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THIAGO SOARES LEITE

FROZEN CONCENTRATED ORANGE JUICE PROCESSED BY HIGH PRESSURE HOMOGENEZATION TECHNOLOGY

PROCESSAMENTO DE SUCO DE LARANJA CONCENTRADO E CONGELADO POR TECNOLOGIA DE HOMOGENEIZAÇÃO A ALTA PRESSÃO

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Dissertation presented to the School of Food Engenieering of University of Campinas in partial fulfillment of the requirements for the degree of Master in food technology

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Orientador: Marcelo Cristianini
Co-orientador: Pedro E. D. Augusto

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Abstract

Brazil is the world's largest orange producer, most of the production being intended for the manufacture of frozen concentrated orange juice (FCOJ). Due to the large export volume and high consistency (apparent viscosity) of FCOJ, knowledge of its rheological properties becomes necessary for a better design of the equipments and processes. In high-pressure homogenization (HAP), a non-thermal processing technology, the product is pressurized and forced through a small gap that dramatically increases its speed, followed by a sudden drop to atmospheric pressure, causing high shear stress and cavitation. These phenomena are able to change the structure of cells and biopolymers, impacting on the particle size and the way they interact, thus changing the rheological characteristics. In the present study this technology was used aiming at decreasing the consistency and other rheological properties. Even small reductions in viscosity can result in great energy savings in various unit operations, such as pumping, given the large volume of juice processed in Brazil. The objective of this work was to evaluate the influence of high pressure homogenization on the rheological steady-state behaviour (time independent and time dependent properties), *Arhenius* activation energy, dynamic rheological behaviour (viscoelasticity), particle size distribution, microstructure and colour of FCOJ, as well as the pulp sedimentation stability of the reconstituted product, the turbidity and colour were also evaluated. The influence of the number of HPH cycles, on rheological behaviour (steady-state) e on particle size distribution of FCOJ was also verified. HPH decreased the consistency of FCOJ as the pressure increased from 0 to 150MPa. At 150MPa the apparent viscosity was decreased to approximately half its original value. The thixotropy and viscoelasticity of the concentrated juice were also reduced during HPH, and the value of the *Arhenius* activation energy had its value increased, indicating that a minor change in temperature produces a significant change in consistency. The particle size was reduced, and the particle distribution changed, showing a higher ratio of small particles over larger ones.

These changes could be observed from the microstructure. The FCOJ or diluted product showed no difference in colour, neither due to affect by the homogenization nor for the storage time. HPH slighty decreased the viscosity of the RTD-juice. None of the above mentioned parameters changed during the storage time. The pulp sedimentation behaviour was not affected by HPH. Multiple HPH cycles at lower pressures are an alternative to a single HPH at higher pressure.

Keywords:

Non thermal process, high pressure homogenization, rheology, frozen concentrated orange juice.

Resumo Geral

O Brasil é o maior produtor mundial de laranja, sendo que a maior parte desta produção é destinada a fabricação do suco concentrado congelado (*Frozen concentrated Orange Juice* - FCOJ). Devido ao seu grande volume de exportação e sua alta consistência (viscosidade aparente), o conhecimento de suas propriedades reológicas se torna necessária para um melhor dimensionamento de equipamentos e processos. Na homogeneização a alta pressão (HAP), uma tecnologia de processamento não térmica, o produto é pressurizado e forçado a passar por um pequeno orifício aumentando drasticamente sua velocidade, fazendo a pressão diminuir para a atmosférica, causando forte cisalhamento e cavitação. Estes fenômenos causam alteração na estrutura de células e biopolímeros, impactando no tamanho das partículas e na maneira como se interagem, alterando assim, as características reológicas do produto. O presente trabalho se baseou na utilização desta tecnologia em FCOJ visando diminuição da sua consistência, e alteração de demais comportamentos reológicos, para gerar uma economia de energia em diversas operações unitárias como bombeamento, congelamento, transporte e estocagem. Reduções mesmo que pequenas na consistência possibilitam grande economia de energia devido aos grandes volumes de suco processado no Brasil. Este trabalho teve como objetivo avaliar a influência da alta pressão de homogeneização (HAP) no comportamento reológico em estado estacionário (independente e dependente do tempo). Foram avaliados também, a energia de ativação de *Arrhenius*, comportamento reológico dinâmico (viscoelasticidade), distribuição de tamanho de partículas, microestrutura e cor do suco concentrado, bem como a estabilidade à sedimentação, turbidez e cor do produto reconstituído. Também foi verificada a influência do numero de ciclos de homogeneização nos comportamentos reológicos (estado estacionário) e tamanho de partícula do suco de laranja concentrado. A consistência do FCOJ foi reduzida com o aumento de pressão no processamento de HAP. Para processos a 150 MPa a viscosidade aparente reduziu para cerca de metade do valor original, a tixotropia e viscoelasticidade também tiveram suas magnitudes reduzidas. A energia de ativação de *Arrhenius* teve seu valor aumentado com a utilização de HAP, indicando que pequenas variações de temperatura acarretam em variações significativas nos valores de consistência. O tamanho de partículas foi reduzido, bem como no perfil de distribuição das mesmas. Houve um aumento na proporção total de partículas pequenas em relação às partículas maiores. Estas mudanças foram possíveis de perceber pela microestrutura observada com microscópio óptico. As cores dos produtos concentrado ou diluído não apresentaram alterações devido à pressão. A HAP não praticamente não reduziu a viscosidade do suco diludo (RTD). Nenhum dos parâmetros estudados apresentou mudanças devido ao tempo de estocagem. O comportamento da sedimentação de polpa também não sofreu alterações com a HAP. Os múltiplos ciclos de HAP a baixas pressões apresentaram ser uma alternativa ao ciclo único com pressão elevada.

Palavras-chave: processos não térmicos, homogeneização a alta pressão, reologia, suco de laranja concentrado e congelado.

“O professor sábio sabe que cinquenta e cinco minutos de trabalho mais cinco minutos de risada valem o dobro do que sessenta minutos de trabalho invariável.”

Gilbert Highet

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Sumário

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Chapter 2:

$\dot{\gamma}$ = shear rate [s^{-1}]

η = viscosity [Pa·s]

η_a = apparent viscosity (= σ / $\dot{\gamma}$) [Pa·s]

σ = shear stress [Pa]

σ_0 = yield stress, Herschel-Bulkley's model (*Equation 2.4*) [Pa]

σ_e= equilibrium stress in the Figoni-Shoemaker model (*Equation 2.3*) [Pa]

σ_i = initial stress in the Figoni-Shoemaker model (*Equation 2.3*) [Pa]

d = particle diameter [μm]

D[4,3] = particle volume-based diameter (*Equation 2.1*) [μm]

D[3,2] = particle area-based diameter (*Equation 2.2*) [μm]

Ea = Activation Energy of Arrhenius equation (*Equation 2.5*) [kJ/K.mol]

k = consistency index, Herschel-Bulkley and Ostwald-de-Waele model (*Equation 2.4* and *Equation 2.8*) [Pa·s^n]

k_0 = Adjustment parameter for Arrhenius (*Equation 2.5*) [Pa·s^n]

k_{FS} = kinetic parameter in the Figoni-Shoemaker model (*Equation 2.3*) [s^{-1}]

n = flow behaviour index, Herschel-Bulkley and Ostwald-de-Waele models (*Equation 2.4* and *Equation 2.8*) [-]

P_H = homogenization pressure [MPa]

t = time [s]

T = absolute temperature [K]

Chapter 3:

α = linear modification of Cox-Merz Rule, *Equation 3.8* [-]

β = linear modification of Cox-Merz Rule, *Equation 3.9* [-]

$\dot{\gamma}$ = shear rate [s^{-1}]

η = viscosity [Pa·s]

η_a = apparent viscosity [Pa·s]

$\eta*$ = complex viscosity [Pa·s]

σ = shear stress [Pa]

σ_0 = yield stress [Pa]

ω = oscillatory frequency [Hz]

G' = storage (elastic) modulus [Pa]

G" = loss (viscous) modulus [Pa]

G* = complex modulus [Pa]

k' = consistency coefficient for the storage modulus power law (*Equation 3.1*) [$Pa.s^{n'}$]

k" = consistency coefficient for the loss modulus power law (*Equation 3.2*) [$Pa.s^{n''}$]

n' = behaviour coefficient for the storage modulus power law (*Equation 3.1*) [-]

n" = behaviour coefficient for the loss modulus power law (*Equation 3.2*) [-]

P_H = homogenization pressure [MPa]

tan δ = loss tangent = G''/G' [-]

Chapter 4:

$\dot{\gamma}$ = shear rate [s-1]

η_a = apparent viscosity (= σ / $\dot{\gamma}$) [Pa·s]

σ = shear stress [Pa]

σ_0 = yield stress [Pa]

σ_e= equilibrium stress in the Figoni-Shoemaker model (*Equation 4.3*) [Pa]

σ_i = initial stress in the Figoni-Shoemaker model (*Equation 4.3*) [Pa]

d = particle diameter [μm]

D[4,3] = particle volume-based diameter (*Equation 4.1*) [μm]

D[3,2] = particle area-based diameter (*Equation 4.2*) [μm]

k = consistency index, Ostwald-de-Waele model (*Equation 4.4*) [$Pa·s^n$]

k_{FS} = kinetic parameter in the Figoni-Shoemaker model (*Equation 4.3*) [s^{-1}]

n = flow behaviour index, Ostwald-de-Waele model (*Equation 4.4*) [-]

PH = homogenization pressure [MPa]

t = time (Equation 4.4) [s]

Chapter 5:

$\dot{\gamma}$ = shear rate [s^{-1}] (Equation 5.5)

μ = viscosity [Pa.s] (Equation 5.5)

IS = (Pulp) Sedimentation index (Equation 5.1) [-]

ISe = Sedimentation index at equilibrium (infinite time) (Equation 5.4) [-]

ISi = Initial value of sedimentation index (time 0) (Equation 5.4) [-]

kS = kinetic parameter in the sedimentation index model (Equation 5.4) [day-1]

PH = homogenization pressure [MPa]

t = time (Equation 5.2) [days]

Capítulo 1. Revisão Bibliográfica e Objetivos

1.1 Introdução

Laranja é um dos dez produtos agropecuários mais produzidos no país, sendo o Brasil o maior produtor do mundo desde a década de 1990. Somente no ano de 2010 a produção superou 19 milhões de toneladas, sendo que cerca de 80% desta produção se destina para a obtenção de sucos industrializados. Somente as exportações de suco de laranja do mesmo ano somaram US$ 1,775 bilhão, aumento próximo de 50% comparado com as exportações do ano de 2003, evidenciando que o mercado continua em crescimento (FAO, 2013; MAPA 2013, Valor Econômico, 2011).

As tecnologias de Homogeneização a Alta Pressão (HAP), alta pressão hidrostática, campo elétrico pulsado (PEF), luz ultra-violeta (UV) e luz pulsada são exemplos de tratamentos não térmicos que estão em constante pesquisa para combinar a mesma eficiência na inativação microbiana que possuem os tratamentos térmicos convencionais, porém com uma maior retenção das características, sensoriais, químicas e físico-químicas do produto se comparadas aos processos tradicionais. Nas indústrias, farmacêutica, cosmética, química e de alimentos, a homogeneização a alta pressão é usada para a preparação ou estabilização de emulsões e suspensões, ou para promover mudanças físicas no produto, como alteração da consistência (Diels e Miches, 2006).

A ciência conhecida como reologia, pode ser definida como o estudo do escoamento e deformação dos materiais, dependendo se o material for líquido ou sólido (Steffe, 1996). O escoamento de materiais líquidos pode se comportar de maneiras diferentes com relação ao fluxo e ao tempo de escoamento, podendo classifica-los dentre vários modelos (Rao, 2005, Steffe, 1996 e Urbecain e Lozano, 1997),

O conhecimento das propriedades reológicas de um produto, como a viscosidade para fluidos newtonianos e os índices de consistência e comportamento e tensão inicial para fluidos não newtonianos é importante, tanto para o processo como para o dimensionamento de bombas e tubulações bem como para a estabilidade e qualidade do produto final e otimização de processos, formulação de produtos e correlação com características sensoriais e consequente aceitação pelo consumidor (Falguera e Ibarz, 2010; Rao, 2005; Tavares et al., 2007; Urbecain e Lozano, 1997 e Vélez-Ruíz et al., 2002).

1.2. Homogeneização a alta pressão (HAP)

A tecnologia de homogeneização a alta pressão se baseia no processo comum de homogeneização usado em produção de leite, porém utilizando pressões maiores (PAQUIN, 1999).

Ao contrário do processo de alta pressão isostática, o processo HAP é contínuo, no qual um produto fluido é bombeado por intensificadores de pressão forçando-o a passar por um orifício estreito (*gap*) em uma válvula de alta pressão composta por um cabeçote e um anel de impacto e um cabeçote de passagem. Neste momento o fluido é acelerado repentinamente pela redução de área de escoamento causando drástica queda de pressão atingindo a pressão atmosférica, desencadeando um processo de alta cavitação, turbulência e alta tensão de cisalhamento. São estes efeitos que causam as alterações no produto e não a elevada pressão como ocorre com a alta pressão isostática (Campos, et al. 2003, Floury, et al., 2004a, Paquin, 1999, Pinho et al., 2011 e Augusto et al., 2012a) A pressão de trabalho é atingida pelo sistema entre os intensificadores e a válvula de homogeneização, onde ocorre à repentina despressurização, o efeito isostático é desprezível, pois o produto fica sob pressão por poucos instantes (Campos, et al. 2003).

A *Figura 1.1* apresenta a esquemática de um tipo válvula de homogeneização:

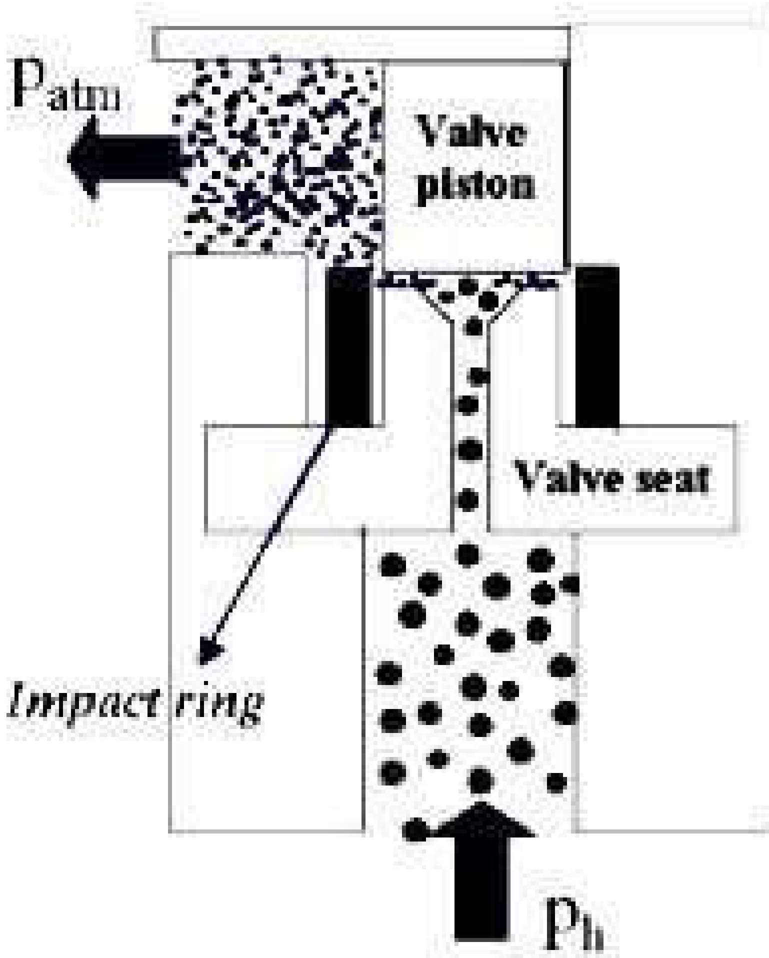

Figura 1.1: Geometria de uma válvula de homogeneização (Floury et al., 2004b).

