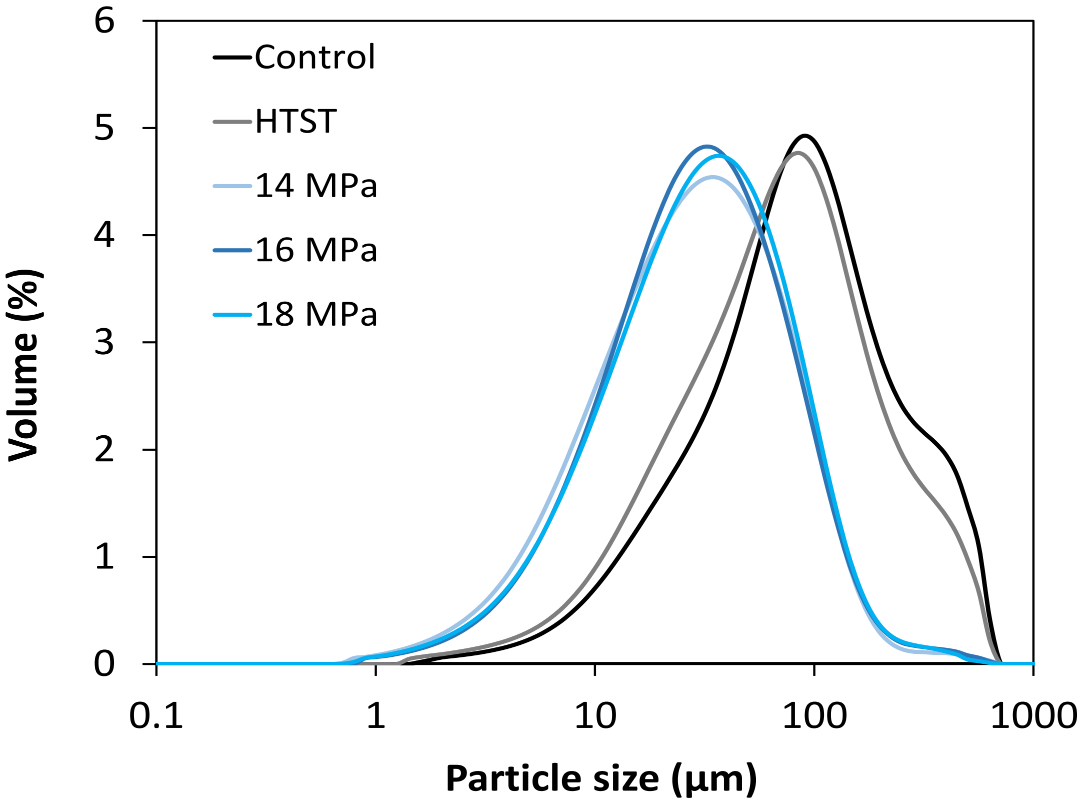

Figure 2. Particle size distribution of whey-grape juice drink without treatment (control), submitted to supercritical carbon dioxide processing (14, 16 and 18 MPa, 35°C/10 min) and conventional heat treatment (HTST, 72°C/15 s).

The lower particle size distribution of the SCCD-treated beverages is due to the homogenization effect promoted by high-pressure CO_2. The microstructure of the beverage ingredients, mainly related to the cellular material coming from grape juice, when submitted to high pressures, undergoes a process of rupture due to the tension

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The *span* expresses the degree of uniformity of the size distribution and the smaller the value, the narrower the distribution, thus implying a more uniform process.

The effect of SCCD on the reduction of mean particle diameter was also observed in apple juice processed at 22 MPa and 60°C for 10 minutes, with lower intensity, with a reduction of approximately 5% in D_{43} and 10% in D_{32} (Xu, Zhang, Wang, at al., 2011). Zhou, Wang, Hu, et al. (2009) evaluated the effect of SCCD on carrot juice subjected to 10, 20, and 30 MPa at different process times (15-60 min). The authors found that the untreated juice and the juice processed in the three pressure conditions, in the longer process time, did not differ statistically in relation to and. They also observed that, regardless of the pressure evaluated, at a process time of less than 60 min, the mean particle diameters were higher than those of the untreated beverage.