A fluidodinâmica envolvida no processo é complexa, ocorrendo intensas mudanças energéticas com o produto que passa de uma zona de alta para uma de menor pressão, como a consequente conversão de energia potencial (de pressão) em energia cinética e, devido ao atrito com o equipamento, em energia térmica; o aumento da temperatura se torna mais evidente conforme o aumento da pressão de trabalho, sendo que se eleva de 2,0 a 2,5°C a cada 10 MPa de pressão (Floury et al., 2004a, Mckay et al., 2011, Popper e Knoor, 1990 apud Diels e Michels, 2006 e Pinho et al., 2011).

O efeito gerado pela homogeneização a alta pressão acarreta na diminuição do tamanho de partículas, rompimento de membranas de microrganismos e até mesmo promovendo alterações em macromoléculas ou coloides (Floury et al., 2004, Floury, et al., 2004b, Paquin, 1999, Campos et al., 2003, Pinho et al., 2011 e Augusto et al., 2012a).

Muitos estudos foram publicados sobre o efeito da homogeneização a alta pressão para a inativação de microrganismos em diversas matrizes, como suco de maçã (Mckay et al. 2011, Maresca et al., 2011, Pathanibul et al. 2009, Bevilacqua et al., 2012), suco de manga (Tribst et al., 2009, Tribst et al., 2011), cerveja (Franchi et al., 2011a e Franchi et al., 2011b), suco de cenoura (Pathanibul et al. 2009), suco de abacaxi (Maresca et al., 2011), produtos de soja (Poliseli-scopel et al., 2012, Torrezan, 2007), polpa de açaí (Alibert, 2009), suco de banana (Calligaris et al., 2012), leite (Pinho et al., 2011, Kheadr et al., 2002) e suco de laranja (Maresca et al., 2011, Belloch et al., 2012, Campos e Cristianini, 2007). Entretanto, existem poucos estudos relacionados às alterações microestruturais, funcionais e tecnológicas em produtos de frutas.

A tecnologia de homogeneização a alta pressão também afeta estruturas muito menores que células de microrganismos, como observado nos estudos com diversos polissacarídeos, tais como CMC, goma guar, hidroxietilcelulose (Villay et al., 2012), amido (Le Thanh-Blicharz et al., 2012), metilcelulose (Floury et al., 2002) e pectina (Augusto et al., 2012a). Observam-se ainda estudos em diversas proteínas, como proteína de soro de leite (Grácia-Juliá et al., 2008), β-lactoglobulina e α-lactalbumina bovinas (Mazri et al., 2012), além de estudos sobre bioacessibilidade in vitro de carotenóides (Svelander et al., 2011), sobre o efeito da HAP na atividade e desnaturação de diversas enzimas, como lipoxigenase de leite de soja (Poliseli-Scopel et al., 2012), Fosfatase alcalina e lactoperoxidase em leite desnatado (Pinho et al. 2011), α-amilase (Tribst e Cristianini, 2012) e proteases (Tribst et al. 2012, Tribst e Cristianini, 2012).

Augusto et al., (2012a), Augusto et al., (2012b) e Augusto et al., (2012c) estudaram o efeito da homogeneização a alta pressão sobre o comportamento reológico em estado estacionário, dependentes do tempo e viscoelasticidade em suco de tomate e em modelo de soro, simulando a porção do suco sem a polpa em suspensão, além do efeito desta tecnologia na distribuição de tamanho de partículas. A HAP resultou no aumento da consistência e comportamento tixotrópico do suco. Em relação aos parâmetros do modelo de Herschel-Bulkey (HB), o índice de consistência (k) decresceu com a pressão de homogeneização, efeito oposto para o índice de comportamento e tensão residual (n e σ_0) acarretando um aumento da viscosidade aparente (η_a). Observa-se que os resultados foram diferentes dos obtidos em trabalhos com outros produtos vegetais, como o de Silva et al. (2010), que estudou o efeito da polpa de abacaxi tratada pela homogeneização a alta pressão, onde ocorreu a redução do índice de consistência e aumento do índice de comportamento resultando na redução da viscosidade aparente, da mesma forma que para brócolis e cenoura Lopez-Sanchez, et al. (2011a).

Portanto, como demonstra Lopez-Sanchez et al. (2011b), cada matriz vegetal pode reagir de formas diferentes ao tratamento por homogeneização a alta pressão, já que algumas células podem precisar de maior tensão de cisalhamento para se romper, como no caso de cenoura, em contrapartida com tomate, cujas paredes celulares são rompidas mesmo em tensões menores.

Em relação ao tamanho de partículas, Augusto et al. (2012a e 2012b) estudaram o efeito da homogeneização nas propriedades no suco de tomate. Embora a viscosidade do soro tenha diminuído, o suco com a fração insolúvel teve o índice de consistência aumentado, mesmo com a redução do tamanho médio das partículas. Tal comportamento se deve ao fato das partículas insolúveis, quando reduzidas, aumentarem a sua área superficial, aumentando assim as interações partícula-partícula e partícula-soro. Portanto, é o balanço entre as alterações nas frações solúveis e insolúveis, que irá ditar o comportamento do fluido. Destaca-se novamente que cada matriz vegetal pode reagir de formas diferentes ao tratamento por homogeneização a alta pressão.

Além do estudo da pressão de homogeneização em diversas propriedades em produtos, também é possível o estudo de múltiplos ciclos de homogeneização como os estudos de Tribst et al., (2013), Caligaris et al., (2012), Welti-Chanes et al., (2009) Maresca et al., (2011), Donsi et al., (2009) and Patrignani et al., (2009). Visa-se, com mais passagens em menores pressões o mesmo efeito de passagens simples em pressões maiores. Equipamentos de menores pressões sofrem menos desgastes, além de possuírem maior capacidade e em geral seu custo fixo é menor.

1.3 Propriedades reológicas do suco de laranja concentrado

Os sucos de frutas são compostos por duas frações: o soro que contem água e todos os compostos solúveis como açúcares, polissacarídeos solúveis, sais e ácidos; e a polpa, que são as fibras e todo material insolúvel disperso, como células integras e seus fragmentos, paredes celulares e polímeros insolúveis (Augusto et al,. 2012a).

Por causa da enorme variação em sua estrutura e composição, os alimentos se comportam de várias formas, podendo ser Newtonianos, não-Newtonianos dependentes do tempo e viscoelásticos. Além disso, um mesmo alimento pode apresentar mais de um tipo de comportamento, dependendo de sua origem, concentração e modificações causadas previamente (Rao, 2005).

A fração solúvel tem comportamento Newtoniano, como para o soro de tomate (Augusto et al., 2012a), ou pseudoplástico comportando-se quase como um Newtoniano (índice de comportamento é próximo de 1,0), como no soro de abacaxi (n=0,94; Silva et al.,2010).

O soro do suco de laranja tende para um fluido newtoniano. Vitali e Rao (1984a) constataram que o soro do suco despectinizado concentrado de laranja (65°Brix) apresentou comportamento newtoniano. Entretanto, mesmo amostras com menos de 1% de pectina já apresentavam comportamento pseudoplástico, com o índice de comportamento entre 0,85 a 0,92.

Vários estudos podem ser encontrados caracterizando o suco de laranja concentrado, cujos comportamentos são semelhantes mesmo se alterando a variedade (Vitali e Rao, 1984a e Vitali e Rao, 1984b).

Telis-Romero et al. (1999) estudaram a mudança dos parâmetros reológicos do suco concentrado de laranja variedade Pera-Rio, com a mudança de temperatura (0,5~62°C) e concentração (34~73% de água). Constatou-se que o fluido apresenta tensão inicial (σ_0) maior quanto menor a temperatura e porcentagem de água. Os autores encontraram que a relação entre a tensão (σ) e a taxa de deformação (γ) não é linear, onde foi ajustado o modelo de Heschel-Bulkley ($\sigma = \sigma_0 + \kappa.\gamma^n$), sendo que o índice de comportamento (n) diminuiu quanto menor o conteúdo de água e maior a temperatura.

Branco e Gasparetto (2003) estudaram as características de um suco concentrado de laranja (64,2°Brix, teor de polpa 10%) em diversas temperaturas (-19,4 até -0,5°C). Os autores verificaram que o suco seguia o mesmo comportamento do observado por Telis-Romero et al., (1999), porém utilizaram o modelo de Casson ($\sigma^{1/2} = K_0 + K_{\chi}.\gamma^{1/2}$). Os autores também encontraram que a tensão residual aumentava com a diminuição da temperatura assim como a viscosidade plástica de Casson (K_c).

Tavares et al,. (2007), estudaram a influência da temperatura (de -18°C a 0°C) e da concentração (de 46,56 a 65,04°Brix) nas características reológicas do suco de laranja variedade Pera-Rio. Ao contrário de Branco e Gasparetto (2003) e Telis-Romero et al., (1999), o suco não apresentou tensão residual, e o modelo utilizado para descrever o comportamento foi o lei da potencia (ou pseudoplástico: $\sigma = k.\gamma^n$); o índice de consistência aumentou com o aumento da concentração e com a diminuição da temperatura. O índice de comportamento não sofreu influencia da temperatura, porém diminuiu com o aumento da concentração.

Para Vitali e Rao (1984a e b) o suco de laranja concentrado com baixo teor de polpa (menor que 7,1%), apresentou comportamento pseudoplástico com tensão residual desprezível, e que a variação da concentração nos níveis de 50 até 65°Brix mantém relação exponencial com o índice de consistência, e não da lei da potência verificada em faixas menores (8 a 35°Brix). Em relação à variação de polpa (3,4%, 5,7% 8,6% e 11,1%), o aumento da concentração da mesma eleva o valor do índice de consistência e diminui o valor do índice de comportamento, ou seja, quanto maior a quantidade de polpa mais consistente e mais distante do comportamento Newtoniano é o suco concentrado. Este fenômeno é atribuído a maior interação partícula-partícula, interação partícula-soro, e competição pela água livre.

Falguera e Ibarz (2010) verificaram a influência da temperatura (de -12 a 30 °C) nos parâmetros reológicos do modelo HB. O índice de consistência diminui com o aumento de temperatura, assim como com o índice de comportamento, porém, em menor grau. Para a tensão residual não foi possível estabelecer alguma relação, porém a viscosidade aparente (em várias taxas de deformações diferentes) diminuiu com o aumento da temperatura.

Alguns estudos também demonstram que o suco de laranja concentrado possui comportamento dependente do tempo (Branco e Gasparetto, 2003). Os autores verificaram a histerese entre as curvas ascendente e descendente, evidência do comportamento dependente do tempo do tipo tixotrópico. Os autores concluem que a tixotropia aumenta conforme a temperatura diminuiu. Porém, como destacado por Tavares et al., (2007), este método não pode ser usado quantitativamente, apenas qualitativamente. Um método para analisar quantitativamente a tixotropia é descrito pelo modelo de Figone-Shoemaker (1983) que possui o modelo: $\sigma = \sigma_\varepsilon + (\sigma_\iota - \sigma_\varepsilon)$. exp(-k.t), que descreve que o fluido não newtoniano tem uma resposta em tensão inicial maior (σ_i) por causa da estrutura intacta que vai quebrando com o tempo de processo (com taxa de deformação constante) até que atinge um valor de tensão de equilíbrio depois de certo tempo (σ_e), sendo que esta variação segue tendência exponencial (k – parâmetro cinético).

Estudando o comportamento tixotrópico do suco de laranja usando o modelo de Figoni-Shoemaker (1983), Tavares et al,. (2007) observaram que ambos os parâmetros tensão de equilíbrio e tensão inicial σ_e e σ_i, aumentam com a diminuição da temperatura, sendo que o parâmetro k, não foi afetado de maneira clara. Para medir o grau de tixotropia, foi usada a diferença ($\sigma_i - \sigma_e$) como medida da destruição da estrutura, que aumenta com a diminuição da temperatura e com o aumento da taxa de deformação.

A tixotropia do suco de laranja concentrado também foi estudada por Ramos e Ibarz (1998). Os autores afirmam que a tixotropia está presente em amostras entre 55 e 60 °Brix, o mesmo não ocorre em amostras com menores concentrações. Além disso, quebra de estrutura diminuiu com o aumento da temperatura. A velocidade de quebra da estrutura (parâmetro cinético, k) aumentou com a diminuição da concentração, ou seja, atingiu o valor de tensão de equilibro mais rapidamente em menores concentrações. Os autores ainda dividem a curva do tixograma em duas partes, com a primeira parte que reduz a estrutura mais bruscamente é causada pela desintegração de polpa irregular, em partículas menores e mais regulares, e a diminuição posterior seria devido ao alinhamento das partículas.

1.4 Alterações no suco de laranja sob o efeito da homogeneização a alta pressão.

Estudos presentes na literatura sobre a HAP aplicada em suco de laranja podem ser classificados em dois grupos: um que visa a inativação de microrganismos e outro que visa a inativação de pectina-metil-esterase (PME) e mudanças físicas do suco de laranja.

Estudos sobre a inativação de *Saccharomyces cerevisiae* e *Lactobacillus plantarum* em suco de laranja (10° Brix) por HAP demonstram que em pressões superiores a 200 MPa, mais de 4 ciclos logarítmicos foram reduzidos para *S. cerevisae* e em pressões superiores a 250 MPa mais de 7 ciclos para o *L. plantarum*. Os resultados provam que a tecnologia pode ser uma alternativa ao uso de pasteurização térmica, garantindo a inocuidade do alimento (Campos e Cristianini, 2007).

Belloch et al. (2012), estudaram também o efeito da HAP na inativação de microrganismos presentes no suco de laranja. Para a *Listeria innocua* foi completamente inativada em 110 MPa ou 5 reduções log em pressões menores. Para *L. plantarum* chegou-se a 5 log de redução em pressões de 150 MPa.

Segundo Maresca et al. (2011), que estudaram o efeito das múltiplas passagens na inativação de *Saccharomyces cerevisiae, Lactobacillus delbruecki e Escherichia coli* em vários sucos, inclusive suco de laranja 10,8°Brix, constataram que tanto com o aumento da pressão, como com aumento do número de passagens o efeito é assintótico.

Lacroix et al. (2005), estudaram o efeito da HAP, na inativação da PME e na estabilização da opalescência/turbidez do suco de laranja. A homogeneização reduziu a atividade da PME em 20% à 170 MPa. Com o uso do pré-aquecimento do suco, a efetividade do HAP foi melhorada reduzindo para 10% de atividade em pH 3,0. Mesmo com a atividade residual de PME no suco processado por HAP ser próxima de um suco processado termicamente, a opalescência se mostrou mais estável, devido ao efeito sinérgico entre a homogeneização, pH e o pré-aquecimento, que causou redução de tamanho de partícula.

Velázquez-Estrada et al. (2012) compararam o efeito da HAP com a pasteurização convencional para inativação da PME, de microrganismos e na vida de prateleira do suco de laranja. Após passagem à pressão de 300 MPa a atividade residual de PME foi equivalente à encontrada no tratamento térmico. Pressões de 200 e 300 MPa obtiveram em média redução de 4 ciclos logarítmicos. Em relação a vida de prateleira, os sucos processados à 200 e 300 MPa, mostraram baixo crescimento microbiológico (menos de 3 ciclos log /mL) após 50 dias.

Em trabalho posterior Velázquez-Estrada et al., (2013), verificaram que os teores de poilfenóis em suco de laranja variedade Valencia processado por HAP, não apresentou diferenças comparando-se ao suco de laranja não processado, porém o suco de laranja pasteurizado, apresentou diferença significativa. Isto é um indicativo de que o processo HAP mantém algumas características nutricionais do suco. Resultado próximo foi encontra por Betoret et al., (2012) para atividade anti-radicais e compostos bioativos, usando HAP em suco de laranja variedade Mandarin.

Avaliando sensorialmente o suco de laranja processado por alta pressão de homogeneização, Cerdán-Calero, et al (2013), constataram que o suco pré-processado à 20 MPa, centrifugado e posteriormente processado à 150 MPa não apresentou diferença em relação ao sabor de fresco, cor, opacidade e composição de aroma se comparado ao suco recém-obtido da fruta. Foi verificado pelo referidos autores que o pré-processamento se faz necessário para a manutenção destas características sensoriais estáveis por até 15 dias em temperatura de refrigeração.

Também visando o efeito da homogeneização na turbidez, polpa, tamanho das partículas suspensas e atividade de PME no suco de laranja (12,4°Brix), Sentandreu et al. (2011), verificaram a redução do tamanho de partícula que foi de 629 μm para 118 μm. Neste caso não houve mudança significativa na redução da PME, porém as condições de pressão de homogeneização não passaram de 20 MPa. Betoret et al., (2009) apresentaram resultados semelhantes para a redução do tamanho de partículas, também não usado pressões maiores que 30MPa. Além disso este tipo de processamento não alterou negativamente a cor do suco. No mesmo estudo a HAP aumentou a turbidez do suco de laranja.

Carbonell et al., (2013) obtiveram que a homogeneização 150 MPa, foi suficiente para se manter estável a sedimentação de polpa (em níveis aceitáveis) durante 3 meses em temperatura de refrigeração. Mesmo considerando a amostra com maior atividade residual de PME.

Segundo Crandall e Davis (1991), o suco concentrado de laranja (65 ºBrix e 50 ºBrix) tem sua viscosidade aparente (100 s^{-1}) diminuída em média 20% quando homogeneizada a 21 MPa. Destaca-se que este trabalho não possuiu uma análise reológica sistemática, além de não verificar em pressões superiores que, devido as magnitudes de energias envolvidas, podem ser bastante significativas.

Considerando-se os trabalhos publicados até o momento, observa-se que a utilização de HAP pode ser uma alternativa interessante para alterar as propriedades reológicas do suco de laranja concentrado. Devido à alta produção de suco concentrado de laranja no Brasil, mesmo pequenas reduções na viscosidade aparente e podem vir a representar relevante economia nos processos de congelamento, estocagem e transporte, assim como, na melhoria da estabilidade do produto reconstituído a ser consumido, sem que as características sensoriais e nutricionais se alterem. Assim, esse faz se necessário a elucidação dos efeitos desta tecnologia emergente nos parâmetros reológicos do suco concentra de laranja (FCOJ).

1.5 Objetivos

Estudar o efeito da alta pressão de homogeneização (HAP) nas propriedades reológicas de suco de laranja concentrado até 150 MPa (1500 atm).

Objetivos específicos:

1. Avaliação do efeito sobre o comportamento reológico em regime estacionário independente e dependente do tempo de suco de laranja concentrado processado por HAP.

2. Verificação do efeito da pressão de homogeneização sobre as propriedades reológicas dinâmicas (viscoelasticidade) em suco de laranja concentrado processado por HAP.

3. Observação das alterações estruturais através de análise de distribuição de tamanho de partículas (PSD) e perfil da microscopia óptica causadas pela HAP.

4. Verificação do efeito do processamento por HAP, nos parâmetros de cor instrumental (CieLAB).

5. Observação do efeito do tempo de estocagem sobre as características listadas acima.

6. Avaliação do efeito de processos múltiplos nas propriedades reológicas de estado estacionário e distribuição de tamanho de partícula (PSD).

7. Verificação das alterações no produto reconstituído, cor e estabilidade a sedimentação de polpa e turbidez.

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Chapter 2. The Use of High Pressure Homogenization (HPH) to Reduce the Consistency of Frozen Concentrated Orange Juice (FCOJ)

Abstract

Frozen Concentrated Orange Juice (FCOJ) is of great economic importance to Brazil but Its consistency is hih due to the degree of concentration, and thus involves a lot of energy to flow. High Pressure Homogenization (HPH) is a promising technology to change the structure and properties of fluid foods in order to obtain tailor-made products. In this work the effects of HPH on the rheological proprieties (steady-state shear and time-dependent flow) of FCOJ (66º Brix) were evaluated up to 150 MPa, and also its PSD and instrumental colour. The evaluation was also carried out during the shelf-life of the product. The HPH process decreased the consistency of the FCOJ, decreasing its consistency index (k) and increasing its flow behaviour index (n). The thixotropy of the FCOJ was also affected, thus HPH decreased both the initial and equilibrium stresses, but the kinetic parameter was not changed. The particle size distribution decreased and changed with HPH, but the colour was not affected. The microstructure was also verified by microscopy, and it was possible to observe the size reduction of some structures in the fluid. The properties were modelled as a function of homogenization pressure, which is useful for a better understanding of the process and for future studies. Furthermore, it was concluded that HPH could be used to reduce the consistency of FCOJ and, consequently, the loss of friction, thus minimizing the amount of energy required to flow during processing and distribution.

Keywords: fruit juices, high pressure homogenization, rheology, friction loss

Resumo

Suco de laranja concentrado e congelado (*Frozen Concentrated Orange Juice* - FCOJ) possui grande importância econômica para o Brasil. FCOJ apresenta grande consistência devido a sua concentração elevada, necessitando de grande quantidade de energia para escoar. O processo de homogeneização a alta pressão (HAP) é uma promissora tecnologia para promover alterações na estrutura ou propriedades de alimentos fluidos, para se obter produtos diferenciados. Neste trabalho avaliou-se o efeito da HAP (até 150Mpa) nas propriedades reológicas (escoamento em estado estacionário e dependência do tempo) de FCOJ (66 °Brix), assim como a distribuição do tamanho de partículas (PSD) e cor. A avaliação também foi realizada durante a estocagem congelada do produto. O processo de HAP reduziu a consistência do FCOJ, apresentando diminuição no índice de consistência (k), e aumento no índice de comportamento (n). A tixotropia do FCOJ também foi afetada onde a HAP reduziu tanto o stress inicial quanto o stress de equilíbrio. Entretanto o parâmetro cinético não foi alterado pela HAP. A distribuição de tamanho de partícula decresceu com a pressão de homogeneização, porém a cor não sofreu alterações. A microestrutura do FCOJ foi verificada por microscopia óptica e foi possível verificar diminuição do tamanho de algumas estruturas dispersas no líquido. As propriedades estudadas foram modeladas em função da pressão de homogeneização, sendo útil para um melhor entendimento do processo e para estudos futuros. Além disso, conclui-se que a HAP pode ser usada para a redução da consistência do FCOJ, e consequentemente, a perda de carga, assim minimizando a energia necessária para o escoamento durante o processo e distribuição.

Palavras-Chave: Sucos de Frutas, Homogeneização a Alta pressão, Reologia, Perca de carga.

2.1 Introduction

Orange is one of the top ten agricultural products produced in Brazil, and Brazil has been the world's largest producer since the 1990s. Just in 2011, the production exceeded 19 million metric tonnes, of which about 80% was destined for the production of industrialized juices. In the same year, orange juice exports totalled US$ 1.775 billion, highlighting its importance for both the food industry and the Brazilian economy (FAO, 2013; MAPA 2012).

High pressure homogenization (HPH) technology consists of pressurizing a fluid to flow through a narrow gap valve, which greatly increases its velocity, resulting in depressurization with consequent cavitation and high shear stress. Thus the macromolecules and suspended particles in the fluid (such as cells and their fragments) are subjected to great mechanical stress, becoming twisted, deformed (Pinho et al., 2011; Floury et al., 2004) and even disrupted.

In fact, the use of HPH as a partial or total substitute for the thermal processing of foods has been studied for various fruit juices. Moreover, apart from being a preservation technique, HPH technology has recently been proposed as a potential unit operation in order to improve the technological properties of food and food components. For example, HPH improved the rheological properties of fruit juices (Augusto et al., 2012b, 2013); reduced sedimentation during storage (Kubo et al., 2013; Poliseli-Scopel et al., 2012; Silva et al., 2010); improved the activity of enzymes applied in food processing (Tribst et al., 2013); reduced fermented milk proteolysis (Oliveira et al., 2013); and improved the properties of polysaccharides (Porto et al., 2012; T. Wang et al., 2012, B. Wang et al., 2012) and proteins (Dong et al., 2011).

The rheological characterization of food products is of great importance, both for process design and optimization (such as pumps, pipelines and equipment) and for product stability and quality prediction, product formulation, correlation with sensory characteristics and consequent consumer acceptance (Falguera & Ibarz, 2010; Rao, 2005; Tavares et al. 2007; Urbecain E Lozano, 1997 and Vélez-Ruíz, 2002).

Several studies have been carried out with orange juice and HPH. However, all of these were carried out with single strength juice, checking for microbial and enzyme inactivation (Croak & Correig, 2006; Campos & Cristianini, 2007; Betoret, et al, 2012; Carbonell, et al, 2013; Cerdán-Calero, et al, 2013), with no study of the impact on product rheology. Also, other studies showed that HPH reduced the mean particle size of tomato juice and carrot based emulsions (Augusto, et al, 2012b, Moelants et al, 2012), with different impacts on product rheology. In fact, each vegetalable matrix reacts differently when processed by this technology, and it is hard to predict how HPH will affect the various parameters of a product. On the other hand, it was shown that HPH reduced the consistency of many polysaccharide solutions, such as those of pectin (Augusto et al., 2012a; Corredig and Wicker, 2001), carboxymethylcellulose (CMC, Floury et al., 2002), flaxseed gum (Wang et al., 2011), xanthan gum (Harte and Venegas, 2010; Lagoueyte and Paquin, 1998), alginate and k-carrageenan (Harte and Venegas, 2010). This suggests that HPH technology could be used to reduce the consistency of concentrated juices with low pulp contents, such as FCOJ. Thus, apart from reducing the consistency, the friction loss during processing and distribution can be reduced, thus minimizing the amount of energy required to flow.

Every vegetable matrix has a unique composition and behaviour, since cell fragmentation exposes and releases internal and wall constituents, such as pectins and proteins, improving the potential for particle–particle and particle–serum interactions. In fact, Lopez-Sanchez et al. (2011b) stated that the effects of the HPH process cannot be easily predicted and that each vegetable matrix has a unique response, since each cell wall has its own structure and consequently its own resistance to shear.

The objective this work was to evaluate the changes caused in the rheology of frozen concentrated orange juice (FCOJ) by high pressure homogenization (HPH).

2.2 Materials and Methods

2.2.1. Frozen Concentrated Orange Juice (FCOJ)

The frozen concentrated orange juice was obtained directly from a local producer (Citrosuco, Limeira, São Paulo, Brazil). It was a commercial blend of juices from the Natal and Valencia varieties, concentrated to 66°Brix, pH 3.8, 3,24% citric acid and 11.7% of pulp, and stored in a conventional freezer at -18°C before and after processing.

2.2.2. High Pressure Homogenization (HPH)

The process was carried out within the range from 0 MPa (control) to 150 MPa (gauge, homogenization pressures – P_H) in a high pressure homogenizer (Panda Plus, GEA Niro Soavi, Italy). The juice was first thawed to 5 °C and then processed The inlet temperature was 25ºC, simulating the conditions just after juice concentration during industrial processing. After the procedure, the samples were stored at -18ºC until all the analyses had been carried out. All processes were carried out in triplicate, and the homogenization pressures were 0, 25, 50, 75, 100 and 150 MPa.

2.2.3. Particle Size Distribution (PSD) Analysis

The particle size distribution was evaluated by light scattering (Malvern Mastersizer 2000 with Hydro 2000s, Malvern instruments Ltd, UK). The mean diameter was also evaluated based on both the particle volume (D[4,3]; *Equation 2.1*) and the particle surface area (D[3,2]; *Equation 2.2*). This is useful since the particles are not ideal spheres, and the D[3,2] is more influenced by the smaller particles, whilst the D[4,3] is more influenced by the larger ones (Lopez-sanchez et al., 2011a; Bengtsson and Tornberg, 2011).

These analyses were carried out immediately after processing and throughout the storage time at -18°C, in triplicate.

$$D[4,3] = \frac{\sum_i n_i d_i^{\,4}}{\sum_i n_i d_i^{\,3}} \qquad (\textit{Equation 2.1})$$

$$D[3,2] = \frac{\sum_i n_i d_i^{\,3}}{\sum_i n_i d_i^{\,2}} \qquad (\textit{Equation 2.2})$$

2.2.4. Rheological Analyses

The rheological analysis were carried out using a controlled stress (σ) rheometer (AR2000ex, TA Instruments, USA), with the temperature maintained constant at -10°C using a Peltier system and a cross hatched plate-plate geometry (40 mm of diameter). The samples were analyzed immediately after HPH and then monthly during the storage period (six months at -18°C)

A gap-independency procedure was used as described by Tonon et al. (2009), which consisted of varying the distance between the plates until the flow curve reached steady state. The gap obtained was 1000 μm.

The rheological evaluation was carried out with fresh samples, which were first placed in the rheometer and maintained at rest for 5 min before shearing. After resting, the samples were sheared at a constant shear rate (300 s−1) for 300 s, while the shear stress was measured for the time-dependent (thixotropic) behaviour evaluation. After the time-dependent shear period, a stepwise linear decreasing protocol (300 s-1 to 0.1 s-1) was used to guarantee steady-state shear conditions for the flow behaviour evaluation.

The time-dependent rheological properties of the FCOJ were evaluated using the first part of the protocol. The shear stress decay was evaluated using the Figoni and Shoemaker model (Figoni and Shoemaker, 1983; *Equation 2.3*).