The greater effect of SCCD on the reduction of particle diameter in the present study when compared to studies on apple juices (Xu, Zhang, Wang, at al., 2011) and carrot (Zhou, Wang, Hu, et al., 2009) may be due to the complexity of whey-grape juice drink from the point of view of its formulation, once it contains whey, xanthan gum, and sugar. Xanthan gum, in particular, is a food additive used as a stabilizing agent to ensure the physical characteristics of emulsions and suspensions. In this sense, it is capable of retarding any type of agglomeration and sedimentation in beverage formulations, thus avoiding the formation of complexes or agglomerates, which can lead to an increase in the particle diameters, as observed in the size distribution of the processed beverage.

Table 1. Color parameters and particle size distribution of whey-grape juice drink*.

Sample	Color Parameters			Particle size distribution						
	L	a *	b *	D_{32}	D_{43}	d_{10}	d_{50}	d_{90}	Span
Control	$13.51^a \pm 0.10$	$7.08^a \pm 0.14$	$-2.58^c \pm 0.2$	$45^a \pm 4$	$136^a \pm 9$	$21^a \pm 2$	$91^a \pm 8$	$331^a \pm 15$	$3.4^a \pm 0.2$
HTST	$13.62^a \pm 0.06$	$6.85^b \pm 0.09$	$-1.56^a \pm 0.34$	$37^b \pm 5$	$115^b \pm 6$	$17^b \pm 2$	$76^b \pm 9$	$272^b \pm 8$	$3.4^a \pm 0.4$
14 MPa	$11.98^b \pm 0.08$	$6.58^b \pm 0.06$	$-2.10^b \pm 0.08$	$16^c \pm 1$	$44^c \pm 1$	$7^c \pm 1$	$30^c \pm 1$	$95^c \pm 4$	$2.9^b \pm 0.02$
16 MPa	$11.76^b \pm 0.35$	$6.50^b \pm 0.25$	$-2.08^b \pm 0.13$	$17^c \pm 1$	$46^c \pm 8$	$8^c \pm 1$	$31^c \pm 4$	$97^c \pm 2$	$2.8^b \pm 0.3$
18 MPa	$11.45^c \pm 0.21$	$6.24^c \pm 0.31$	$-1.98^b \pm 0.05$	$17^c \pm 1$	$47^c \pm 3$	$8^c \pm 1$	$33^c \pm 3$	$98^c \pm 1$	$2.8^b \pm 0.1$

* Results are presented as the mean ± standard deviation. Control (without processing), HSTS (72°C/15 s) and SCCD technology (14, 16 and 18 MPa; 35°C/10 min). L, a*,b* are dimensionless. D_{32}, D_{43}, D_{10}, D_{50}, D_{90} and span are expressed in µm. [abc] Different letters in the same column denote difference according the Tukey test (p-value < 0.05).

Table 2. Rheological characteristics (apparent viscosity, consistency indices and flow behaviour index) and statistical parameters of the whey-grape juice drink*.

Sample	Apparent viscosity at 101 s^{-1}	Consistency index (k)	Flow behaviour index (n)	R^2	E (%)
Control	$2.9^a \pm 0.6$	$9^{ab} \pm 3$	$0.76^{ab} \pm 0.02$	0.999	4.3
HTST	$3.0^a \pm 0.2$	$12^a \pm 1$	$0.71^c \pm 0.01$	0.998	4.5
14 MPa	$2.0^a \pm 0.3$	$5.0^b \pm 0.4$	$0.81^a \pm 0.02$	0.998	5.6
16 MPa	$1.9^a \pm 0.1$	$4.3^b \pm 0.3$	$0.82^a \pm 0.02$	0.998	5.4
18 MPa	$1.9^a \pm 0.3$	$4.4^b \pm 0.4$	$0.82^a \pm 0.01$	0.998	5.5

* Results are presented as the mean ± standard deviation. Control (without processing), HSTS (72°C/15 s) and SCCD technology (14, 16 and 18 MPa; 35°C/10 min). Apparent viscosity at 100 s^{-1} and consistency index is expressed in mPa.s. Flow behavior index is dimensionless. R^2 = coefficient determination and E is the mean relative percentage deviation modulus [abc] Different letters in the same column denote difference according the Tukey test (p-value < 0.05).