The steady-state shear rheological properties were evaluated using the second part of the protocol. The product flow behaviour was modelled using the

Herschel-Bulkley model (*Equation 2.4*), which comprises the Newton, Bingham and Ostwald-de-Waele (power law) models.

Both the time-dependent and steady-state shear procedures were carried out at -10ºC, in order to evaluate the rheological properties of the product undo the storage and transportation conditions.

$$\sigma = \sigma_e + (\sigma_i - \sigma_e)\cdot \exp(-k_{FS} \cdot t) \quad \text{(Equation 2.3)}$$

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^n \quad \text{(Equation 2.4)}$$

An additional rheological characterization was carried out at different controlled temperatures (-10°C, 0°C, 10°C, 20°C and 30°C) using the same protocol, in order to evaluate the effect of HPH on the temperature dependency of the FCOJ flow properties. The product consistency and flow behaviour indexes (k and n, respectively; *Equation 2.4*) were obtained at each temperature and homogenization pressure, and the activation energy (Ea) of Arrhenius's equation (*Equation 2.5*) obtained. Thus the impact of HPH on the temperature dependency of the FCOJ consistency could be verified.

$$k = k_0 \cdot e^{(Ea/\ RT)} \quad \text{(Equation 2.5)}$$

The parameters of each model were modelled as a function of the homogenization pressure (P_H) using linear or nonlinear regression with the software CurveExpert Professional (v.1.6.3, http://www.curveexpert.net/, USA) at a significant probability level of 95%.

2.2.5 Instrumental Colour

The instrumental colour was obtained using an ULTRA PRO colorimeter (Hunter Associates Laboratory, USA), with illuminant D65 and angle of 10°, previously calibrated with a RSIN white reference (L*=92.03, a*=−0.88, b*=0.63), as described by Sánchez-Moreno et al. (2006) and Rodrigo et al. (2007). The samples were placed in glass cuvettes, and three readings were obtained for each replicate.

The CIELab technique was used for the evaluation, where the values for L* (lightness), a* (redness: green to red) and b* (yellowness: blue to yellow) were first obtained and could then be used to express the colour changes.

2.2.6. Microstructure

The optical microstructure was observed using an optical microscope (Carl Zeiss Jenaval, Carl Zeiss Microimaging GmbH, Germany) with an X25 objective and X1.25 optovar, coupled to a digital camera and software (EDN2 Microscopy Image Processing System). Before the observation, 30 µL of sample were carefully placed on a glass slide, and the droplet covered with a cover glass, which was carefully rotated at 45°C in order to guarantee the same orientation for the samples, as described by Bayod and Tornberg (2011) and Mert (2012).

2.3 Results and discussion

2.3.1. Particle Size Distribution (PSD)

The effect of HPH on the frozen concentrated orange juice PSD immediately after the process is shown in *Figure 2.1*. The mean particle size decreased with increase in homogenization pressure (P_H), similar to that observed by other authors with different vegetalable matrices (Augusto et al, 2012b, Silva et al, 2010, and Moelants et al, 2012). It can also be seen that the 0 MPa sample had the same distribution as the non-homogenized sample, as observed by Kubo et al (2013) for tomato juice, justifying the use of the 0 MPa sample as the control sample.

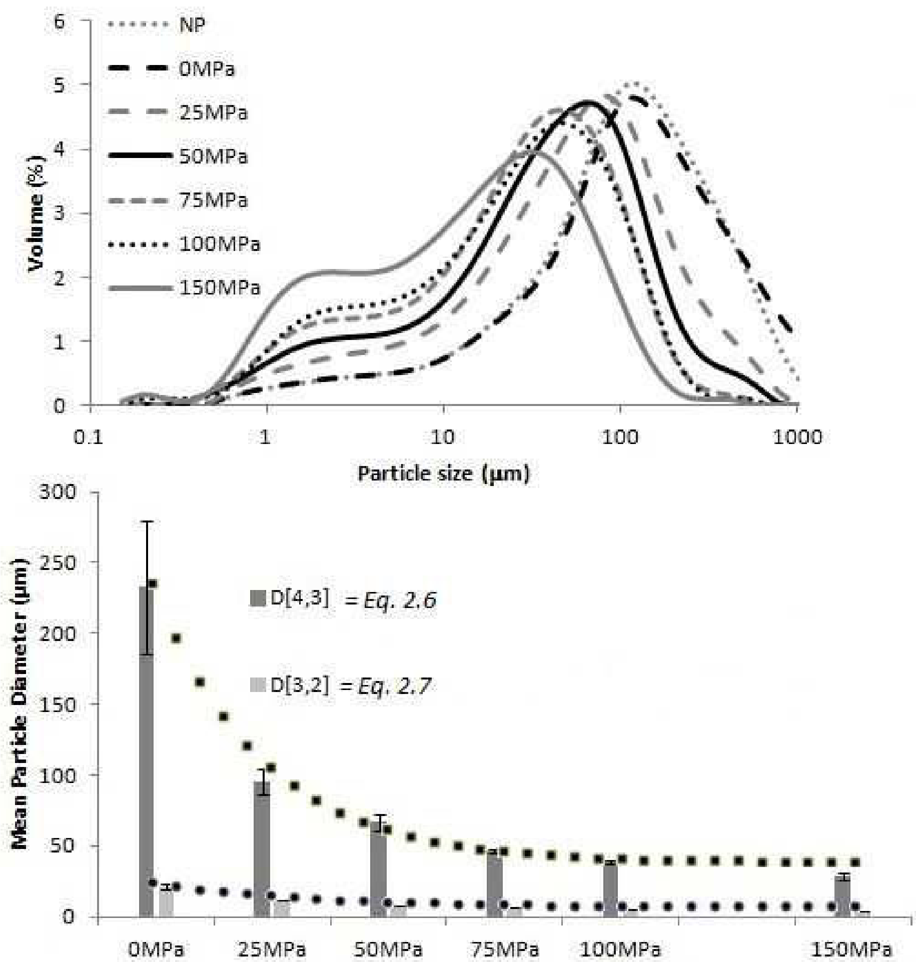

Figure 2.1: Effect of HPH on the particle size distribution (PSD) and mean particle diameters (D[4,3] and D[3,2]) of FCOJ immediately after processing dots are the model. (Vertical bars are standard deviation)

The number of larger particles was larger than that of smaller ones, which can be confirmed in *Figure 2.1*. This explains the fact that the D[4,3] showed a tenfold mean diameter value higher than that of the D[3,2], for all the samples. Both the mean particle diameter D[4,3] (*Equation 2.1*) and D[3,2] (*Equation 2.2*) were asymptotically reduced with increase in P_H (*Figure 2.1*). The mean diameter dropped to a value equivalent to ~40% (25 MPa) and ~12% (150 MPa) for D[4,3] and D[3,2], of the initial diameter (0 MPa), when processed by HPH.

The PSD also changed in shape with the increase in P_H. the sample processed at 0 MPa showed an almost monomodal distribution, with a mean value close to 100 μm, while all the other samples showed a plateau of small particle sizes (1 to 5 μm). In addition, the number of particles on this plateau increased in relation to the homogenization pressure, demonstrating that the larger particles were broken into smaller ones just inside this range. For the pressure range used (up to 150MPa), the particles could not be broken any smaller than this range regardless of the initial size. This was certainly caused by a physical limitation of the equipment related to the gap dimensions and the resultant shear stress and the particle properties, since the stress required to disrupt the smaller particles is greater than that delivered by the equipment. Also, an asymptotic behaviour can be observed in relation to the effect of HPH, i.e., the higher the homogenization pressure, the less the increase impacted on the PSD. Equivalent results were achieved by Augusto et al., (2012b) and Silva et al., (2010), for tomato juice and pineapple pulp, respectively.

Figure 2.1 also shows the model of D[4,3] (*Equation 2.6*, R^2=0.993) and D[3,2] (*Equation 2.7*, R^2=0.991) as a function of homogeneization pressure. This model present the initial value of the respective mean diameter, related to the unprocessed FCOJ. Also present the minimum mean diameter, which can be related to the gap size limitation and, finally a constant that represent how susceptible to breaking is the particles with the increase of pressure. These models can be used to process and product tailoring.

$$D[4,3] = 35.266 + (231.739 - 35.266) \cdot \exp(-0.0430 \cdot P_H) \quad \text{(\textit{Equation 2.6})}$$

$$D[3,2] = 4.246 + (21.074 - 4.246) \cdot \exp(-0.0297 \cdot P_H) \quad \text{(Equation 2.7)}$$

Figure 2.2 only shows the PSD of the 0 MPa and 150 MPa samples over the six month storage period at -18ºC (the other pressure processed samples showed an analogous behaviour). This figure shows that the PSD did not change during storage (p<0.05). This indicates that the changes caused by the HPH process were not reversible.

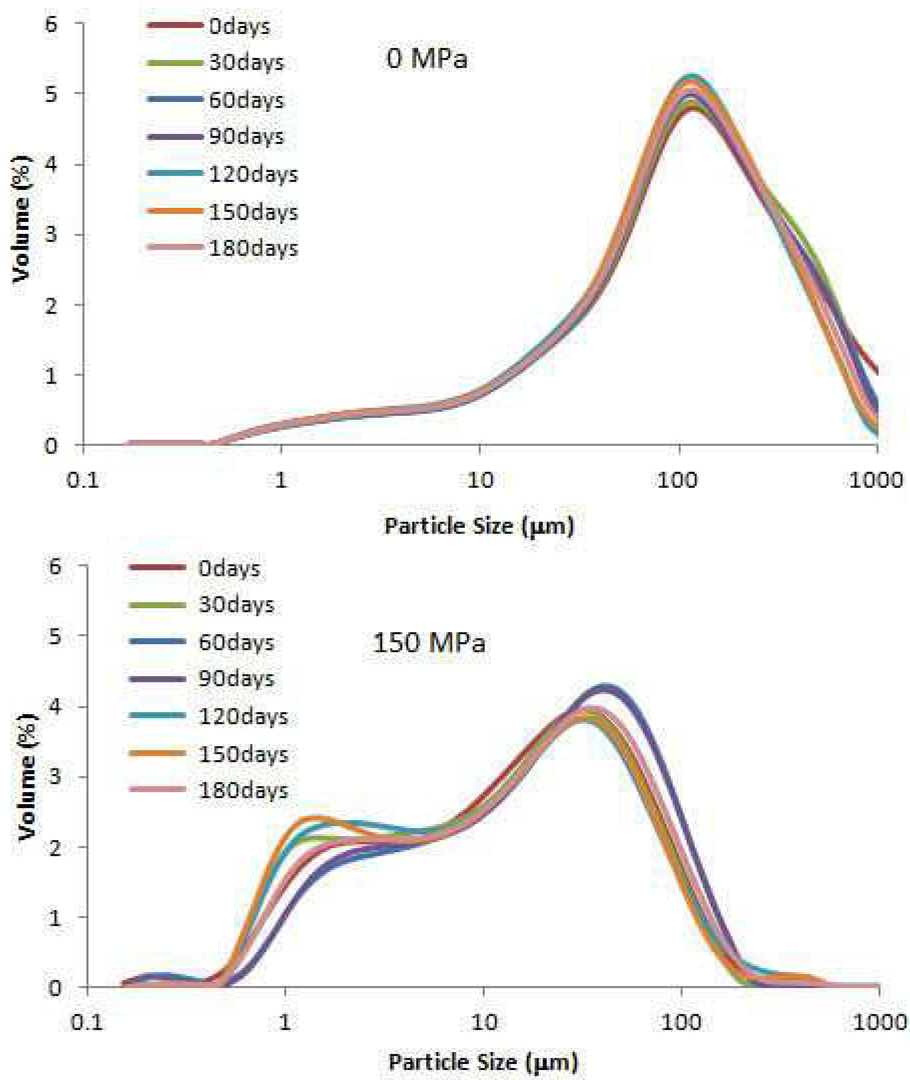

Figure 2.2: Particle size distribution (PSD) of FCOJ processed at 0 and 150 MPa during the storage time at -18ºC.

2.3.2. Microstructure

Figure 3.3 shows the microstructure of the FCOJ for the samples processed at 0 MPa [A and B] and at 150 MPa [C and D]. The control sample (processed 0 MPa) shows some cell fragments, pectin chains and some agglomerates of orange pigments. No intact cells could be found, which was to be expected due to the aggressive process the juice was submitted to during concentration, thermal processing and pumping.

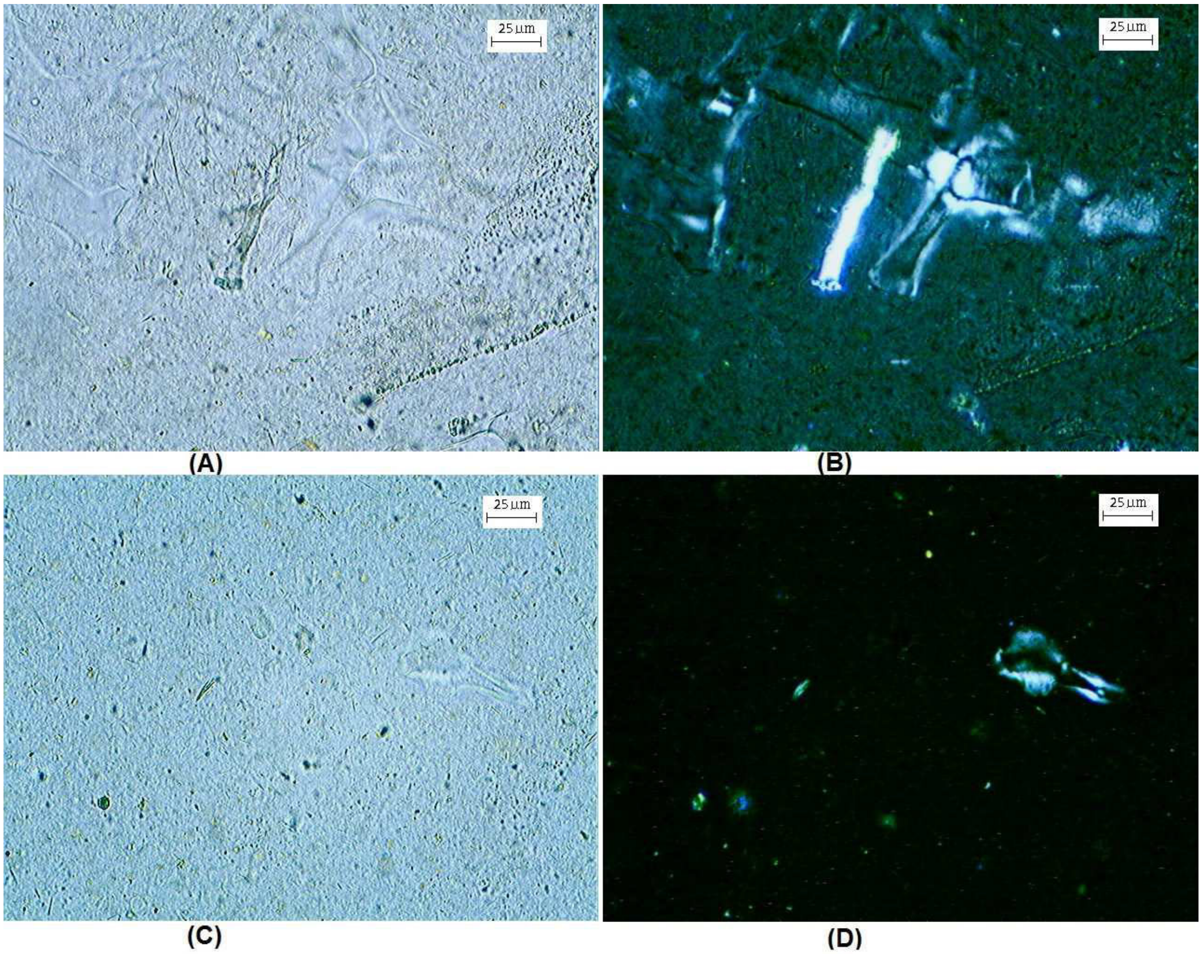

Figure 2.3: Microstructure of FCOJ as registered by optical microscopy: (A) 0 MPa/Direct light; (B) 0 MPa/Polarized Light; (C) 150 MPa/Direct light; (D) 150 MPa/Polarized light (Horizontal bar refers to 25 μm).