3.2.3 Rheology parameters

The rheological flow curves were adjusted to the Power Law model (Table 2), commonly used to describe the rheological behavior of dairy beverages (Cruz, Cavalcanti, Guerreiro, et al., 2013). The results indicated a good fit of the model in the description of the experimental data, with a high coefficient of determination ($R^2 \geq 0.998$) and low mean relative percentage deviation modulus ($E \leq 5.6$).

The different treatments did not affect the apparent viscosity of the beverages (p-value> 0.05). However, the consistency index (K) and the flow behavior index (n) were influenced by the treatments (p-value < 0.05). All samples presented a pseudoplastic behavior, evidenced by n values lower than 1. Pseudoplastic fluids are characterized by the decrease of apparent viscosity as a function of the deformation rate applied.

The conventional HTST pasteurization led to an increase in the consistency index of the beverage and conferred a more pseudoplastic character to the whey-grape juice drink. Possibly this effect is related to a higher molecular interaction between the ingredients due to the increase in temperature of the heat treatment, which may allow greater molecular mobility, resulting in a greater interaction of macromolecules in the product (xanthan gum, sugars, whey proteins). These interactions may lead to the formation of aggregates, with an increase in k value and a decrease in n value.

On the other hand, the treatments with CO_2 at high pressure resulted in lower k values and higher n values. As previously discussed, the stress applied to the beverage during SCCD processing led to the rupture of aggregates, leading to smaller structures with lower diameter, resulting in a beverage with lower k and rheological behavior relatively closer to that of a Newtonian fluid.

Liu, Hu, Zhao, et al. (2012) evaluated the effect of SCCD pasteurization on watermelon juice when compared to thermal pasteurization. The authors observed no difference in the apparent viscosity of watermelon juice subjected to CO_2 processes under pressure conditions of 10 and 20 MPa when compared to the untreated juice. However, they found that the heat treatment (95°C/1 min) led to a significant increase in this parameter. On the other hand, different results were recently described by Zou, Lin, Bi, et al. (2016), studied the rheological properties of mulberry juice subjected to HHP (500 MPa/5 min), SCCD (15 MPa/55°C/10 min), and HTST (110°C/8.6 s) when compared to the untreated juice. The authors reported no changes in apparent viscosity and k and n values in SCCD-treated mulberry juice when compared to the control, while the HTST-treated juice maintained the same n value, with a significant reduction in viscosity and k value.

Those findings corroborate the present study, which clearly shows that food products are complex systems characterized by the presence of components with different physicochemical properties. Thus, the modeling of these systems for use in other products will always be error-prone, since changing a single component of the formulation can lead to a completely opposite to expected behavior.

Although similar studies with dairy products have not been reported, Zhong and Jin (2008) have observed that the gelling properties of whey proteins (whey protein isolate and whey concentrate) in water or powder have improved with SCCD treatment (10 and 30 MPa, 65°C), indicating that high-pressure CO_2 processing can cause structural changes in milk proteins, thus contributing to changes in the rheological behavior as observed in the whey-grape juice drink.

3.2.4 Physical stability

Figure 3 shows the results of the physical stability regarding the phase separation of the whey-grape juice drink submitted to the different pasteurization processes. The results were obtained by monitoring the backscattering profile (BS) as a function of height (mm) of glass tubes containing the beverages, considering the initial time of 0 h (immediately after treatment) and after 24 h of storage at 25°C.

The equipment detection system performs a sweep from the bottom to the top of the glass tube, recording the BS data (%) as a function of height (mm). The principle of the measurement is based on the fact that the BS is dependent on the variation of the volumetric fraction or particle diameter. For particles that do not absorb light at 880 nm, the BS profile depends solely on the concentration and particle diameter and the refractive index of the mixture (Pizzino, Rodriguez, Xuereb, et al., 2007).