The polarized light images, which showed some fluorescent structures, were useful to better visualize the pectin chains and/or fibres. Apple pectin was verified using the polarized light microscopy in a study by Turakhozhaev, et al. (1997), and showed a similar structure to that of the 0 MPa samples (Figure 3B).

On the other hand, the structures became smaller in the samples processed at higher pressures, as the homogenization pressure increased. However the differences between the samples homogenised at different pressures (25MPa to 150MPa) were not easily distinguishable, and only the picture obtained at 150MPa was shown in Figure 3. Using polarized light, some smaller fluorescent structures could be seen in the sample processed at 150MPa. Furthermore, all the images showed the same amount of pigment agglomerates, which means there was no additional release of pigments into the medium.

2.3.3. Instrumental Colour

Figure 2.4 shows the instrumental colour parameters of the CieLAB method (L*, a*, b*) immediately after the HPH process and throughout six months of storage at -18ºC.

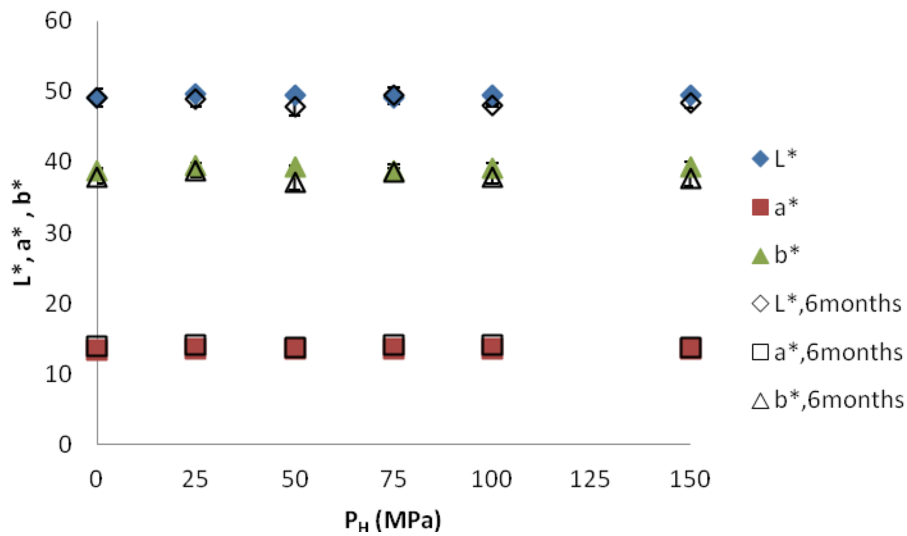

Figure 2.4: Primary parameters for instrumental colour by the CieLAB method for FCOJ processed at various homogenization pressures, immediately after processing and after 6 months of storage at -18°C); dots are means of experimental data, and vertical bars are the standard deviation)

The HPH process showed no impact on any of the primary instrumental colour parameters, or on the derived parameters such as hue and chroma (data not shown), with no significant difference ($p<0.05$). Since the FCOJ was a product that suffered major changes during production, most of the pigmented cell components were already disrupted. Thus there was no additional pigment liberation after the HPH process. It is interesting to note that this is a desirable result, since it infers that HPH does not change the visual perception of FCOJ by the consumer.

It was also observed that HPH had no influence on the instrumental colour parameters throughout the storage time ($p<0.05$). Since the product was stored at subzero temperatures, it was to be expected that the colour of the product would show no major differences. As stated by Mannheim et al. (1988), non-enzymatic browning is mainly controlled by the storage temperature. Once again this is interesting since the consumers will see no changes in the processed juice.

2.3.4. Rheological Behaviour

HPH effects on Flow Behaviour

Figure 2.5 shows the flow behaviour of the FCOJ at -10°C. The juice showed shear thinning behaviour without yield stress, and could be well described by the Ostwald-de-Waele model (power law; *Equation 2.8*), which is a simplification of E*quation 2.4* without the yield stress (σ_0). *Table 2.1* compares the values obtained with those reported in the literature.

$$\sigma = k \cdot \dot{\gamma}^n \qquad \text{(}\textit{Equation 2.8}\text{)}$$

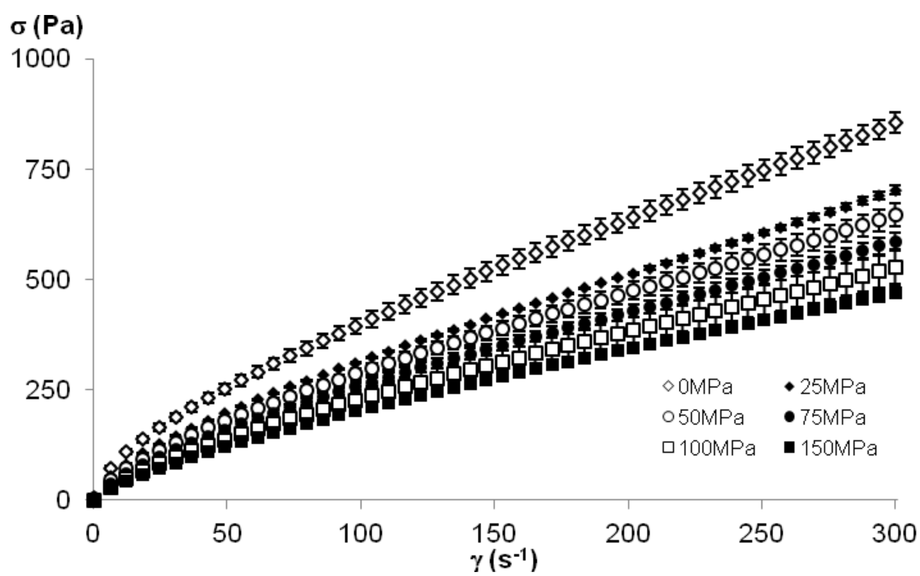

Figure 2.5: Flow curves at -10°C for FCOJ processed by HPH (dots are the mean values, and the vertical bars are the standard deviation).

Table 2.1: Values for the rheological parameters of FCOJ found in the literature

Product	T (°C)	σ_0 (Pa)	k ($Pa.s^n$)	n	Reference
FCOJ (0 MPa) - Natal/Valencia var.	-10 25	- -	17.9 6.1	0.67 0.59	Present Work
Concentrated orange juice - Pera var.	-10 20	- -	8.8 1.25	0.791 0.774	Vitali and Rao (1984b)
Concentrated orange juice - Natal var.	-10 20	- -	10.8 1.64	0.743 0.721	Vitali and Rao (1984b)
Concentrated orange juice	25 -9	2.2 1.5	3.13 12.6	0.64 0.685	Falguera and Ibarz (2010)
Concentrated orange juice - Pera-rio var	-10	-	57.71	0.69	Tavarez et al., (2007)

The flow behaviour of the FCOJ at -10°C was affected by the HPH process, as shown in *Figure 2.5*, reducing the juice consistency and apparent viscosity (η_a). This can be seen by fixing a specific shear rate ($\dot{\gamma}$) and observing the smaller values of the correspondent shear stress (σ) in the homogenized samples (*Figure 2.5*). The apparent viscosity (η_a) of the product was plotted in *Figure 2.6*, considering the properties at -10ºC. It can be seen that the non processed sample (0 MPa) showed a higher apparent viscosity independent of the shear rate. *Table 2.2* shows the values for apparent viscosity at some fixed shear rates for the processed and unprocessed samples (i.e., η_{a_PH} / η_{a_0MPa} = f(P_H)). The impact of HPH on the FCOJ can be clearly seen, since the apparent viscosity decreased by ~50% of its initial value when processed at 150 MPa. Since the apparent viscosity represents the resistance of the fluid to flow, the results showed that the product would be easier to pump and, consequently less expensive.

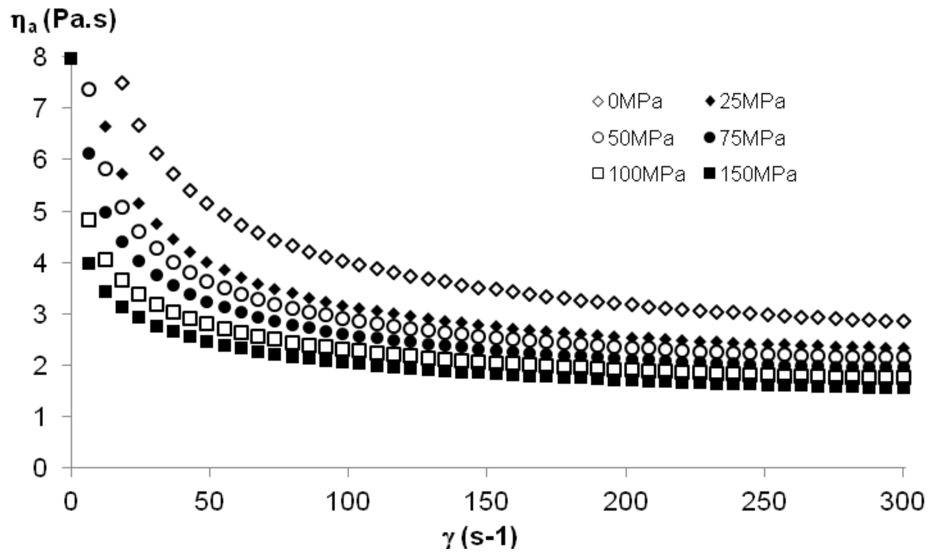

Figure 2.6: Apparent Viscosity at -10°C for FCOJ processed by HPH (dots are the mean values).

Table 2.2: Apparent viscosity (η_a) at -10°C of FCOJ processed by HPH at various shear rates

Shear Rate	50 s^{-1}		100 s^{-1}		300 s^{-1}	
P_H (MPa)	η_a (Pa.s)	$\eta_{a-PH}/\eta_{a-0\,MPa}$	η_a (Pa.s)	$\eta_{a-PH}/\eta_{a-0\,MPa}$	η_a (Pa.s)	$\eta_{a-PH}/\eta_{a-0\,MPa}$
0	5.14	1.00	4.02	1.00	2.85	1.00
25	4.01	0.78	3.17	0.78	2.34	0.82
50	3.64	0.70	2.91	0.72	2.15	0.75
75	3.24	0.63	2.62	0.65	1.95	0.68
100	2.80	0.54	2.31	0.57	1.75	0.61
150	2.46	0.47	2.06	0.51	1.56	0.55

Moreover, asymptotic behaviour can also be noted in relation to the effect of the homogenization pressure (P_H), in accordance with the reduction observed for the PSD (*Figure 2.1*).

The rheological behaviour described and the effect of HPH were similar for the other temperature conditions evaluated (0, 10, 20, 25 and 30°C). Therefore, only the results at -10ºC, the most consistent condition, were shown (*Figures 2.5*, *Figure 2.6* and *Table 2.2*).

Figure 2.7 and *Table 2.3* show the effect of HPH on the Ostwald-de-Waele model parameters for the FCOJ at -10°C. The consistency index (k) decreased in an asymptotic way with the increase in pressure, while the flow behaviour index (n) showed the opposite behaviour. The equation of the model of k as a function of is presented on *Equation 2.9* and the equation of the model of n as a function of P_H is presented on *Equation 2.10*.

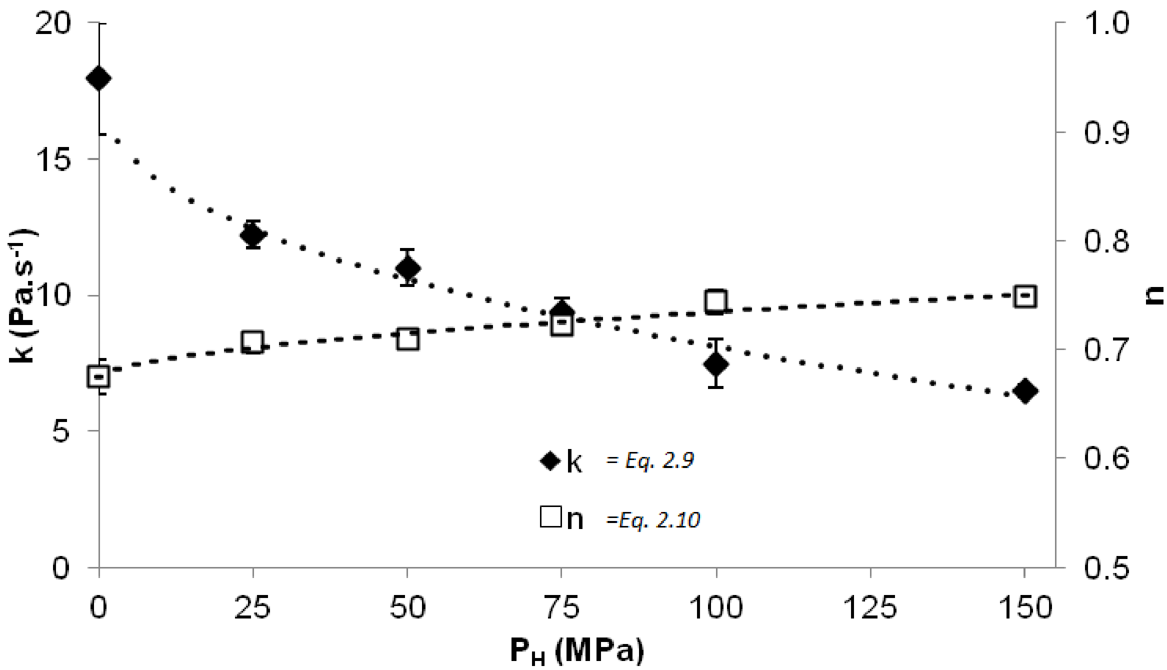

Figure 2.7: Consistency index (k) and flow behaviour index (n) for FCOJ at -10°C as a function of the homogenization pressure (dots are the mean value, vertical bars are the standard deviation, curves are the models).

$$k = 17.9 - 1.44 {P_H}^{0..417} \quad R^2=0.992 \qquad (\textit{Equation 2.9})$$

$$n = 0.675 + 0.004 {P_H}^{0..579} \quad R^2=0.992 \qquad (\textit{Equation 2.10})$$

Table 2 3: Effect of homogenization pressure (P_H) on the flow properties of FCOJ: Parameters of the Ostwald-de-Waele model at -10°C

P_H	k	n
0	17.96 ± 2.049	0.67 ± 0.016
25	12.21 ± 0.491	0.70 ± 0.008
50	11.00 ± 0.685	0.71 ± 0.005
75	9.34 ± 0.561	0.72 ± 0.006
100	7.48 ± 0.895	0.74 ± 0.011
150	6.48 ± 0.216	0.74 ± 0.005

As the pressure increased from 0 MPa to 150 MPa, the consistency index (k) at -10°C dropped from 17.96 to 6.48 Pa.s^n, which represents a reduction to 36% of the original value. On the other hand, the flow behaviour index (n) at -10°C increased from 0.68 to 0.75, or an increase of 11%, in the same range in variation in the homogenization pressure. These results are also similar to those reported by Silva et al. (2010) for pineapple pulp, whose value for k decreased to 7% of the original value, and value for "n" increased by 89% as the homogenization pressure increased from 0 to 70 MPa.

Working with tomato juice at 25°C Augusto et al. (2012b) also observed this decreasing behaviour of k from 11.0 to 5.0 Pa.s^n, and increasing behaviour of n from 0.4 to 0.9, with increasing P_H (0-150 MPa). Although a direct comparison cannot be made due to the Herschel-Bulkley behaviour of tomato juice with a characteristic yield stress (σ_0), a general trend can be considered.

Fruit juices and pulps are composed of two phases. The first is the serum, which is mainly the water plus all the other soluble compounds, such as acids, sugars and polysaccharides. The second is the dispersed phase, which is all the insoluble material, such as fibres, whole, disrupted or fragmented cells and chains or clusters of insoluble polymers suspended in the first phase (Augusto et al., 2012a).

The serum shows Newtonian behaviour (Vitali and Rao, 1984a; Augusto et al., 2012a). The dispersed phase, as demonstrated by Vitali and Rao (1984a), Vitali and Rao (1984b) and Augusto et al. (2011) gives the non-Newtonian behaviour to the fluid. The rheology of these products will change based on how these phases interact with each other and how some of the components interact with other components in the same phase, as well as according to the different effects of HPH on each phase (Augusto et al, 2012b).

The rheological properties of the serum are only slightly affected by HPH, as demonstrated by Augusto et al. (2012a), with a reduction in viscosity due to polysaccharide disruption (Corredig and Wicker, 2001, Floury et al., 2002, Wang et al., 2011, Lagoueyte and Paquin, 1998 and Harte and Venegas, 2010). However, even small changes can be important in concentrated products such as the FCOJ evaluated here, partially explaining the behaviour observed.

The changes in PSD can also be explained by the decrease in FCOJ consistency. The original juice showed a monomodal distribution, with a narrower distribution when compared to that of the processed product (*Figure 2.1*). Furthermore, after the HPH process, the FCOJ showed a significant accumulation of small particles, with the plateau previously described and a bimodal distribution. The small particles can occupy the spaces amongst the larger ones, resulting in an important lubricant effect, decreasing the resistance to flow and leading to a reduction in the consistency index (k) (Servais et al, 2002).

The homogenized samples showed a flow behaviour closer to a Newtonian one, moving away from shear-thinning behaviour as the flow behaviour index (n) got closer to 1.0 (*Figure 2.7* and *Table 2.3*). As the suspended particles became smaller and more uniform, the alignment to flow tended to be less expressive.

Since the FCOJ did not show yield stress (σ_0), even when the suspended particles were disrupted into small ones resulting in a higher surface area, it can be inferred that the inter-particle attraction was very low, and there was no eligible particle network (i.e., arrangement of suspended particles and molecules when at rest or very small shear rates, resulting in a network structure – and, consequently, yield stress). Since the product concentration was high, the molecules were closer, with less water between them, which would increase the interactions amongst them. Thus since no σ_0 was presented, this shows that the observed high consistency of the product was mainly related to the serum drag through the dissolved and suspended particles.

In addition, the serum phase itself is affected by the HPH, since it contains several polysaccharides that can also be affected by HPH, such as carboxymethylcellulose (CMC, Floury et al., 2002) and xanthan gum (Lagoueyte and Paquin, 1998), showing a reduction in the consistency index and an increase in the behaviour index. The effect of HPH on a serum model (pectin solution) was studied by Augusto, et al. (2012a), showing a reduction in fluid viscosity. These studies corroborate the results obtained in the present work, in other words, HPH changes the rheology of FCOJ by changing the size of the macromolecules in the serum.

Therefore both the consistency index (k) and the flow index (n) were modelled as a function of the homogenization pressure (P_H, *Figure 2.7*). The models obtained showed asymptotic behaviour for both the parameters with the increase in pressure. These models can be used for industrial applications and process optimization. The flow behaviour of the FCOJ was also verified throughout 6 months of storage at -18ºC. Both consistency index (k) and the flow behaviour index (n) were unaffected by the storage time, with no statistical difference ($p>0.05$; data not shown). Therefore, it can be concluded that the changes caused by HPH in FCOJ are permanent, with no recovery during storage, which is of great interest.

Since the fluid consistency is related to the friction loss during processing, the reduction in consistency of the FCOJ due to HPH is highly desirable, since it represents a reduction in energy consumption during pumping. It also impacts other unit operations, such as facilitating the heat exchange operations by increasing the convective heat transfer coefficient (h). Therefore the use of HPH can improve the handling and processing of the FCOJ by reducing both the costs of the energy required during processing (pumping and heat transfer) and the equipment costs (smaller and cheaper). When larger FCOJ productions are considered, the savings are emphasized.

Effect of HPH on Arrhenius Activation Energy

Figure 2.8 shows the parameters of the Ostwald-de-Waele equation for the FCOJ at various temperatures, for the samples processed at 0 MPa and 150 MPa. As expected, the higher the temperature, the smaller the consistency index (k), since the resistance to flow is reduced at higher temperatures. Furthermore, the temperature did not affect the flow behaviour index (n), which is common in this range of temperatures. Therefore only the Arrhenius's activation energy (Ea) for the consistency index (k) was evaluated.

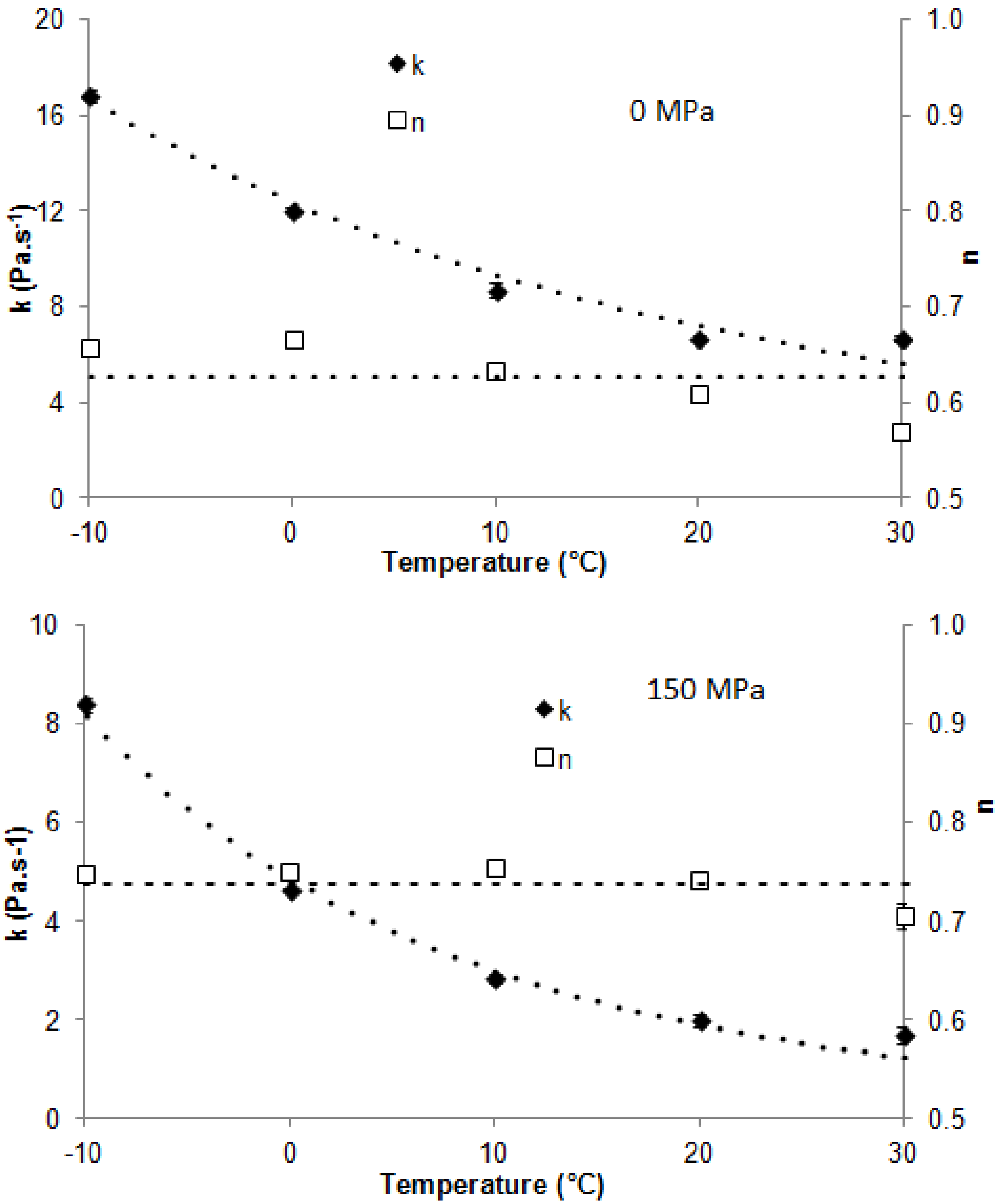

Figure 2.8: Ostwald-de-Waele parameters for FCOJ at 0 MPa and 150 MPa and various temperatures (dots are the mean values of experimental data, vertical bars are the standard deviation, curves are the models).

The Ea for the consistency index of each sample is presented in *Figure 2.9*. The Ea value for the juice processed at 150 MPa (31.10 kJ/K·mol) was almost twice as high as that of the control sample (17.82 kJ/K·mol), indicating that the variation in consistency index (k) of the FCOJ in relation to temperature is higher for HPH processed juices (higher activation energy values indicate a greater variation in consistency with temperature – dk/dT). The HPH process reduced the size of the suspended particles (*Figure 2.1*), resulting in a minor alignment effect during flow, which can be confirmed by the increase in the flow behaviour index (n). Thus, as stated before, the fluid starts to behave more like a Newtonian fluid. According to Saravagos (1970), the Ea values of Newtonian fluids are higher than those of non-Newtonian fluids, considering the same solids contents.

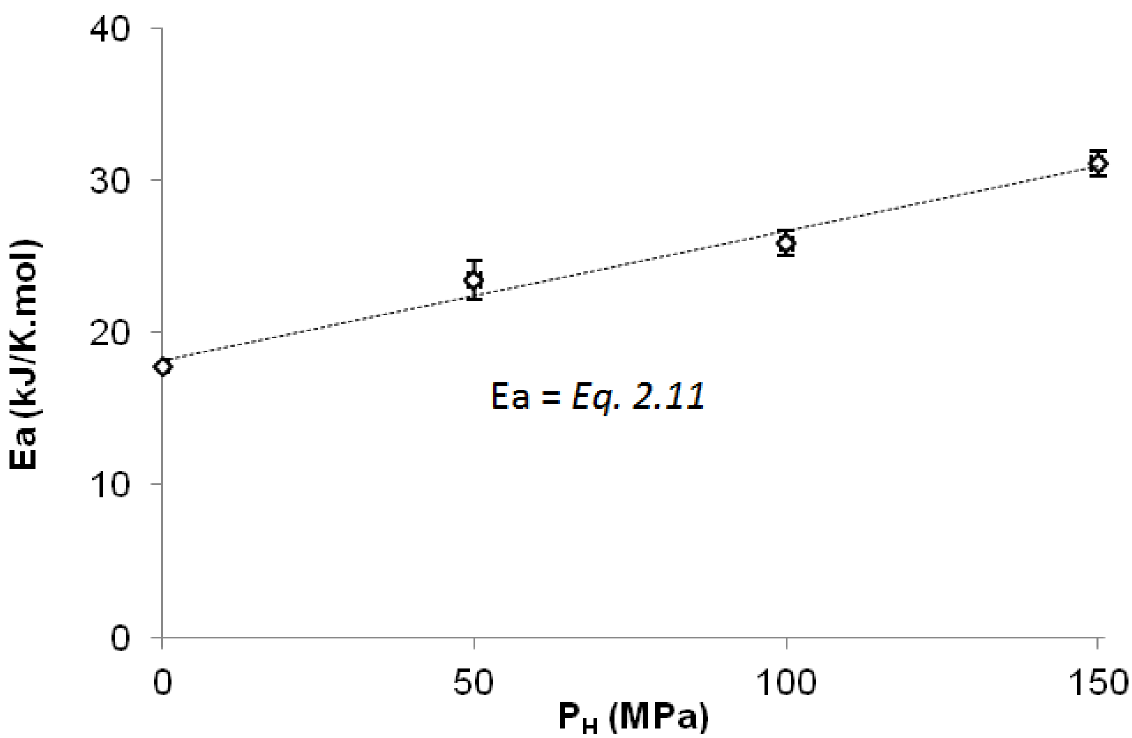

Figure 2.9: Consistency index energy of activation (Ea, Equation 5) of FCOJ processed by HPH (dots are the mean value of experimental data, vertical bars are the standard deviation, the curve is the model)

This can probably be related to the amount of energy that each fluid needs to flow. For Newtonian fluids, the resistance to flow is only related to the inertial tendency to stay at rest, opposing the motion. At higher temperatures, with a higher internal energy and molecular movement, this resistance is reduced. Thus the thermal energy provided to Newtonian fluids directly impacts its viscosity, being characterized by higher activation energy (Ea) values.

On the other hand, the resistance to flow of pseudoplastic fluids is related to the Newtonian phase drag through the dispersed phase and interactions between the phases (in this case, the suspended juice particles in the serum). Furthermore, in these products, the effect of the alignment of the dispersed phase to the flow is also important. Thus, the energy provided is distributed to the serum and to the suspended phase, whose relative motion does not necessarily change the overall resistance of the fluid to flow. For example, a change in a polysaccharide conformation, with a small amount of unfolding, can be evaluated. Although this can facilitate the molecular alignment, reducing the pseudoplastic behaviour of the product, the serum drag through the molecule is not reduced and nor is product consistency. Therefore the non-Newtonian fluids need higher temperatures (internal energy) to show the same resistance to reduction as the Newtonian fluids, i.e., the dependence of product consistency on the temperature, and also the activation energy (Ea), are lower.