In general, similar the BS profiles were observed for time 0 h of all samples immediately after processing, except for the lower part of the tubes containing the HTST pasteurized beverage and the beverage subjected to 18 MPa, which showed slightly higher BS values (%). This result may be due to the higher concentration of particles in the region due to the beginning of the phase separation process.

After 24 h of storage, a reduction of BS (%) values from approximately 20% to 7% was observed in the region of 0 to 7 mm for all beverages, except for HTST-treated sample, which presented a reduction up to 4%. The abrupt fall of BS (%) in the region of 0 to 7 mm evidenced the phase separation due to the sedimentation phenomenon. Figure 4 shows the visual appearance of the beverages immediately after the SCCD or HTST processing, and after the 24 h-storage periods. It is worth noting that regardless of the process, all beverages had the same visual appearance after processing and storage, with no significant differences between them. The reduction of BS (%) values up to 4% in the HTST-treated sample may be due to a higher concentration of particles with a larger diameter at the bottom of the tube. Although the control beverage had particles with a larger diameter, heat from the HTST may have favored the formation of aggregates, as previously discussed, resulting in lower BS (%) when compared to the other samples due to the concentration at the bottom of the tube.

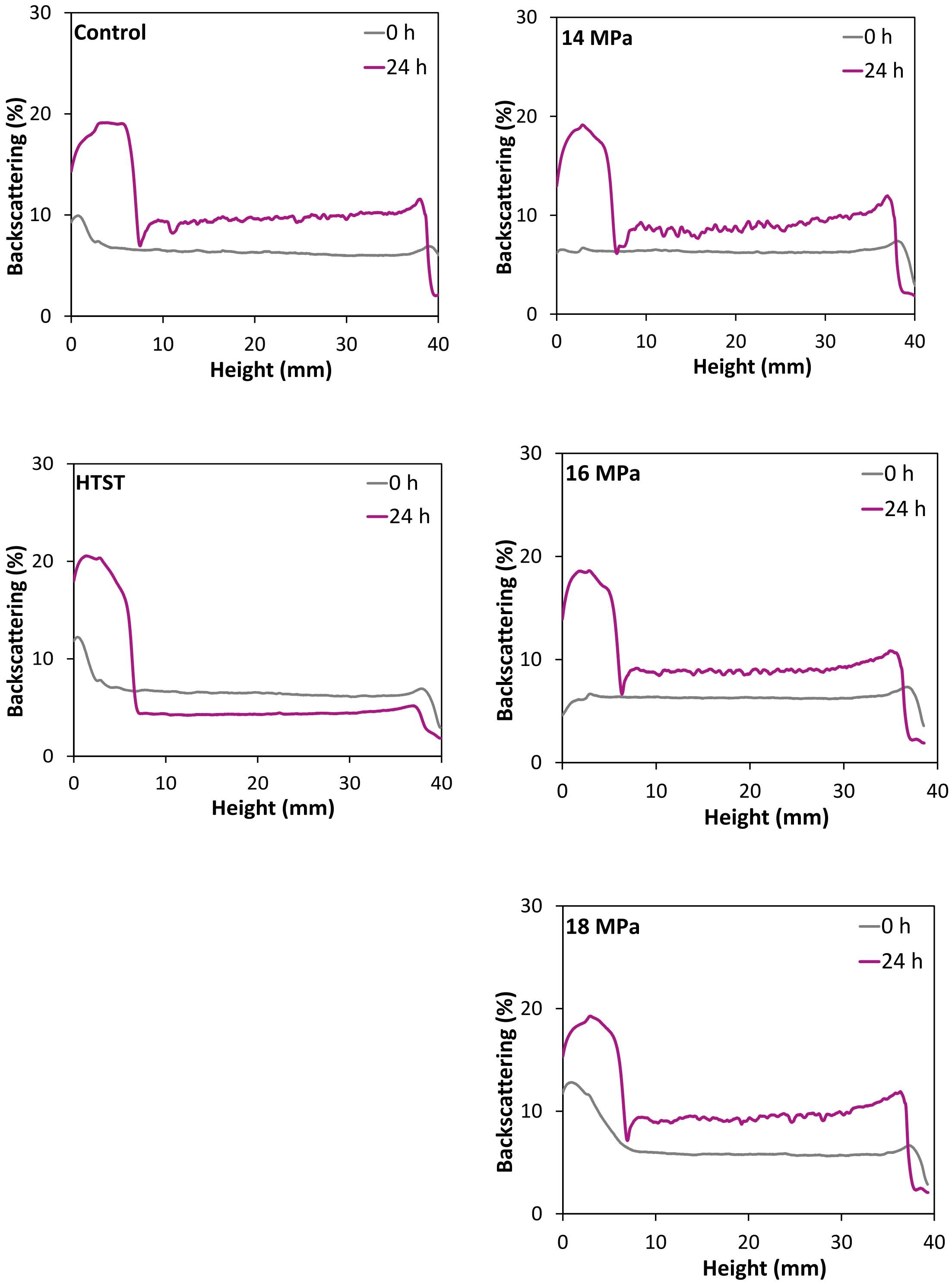

Figure 3. Backscattering profile of whey-grape juice drink without treatment (control), submitted to supercritical carbon dioxide processing and conventional heat treatment (HTST) immediately after processing (0 h) and after refrigerated storage (24 h).

Fresh beverage **After storage (24 h)**

Figure 4. Visual aspect of whey-grape juice drink immediately after processing (0 h) and after refrigerated storage (24 h) at 25°C.

3.3 Sensory Evaluation

No significant differences (p-value> 0.05) were observed for all sensory attributes evaluated (Table 3). However, the HTST-treated sample exhibited the lowest scores in almost all attributes. The beverage subjected to SCCD at 16 MPa scored better on most of the attributes, with the highest scores for appearance, flavor, and texture. The sample subjected to 18 MPa received the highest score for the attribute flavor, while the sample treated at 14 MPa stood out in the overall impression. In general, the scores for appearance, aroma, flavor, and overall impression were 6, represented by "I liked it lightly", while the texture scores ranged from 6 represented by "I liked it lightly" to 7 "I liked it moderately".

Table 3. Consumer test of whey-grape juice drink*.

Sample	Appearance	Aroma	Flavor	Texture	Overall acceptance
HTST	5.88 ± 1.84	6.15 ± 1.56	6.15 ± 2.09	6.35 ± 1.78	5.88 ± 1.98
14 MPa	6.13 ± 1.79	6.33 ± 1.37	6.33 ± 1.80	6.78 ± 1.51	6.45 ± 1.52
16 MPa	6.18 ± 1.50	6.18 ± 1.47	6.38 ± 1.88	6.78 ± 1.67	6.35 ± 1.51
18 MPa	6.00 ± 1.55	6.40 ± 1.26	6.03 ± 1.70	6.60 ± 1.53	6.10 ± 1.69
Pr > F	0.856	0.835	0.828	0.609	0.422
Significant	No	No	No	No	No

* Results are presented as the mean ± standard deviation. HSTS (72°C/15 s) and SCCD technology (14, 16 and 18 MPa; 35°C/10 min). Mean data from 50 consumers and based on a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). The results were not statistically different, according to the Tukey test (p-value < 0.05).

The results of this study corroborate with those reported by Pozo-Insfran, Balaban, and Talcott (2006), who studied the differences between grape juice subjected to SCCD (34.5 MPa at 8 and 16% CO2, 6.25 min, and 30°C) and juice treated with

HTST (75°C/15 s) using a 10-point hedonic scale and 30 assessors. The authors reported that the SCCD-treated grape juices received higher scores for overall acceptance when compared to HTST-treated juices, indicating a higher preference for juices processed by SCCD. Similarly, Cappelletti, Ferrentino, Endrizzi, et al. (2015) verified through a panel of 16 trained assessors, that coconut water treated with high pressure CO_2 (12 MPa/40°C/30min) was similar to the coconut water in natura (untreated), which was significantly different from the HTST-treated coconut water (90°C/1 min).