This result is of particular advantage since it is possible to reduce the consistency of a processed sample by a small temperature change, preventing possible deterioration due to a larger rise in temperature.

Linear correlation (*Equation 2.11)* could be obtained between the Ea and the homogenization pressure, and the model was plotted in Figure 9. This model is useful to predict the Ea under other conditions within the range, which is an important tool in process optimization.

$$Ea = 18.214 + 0.0845 P_H \quad R^2=0.9797 \qquad (\textit{Equation 2.11})$$

Effects of HPH on the time dependent properties

The effect of HPH on the thixotropy of FCOJ was evaluated (*Figure 2.10*) at a temperature of -10ºC. The thixotropy of the product could be well modelled by the Figoni-Shoemaker model, and the parameters are shown in *Table 2.4* in comparison with other fruit juices. The shear stress decreased since shearing at 300 s^{-1} (σ_i – σ_e) was ~80% of its initial value (-10ºC), reinforcing the importance of evaluating the time-dependent properties of the FCOJ.

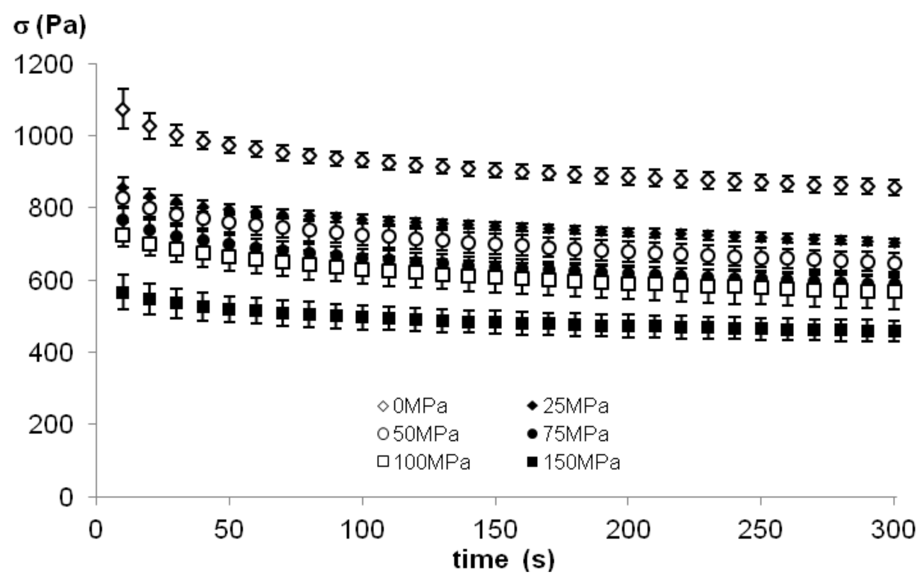

Figure 2.10: Shear stress with time (thixogram) during shearing at 300 s^{-1} and -10°C for FCOJ previously processed by HPH (dots are the mean value, vertical bars are the standard deviation).

Table 2.4: Values found in the literature for the Figoni-Shomaker model kinetic parameters of some products

Product	T (°C)	$\dot{\gamma}$ (s^{-1})	σ_i - σ_e (Pa)	k_{FS} (s^{-1})	References.
FCOJ (0MPa)	-10	300	220.04	0.011	Present Work
FCOJ (0MPa)	25	300	34.21	0.0058	Present Work
Gilaboru (43 °Brix)	20	100	5.33	0.0029	Altan et al., (2005)
Pineapple Jam	-	10 - 100	-	0.0024 - 0.0094	Basu et al., (2007)
Chickpea flour dispersion	20	100	40	0.0055	Ravi and Bhattacharya (2006)
Tomato juice	25	300	0.81	0.0056	Augusto et al., (2012c)
Concentrated orange juice	-10	70	236.4	0.71	Tavares et al., (2007)

The time-dependent properties described and the effect of HPH at -10ºC, were similar to those evaluated at other temperatures (0, 10, 20, 25 and 30°C). Therefore, only the results at -10ºC (the highest initial stress (σ_i) condition) are shown (*Figure 2.10*).

HPH decreased the thixotropy of FCOJ by reducing both the correspondent shear stress at 300 s^{-1} during shearing ($\sigma_{3001/s}$ = ($t_{shearing}$)) and the difference between the initial and equilibrium shear stresses (σ_i –σ_e; *Figure 2.11*). At -10°C the sample processed at 150 MPa showed an initial value for stress (σ_i) of 567.9 Pa, reaching equilibrium at 456.1 Pa, which is almost half the value of the unprocessed sample.

The effect of HPH on the parameters of the Figoni-Shoemaker model can also be observed in *Figure 2.11* and *Table 2.5*, which shows these parameters at -10°C plotted as a function of the homogenization pressure. The equations of the three Figoni-shoemaker models are listed on *Equation 2.12, 2.13* and *2.14*.

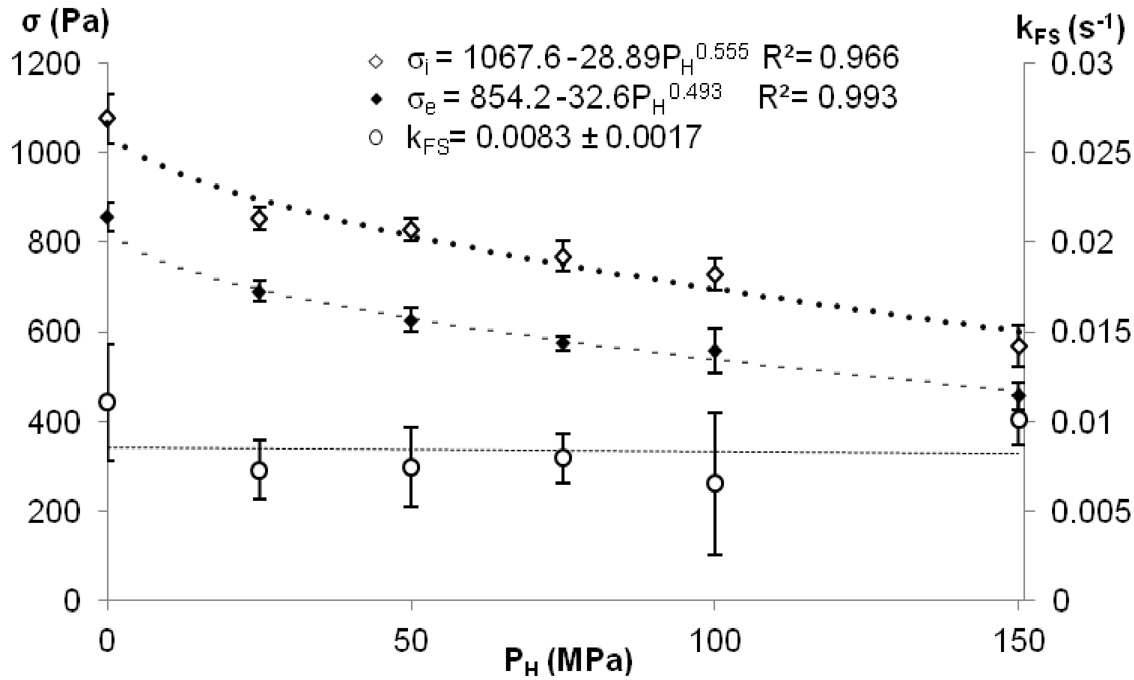

Figure 2.11: Modelling of the initial and equilibrium stresses (σ_i ; σ_e) and the rate of decay (k_{FS}) for the Figoni-Shoemaker model at -10°C as a function of the homogenization pressure (dots are the mean value of experimental data, vertical bars are the standard deviation, curves are the models).

$\sigma_i = 1067.6 - 1.44 P_H^{0..555}$ R^2=0.966 *(Equation 2.12)*

$\sigma_e = 854.2 - 132.6 P_H^{0..493}$ R^2=0.993 *(Equation 2.13)*

$k_{FS} = 10.0083 \pm 0.0017$ *(Equation 2.14)*

Table 2.5: Effect of homogenization pressure (P_H) on the FCOJ flow properties: Parameters of the Figoni-Shoemaker model according to the homogenization pressure at -10°C

P_H (MPa)	σ_i (Pa)	σ_e (Pa)	$\sigma_i - \sigma_e$ (Pa)	k_{FS} (s^{-1})
0	1075.34 ± 55.33	855.30 ± 32.08	220.04[a]	0.0111 ± 0.0032
25	851.97 ± 25.64	690.25 ± 22.77	161.72bc	0.0073 ± 0.0016
50	827.49 ± 23.94	626.29 ± 26.44	201.20[a]	0.0075 ± 0.0022
75	768.87 ± 32.56	575.04 ± 16.00	193.83ab	0.0080 ± 0.0014
100	728.19 ± 34.27	558.37 ± 49.73	169.82bc	0.0065 ± 0.0040
150	567.98 ± 47.90	456.13 ± 31.56	111.84c	0.0101 ± 0.0014

The thixotropic behaviour is related to the structural changes caused by shear (Ramos and Ibarz 1998), i.e., the destruction of the internal structure during flow (Cepeda et al. 1999). This can be seen from the decrease in apparent viscosity (σ_a) or shear stress (σ) with time at a specific shear rate, due to the change in structure during shearing (Ramon and Ibarz, 1998 and Rao, 2005). Consequently, a time-dependent rheological characterization is extremely important for a better understanding of the product changes that occur during the process.

The initial structure can be related to inter-particle and intermolecular interaction, forming aggregates and/or a network with a higher initial resistance to flow. When the product is sheared this structure collapses, reducing the product resistance. These changes can be reversible or non-reversible.

The tendency to form particle aggregates is related to the inter-particle forces. As these forces are negligible at high shear rates (Genovese et al, 2007), they cannot maintain the network during shearing and the aggregates collapse. Thus the shear stress related to flow drops from the initial stress value (σ_i) to the equilibrium value (σ_e) after a certain flow time. This structural change can also be reversible or otherwise. Another explanation of the time-dependent behaviour is the tendency of the suspended particles to remain still (inertia) or to maintain their inertial alignment/entanglement. Thus as the product starts to flow, there is a gradual change to an optimal alignment. When shearing is interrupted, particle interaction becomes relevant again and the sample can re-aggregate or return to its initial random distribution (non-alignment) due to Brownian movement.

The present results showed a decrease in both the initial (σ_i) and equilibrium (σ_e) shear stresses, as well as in the difference between them ($\sigma_i - \sigma_e$), but not in their rate of decay (k_{FS}). From the PSD analysis, it can be seen that the suspended particles were disrupted into smaller particles, which explains the reduction in both stresses, since smaller particles are less resistant to shear and show less friction during flow.

On the other hand, small particles can show greater interaction as a result of their larger surface area. However, an increase in the inter-particle/intermolecular forces would result in an elevation/development of yield stress (σ_0) or a greater difference between the stresses ($\sigma_i - \sigma_e$), which was not observed for the processed FCOJ. This indicates that interaction between the FCOJ particles was small. As stated before, changes in the serum phase have an important impact on product rheology, and a reduction in the molecular size in the serum phase can reduce both the initial and equilibrium stresses.

The destruction rate of this network during shearing is not affected by HPH, as evidenced by the lack of a major impact on the k_{FS}

Finally thixotropy was also verified throughout the storage time at -18ºC. Similar to the flow behaviour results, the storage time did not affect ($p>0.05$) any of the Figoni-Shoemaker model parameters (data not shown). Thus, it can be concluded that the changes promoted by HPH were maintained during storage without any structural recovery, since the samples were stored at subzero temperatures.

The changes in thixotropy of the FCOJ are not negligible in industrial processing, due to the high consistency of the product and the differences between the initial and equilibrium stress values. Pumping, or any other process of this product, is a relevant issue in the daily operational routine of an industry and to process design. Therefore the time dependent properties were modelled as a function of the P_H (*Figure 2.11* and *Table 2.5*). As can be seen, the models obtained described the effect of HPH on FCOJ thixotropy well, and are of interest for future studies and process design.

2.4 Conclusions

The HPH process disrupted the suspended particles, reducing the mean particle diameter and changing the PSD, with the formation of a plateau of small particles. HPH changed the rheological behaviour of the product, reducing its resistance to flow (reducing its consistency index - k), which thus approximated that of Newtonian behaviour (increasing its flow behaviour index - n). Also the Arrhenius activation energy of the consistency index was verified and showed an increase in its value. Furthermore, HPH reduced the thixotropy of the FCOJ, decreasing both the initial stress (σ_i) and the equilibrium stress (σ_e). These changes were discussed and modelled as a function of the homogenization pressure (P_H).

HPH showed no impact on the colour of the product, even with storage time. The reduction in apparent viscosity of the FCOJ led to reduced energy consumption during unit operations such as pumping. Therefore, the use of HPH could promote the use of smaller and cheaper equipment, with less energy consumption and more efficient FCOJ processing.

2.5 Acknowledgments

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Chapter 3. Processing Frozen Concentrated Orange Juice (FCOJ) By HPH Technology: Changes in the Viscoelastic Properties

Abstract

The rheological properties of a fluid have an important role in process development and optimization. Due to its high concentration, Frozen Concentrated Orange Juice (FCOJ) shows viscoelastic behaviour, especially at low temperatures. In this study, high pressure homogenization (HPH) processing (up to 150 MPa) was used to change the viscoelastic properties of FCOJ. The rheological behaviour of the FCOJ, before and after HPH processing, was evaluated using dynamic frequency sweep procedures. The storage (G') and loss (G") moduli were modelled as a function of the oscillatory frequency using the Power Law. The sample processed at 0 MPa showed G'>G". On the other hand, the processed samples presented G">G'. Both the elastic and viscous behaviours decreased with increasing homogenization pressure. The power law parameters were then modelled as a function of the homogenization pressure using exponential functions. Also the Cox-Merz rule was verified for all the samples, showing good applicability when a linear mathematical modification was applied. The reduction in the elastic and viscous components was explained by the reduction in particle size and molecular size of the serum constituents. A decrease in viscoelasticity leads to less resistance to flow, and therefore to lower energy costs for the FCOJ process. These results are useful to understand the phenomena leading to an industrial application of this technology.

Keywords: Fruit Juices, High Pressure Homogenization, Rheology, Viscoelasticity, Cox-Merz Rule

Resumo

As propriedades reológicas de um fluido tem um papel importante no desenvolvimento e otimização de processos. O suco de laranja concentrado e congelado (FCOJ), devido a sua elevada concentração, apresenta comportamento viscoelástico, especialmente em baixas temperaturas. Neste estudo, a homogeneização a alta pressão (HAP) foi utilizada até 150MPa, para alterar as propriedades viscoelásticas do FCOJ. O comportamento reológico foi avaliado, antes e após o processamento, utilizando varredura dinâmica de frequência. Os módulos de armazenamento (G') e dissipação (G") foram modelados em função da frequência oscilatória usando a Lei da Potência. A amostra não processada (0 MPa) apresentou G'>G". Por outro lado, as amostras processadas apresentaram G">G'. Ambos os comportamentos elástico e viscoso decresceram com o aumento da pressão de homogeneização. Os parâmetros de lei da potência foram modelados em função da pressão de homogeneização, usando funções exponenciais. A regra de Cox-Merz foi verificada para todas as amostras, mostrando uma boa aplicabilidade quando utilizada uma modificação matemática linear. A redução nos componentes elásticos e viscosos são explicados pela redução do tamanho de partículas e tamanho molecular de constituintes do soro. A redução da viscoelasticidade leva a uma menor resistência para o escoamento e, portanto, a um menor custo de energia durante o processo de FCOJ. Os resultados são uteis para o entendimento do fenômeno, levando a uma aplicação bastante útil desta tecnologia.