The intention to purchase was assessed using a logistic regression, based on the binomial scale (0/1), where the scores of 1, 2, and 3 were coded as 0 (zero) and the scores 4 and 5 as one (1), as proposed by Cruz, Cadena, Faria, et al. (2011). According to the results (Table 4), the logistic regression indicated that the attributes did not significantly affect the purchase intention of the SCCD-treated samples, while the attribute texture contributed most significantly (p = 0.052) to the purchase intention of the HTST-treated sample.

Table 4. Parameter estimates, probability, and odds ratio estimates to predict purchase intent of whey-grape juice drink*.

Sample		**Appearance**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptance**
HTST	Parameter	-1.769	1.327	1.547	2.682	0.207
	Pr > x2	0.067	0.213	0.072	0.052	0.766
	odds ratio	0.170	3.771	4.697	14.620	1.230
14 MPa	Parameter	0.446	-0.884	0.353	0.470	0.429
	Pr > x2	0.267	0.071	0.315	0.180	0.368
	odds ratio	1.563	0.413	1.423	1.599	1.535
16 MPa	Parameter	-0.718	0.616	0.366	1.075	0.649
	Pr > x2	0.176	0.272	0.392	0.057	0.350
	odds ratio	0.488	1.851	1.442	2.929	1.913
18 MPa	Parameter	-0.371	0.031	0.470	0.269	1.161
	Pr > x2	0.375	0.938	0.282	0.517	0.090
	odds ratio	0.690	1.031	1.599	1.309	3.192

* Based on the logistic regression analysis, using a full model with five sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Significance of parameter estimates was based on the Wald X^2 value at $P < 0.05$. HSTS (72°C/15 s) and SCCD technology (14, 16 and 18 MPa; 35°C/10 min).

4 CONCLUSION

The results obtained in this study showed that the use of supercritical CO_2 as a non-thermal pasteurization process is an effective alternative for the production of whey-grape juice drink. The processing of the beverage with high-pressure CO_2 resulted in a beverage with a lower particle diameter, a lower consistency index, and a less pseudoplastic character when compared to the HTST-treated beverage (conventional process). These results showed that the supercritical pasteurization acted as a process of homogenization of the beverage. The sensory attributes of the beverage were not affected by the CO_2 pressure levels (14, 16, and 18 MPa). Consumers did not discriminate beverages treated with supercritical CO_2 when compared to the HTST-treated beverages. Our findings suggest the supercritical carbon dioxide technology can be used for the processing of whey-grape juice drink since no significant differences in the sensory acceptance were found between the CO_2-treated beverages and the beverage obtained by the conventional pasteurization process.

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CONCLUSÃO GERAL

A tecnologia do DCSC é uma técnica inovadora e promissora para o tratamento de leite e produtos lácteos e tem o potencial de melhorar a formulação de produtos lácteos que afetam de forma positiva o valor nutricional, ao mesmo tempo em que fornece a segurança dos produtos finais.

A tecnologia empregada no grape whey drink não alterou as propriedades físico-químicas das bebidas, que permaneceram intactas quando comparados ao tratamento térmico convencional HTST. Os atributos sensoriais da bebida também não foram afetados.

O perfil volátil da bebida foi alterado, embora essa alteração não tenha sido suficiente para efetivamente modificar as características do produto final. A principal resposta fisicoquímica observada no tratamento com DCSC foi o efeitos positivos na atividade inibitória da enzima conversora de angiotensina, uma vez que se observou um efeito linear positivo na eficácia da inibição ao aumentar a pressão do CO_2. Além do fato da pasteurização supercrítica ter atuado como um processo de homogeneização da bebida.

Nossas descobertas sugerem que a pasteurização usando tecnologia supercrítica de dióxido de carbono é uma alternativa efetiva para a produção de grape whey drink.