Palavras-chave: Suco de frutas, Homogeneização a Alta Pressão, Reologia, Viscoelasticidade, Relação de Cox-Merz

3.1 Introduction

Characterization of the food rheological properties is important from processing to consumption, considering the design of the equipment and unit operations, the sensory characteristics and the final product quality and stability, which have an impact on consumer acceptance. Furthermore, the energy consumption of many unit operations is mainly dependent on the rheological properties of a given product (Urbecain & Lozano, 1997; Vélez-Ruíz, 2002; Rao, 2005; Tavares et al. 2007 and Falguera & Ibarz, 2010).

The rheological properties of some products are defined by a complex interaction of viscous and elastic properties which is called viscoelasticy. Although the characterization of flow is an important tool to understand the viscous response of the product to shear, the elastic behaviour is not taken into consideration, although it is important in order to understand product behaviour in low shear situations, such as in particle sedimentation. Dynamic viscoelastic assays are a useful tool to describe both behaviours and provide the storage (G’) and loss (G”) moduli. The first is related to the elastic properties, being the amount of deformation energy that is accumulated by the product structure during strain. The latter is related to the viscous properties, being the amount of deformation energy that is lost during strain (Steffe, 1996; Rao, 2005; Ahmed and Ramaswamy 2006 and Alvarez, 2013).

According to Gunasekaran and Ak (2000), the evaluation of steady-state shear can promote slippage and migration of sample constituents during the procedure. This is particularly true in some food products such as fruit juices, implying in several limitations to this procedure. In fact, in this procedure, the sample structure can change due to the great amount of energy transmitted to the sample by the application of deformation. When low shear conditions are required, such as in the simulation of particle sedimentation, these procedures are not capable of providing accurate results.

A non-destructive alternative could be the use of dynamic rheological procedures, which are carried out using small amplitude oscillatory frequencies (Rao 2005; Dogan and Kokini 2007). Since it is a non-destructive technique, it is possible to change the experimental conditions and to carry out multiple evaluations on the same sample (Dogan and Kokini 2007). On the other hand, dynamic rheology has its own limitations. Due to the measurements of small oscillatory amplitudes, the low rates and strain at which the procedures are carried out are not those involved in many practical or daily operations (Dogan and Kokini 2007; Steffe 1996). In these cases, steady-state shear evaluations (flow behaviour) must be carried out (Gunasekaran and Ak 2000). Furthermore, it is interesting to observe that the thixotropic fluid flow behaviour can only be measured after a pre-shearing period.

High Pressure Homogenization (HPH) is a non-thermal technology which was initially studied as an alternative to conventional thermal processing. In recent years, this technology has successfully been used for several other objectives, such as improving the rheological properties of fruit juices (Augusto et al., 2012b, 2013b); reducing pulp sedimentation during storage (Kubo et al., 2013; Poliseli-Scopel et al., 2012; Silva et al., 2010); increasing the activity of enzymes applied in food processing (Tribst et al., 2013); reducing the proteolysis in fermented milk (Oliveira et al., 2013; and improving the properties of polysaccharides (Porto et al., 2013; T. Wang et al., 2012, B. Wang et al., 2012) and proteins (Dong et al., 2011).

This technology is based on the continuous pumping of a fluid through a narrow gap. Due to the mass and energy conservation laws, the fluid drastically increases its velocity during this process, which also results in a great pressure drop to atmospheric pressure, resulting in high shear stress, high turbulence and cavitation. These modifications are the main causes of product changes.

Frozen concentrated orange juice (FCOJ) is the juice most produced in the world, Brazil being the leader of its production. In 2011 Brazilian exportation reached a value of US$1.775 billion (FAO, 2013), hence the economic importance of FCOJ to Brazil, as well to the industry, is clear.

This product is highly consistent, especially at low temperatures, due to its high concentration, requiring a great amount of energy to be pumped. Furthermore, it also shows non-Newtonian behaviour with shear thinning (Vitali and Rao, 1984; Tavarez, et al, 2007; Falguera and Ibarz, 2010) and viscoelastic behaviour.

However, no studies regarding the viscoelasticity of FCOJ are available in the literature.

The effect of HPH on the microbial and enzyme inactivation of single strength orange juice has been widely studied and reported in the literature, such as the studies of Croak & Correig (2006); Campos & Cristianini (2007); Betoret et al. (2009), Sentandreu et al. (2011), Betoret et al. (2012); Carbonell et al. (2013); and Cerdán-Calero et al., (2013).

Chapter 2 reported the effect of HPH on FCOJ rheology, but only considering the steady-state shear properties (i.e., the viscoelastic properties were not covered). Therefore, the objective of this work was to evaluate the viscoelastic properties of FCOJ and the applicability of the Cox-Merz Rule, and also evaluate the effect of high pressure homogenization (HPH) on these properties.

3.2 Materials e Methods

3.2.1. Frozen Concentrated Orange Juice (FCOJ)

The frozen concentrated orange juice was obtained directly from a local producer (Citrosuco, Limeira, São Paulo, Brazil). It was a commercial blend of juices from the Natal and Valencia varieties, concentrated to 66°Brix, pH 3.8, 3,24% citric acid and 11.7% of pulp, and was stored in a conventional freezer at -18°C before processing.

3.2.2. High Pressure Homogenization (HPH) Processing

The process was carried out within the range from 0 MPa (control) to 150 MPa (gauge, homogenization pressures – P_H) in a high pressure homogenizer (Panda Plus, GEA Niro Soavi, Italy). The juice was first thawed to 5 °C and then processed, using an inlet temperature of 25ºC in order to simulate the conditions just after juice concentration during industrial processing. After the procedure, the samples were stored at -18ºC until all the analyses had been carried out. All processes were carried out in triplicate, and the homogenization pressures were 0, 25, 50, 75, 100 and 150 MPa.

3.2.3. Rheological Analyses

The Rheological analyses were carried out using a controlled stress (□) rheometer (AR2000ex, TA Instruments, USA), with the temperature maintained constant at -10 °C using a Peltier system and a cross hatched plate-plate geometry (40 mm diameter). This temperature was chosen to simulate the industrial condition after freezing, especially during pumping.

Initially, the gap independency procedure was carried out, as described by Tonon et al. (2009). This consists of varying the distance between the plates until the flow curve shows no further change. In this way the gap obtained was set at 1000 μm. For the viscoelastic evaluation, a shear stress sweep procedure was carried out between 0.1 and 100 Pa, with a fixed frequency of 1Hz, in order to define the linear viscoelastic range. The value obtained for shear stress was 1.0 Pa.

The rheological evaluation was always carried out with fresh samples using a four-step protocol. The samples were first placed in the rheometer and maintained at rest for 5 min before starting the evaluation. After resting, the frequency sweep procedure was started in order to evaluate the viscoelastic properties. The frequency sweep was carried out between 0.01 and 100 Hz, and subsequently the samples were sheared at a constant shear rate (100 s^{-1}) for 300 s to eliminate product thixotropy. Finally, a linear decreasing stepwise protocol (100 s^{-1} to 0.01 s^{-1}) was used to guarantee steady-state shear conditions for the flow behaviour evaluation. This last step was necessary to evaluate the applicability of the Cox-Merz rule.

The viscoelastic properties of the FCOJ (Storage and Loss Moduli) were evaluated by the power law model (*Equation 3.1 and 3.2*), whose parameters (k', k'', n', n'') were modelled as a function of the homogenization pressure (P_H).

$$G' = k' \cdot \dot{\gamma}^{n'} \qquad (\textit{Equation 3.1})$$

$$G'' = k'' \cdot \dot{\gamma}^{n''} \qquad (\textit{Equation 3.2})$$

A correlation between the steady-state shear and dynamic oscillatory experiments could be useful, due to the characteristics and limitations of each kind of experiment. . The complex viscosity (η*), which is obtained from the dynamic viscoelastic characterization, could be related to the apparent viscosity (η_a), which is obtained from the steady-state flow characterization using the Cox-Merz rule. It can be applied when in a given specific oscillatory frequency equal to the shear stress, the values for the complex and apparent viscosities are the same (*Equation 3.3*, Steffe, 1996; Rao, 2005).

$$\eta^*(\omega) = \eta_a(\dot{\gamma})\Big|_{\omega=\dot{\gamma}} \qquad \text{(\textit{Equation 3.3})}$$

The Cox-Merz rule can be applied directly to different polymer dispersions, but this rule usually needs modifications for complex products such as foods (Steffe, 1996; Rao, 2005).

The parameters for each model were obtained by linear or nonlinear regression using the software CurveExpert Professional (v.1.6.3, http://www.curveexpert.net/, USA) with a significant probability level of 95%.

3.3 Results e Discussion

3.3.1. Viscoelastic behaviour of FCOJ

Figure 3.1 shows the viscoelastic behaviour of FCOJ at -10°C, represented by the storage (G'; the elastic response) and the loss (G"; the viscous response) moduli. *Figure 3.1* also shows the product loss tangent (tanδ), which is the ratio between the loss and storage moduli (=G"/G'). Values for tanδ above 1.0 indicate that the viscous properties are dominant over the elastic ones.

Both characteristics were highly dependent on the oscillatory frequency (ω). Both moduli (G', G'') showed a rising tendency in relation to the oscillatory frequency (ω), with similar values throughout the entire frequency range. Thus it can be affirmed that FCOJ stores and dissipates shear energy at the same rate. Even so, at lower frequencies (below 2 Hz) the storage modulus was greater than the loss modulus (tanδ <1) and the opposite occurred at higher frequencies. As stated before, both moduli showed similar values, which is not common in many food products. The experimental conditions were in the range from 0.01 to 100 Hz, however, the linear viscoelasticy zone went up to ~30Hz.

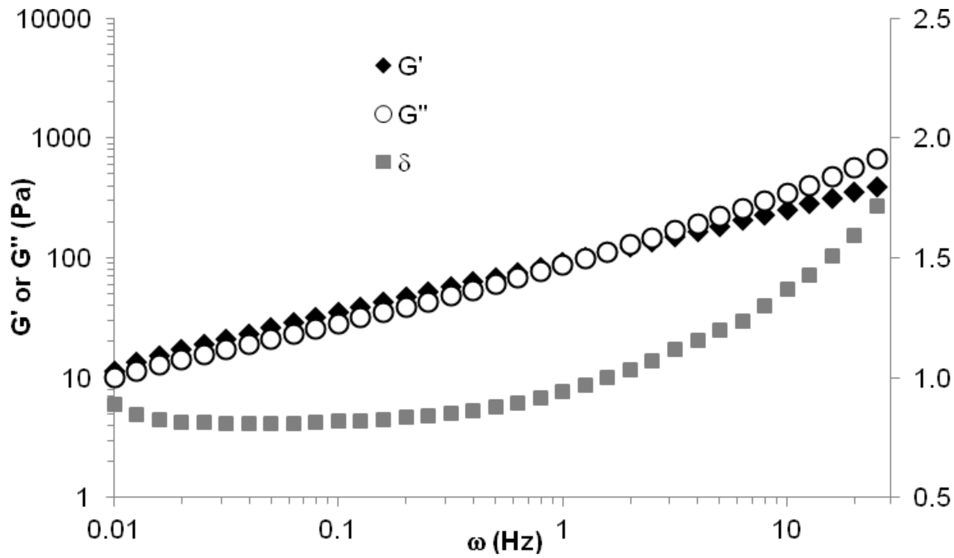

Figure 3.1: Viscoelastic behaviour of FCOJ at -10°C (dots are the mean values, and the vertical bars are the standard deviation).

At the temperature evaluated, FCOJ shows a large viscous component (G”) when compared with the majority of food products, such as peach puree (Massa et al., 2010), açai pulp (Tonon et al., 2009), jabuticaba pulp (Sato and Cunha 2009), umbu pulp (Pereira et al., 2008), baby foods (Ahmed and Ramaswamy, 2006; Ramamoorthi et al., 2009), potato puree (Alvarez et al., 2004), peach juice with fibres (Augusto et al., 2011) and tomato products (Augusto et al. 2013a; Bayod et al. 2008; Valencia et al. 2002; Yoo and Rao 1996). While these products are classified as weak gels, with G’>G’’ throughout the whole mechanical spectra, FCOJ is considered to be a viscoelastic liquid (Rao, 2005). In fact, this is a consequence of its low pulp content and small pulp interaction, consequently showing less elastic interactions.

The higher storage modulus (G') at low frequencies can be explained by the inertial resistance to flow, and the interaction between the particles and the soluble molecules. However, this resistance reduces as the frequency gets higher, due to alignment to the flow. Thus, the viscous fluid aspect (G'') becomes more noticeable at higher frequencies.

Both the storage (G') and loss (G") moduli could be well described by the power law model (*Equation 3.1* and *Equarion 3.2*; R^2 higher than 0.99), and these parameters are presented in E*quation 3.4* and *Equation 3.5.*

$$G' = 91.87 \cdot \dot{\gamma}^{0.418'} \qquad (\textit{Equation 3.4})$$

$$G'' = 76.12 \cdot \dot{\gamma}^{0.675} \qquad (\textit{Equation 3.5})$$

The viscous behaviour of FCOJ was more affected by the oscillatory frequency (ω) than by the elastic one, as can be verified by the fact that the value for n" was higher than that for n'. This showed that the elastic interactions amongst the suspended particles, serum and ice crystals were less affected by the oscillatory frequency (ω) than by the alignment effect of the suspended particles and ice crystals or the drag of the suspended particles through the serum.

The microstructure of a product can be correlated with its rheological behaviour and the viscoelastic properties are very useful in the design and prediction of the stability of stored samples (Ibarz and Barbosa-Cánovas 2003). Moreover, the viscoelastic products may exhibit some interesting behaviour such as the Weissenberg and Barus effects (Ibarz and Barbosa-Cánovas 2003; Steffe 1996). Thus the study and description of the viscoelastic properties of liquid foods is important for a better understanding of the behaviour observed during processing, storing, and consumption.

Figure 3.2 shows a comparison of the complex viscosity (η^*) of FCOJ, such as the function of the oscillatory frequency (ω) and the apparent viscosity (η_a) as a function of the shear rate ($\dot{\gamma}$). The complex viscosity (η^*; *Equation 3.6*) was obtained using the dynamic viscoelastic procedure, while the apparent viscosity (η_a; *Equation 3.7*) was obtained using the steady-state flow procedure.

$$\eta^* = \frac{G^*}{\omega} = \frac{\sqrt{(G')^2 + (G'')^2}}{\omega} \qquad (\textit{Equation 3.6})$$

$$\eta_a = \frac{\sigma}{\dot{\gamma}} = k \cdot \dot{\gamma}^{n-1} \qquad (\textit{Equation 3.7})$$

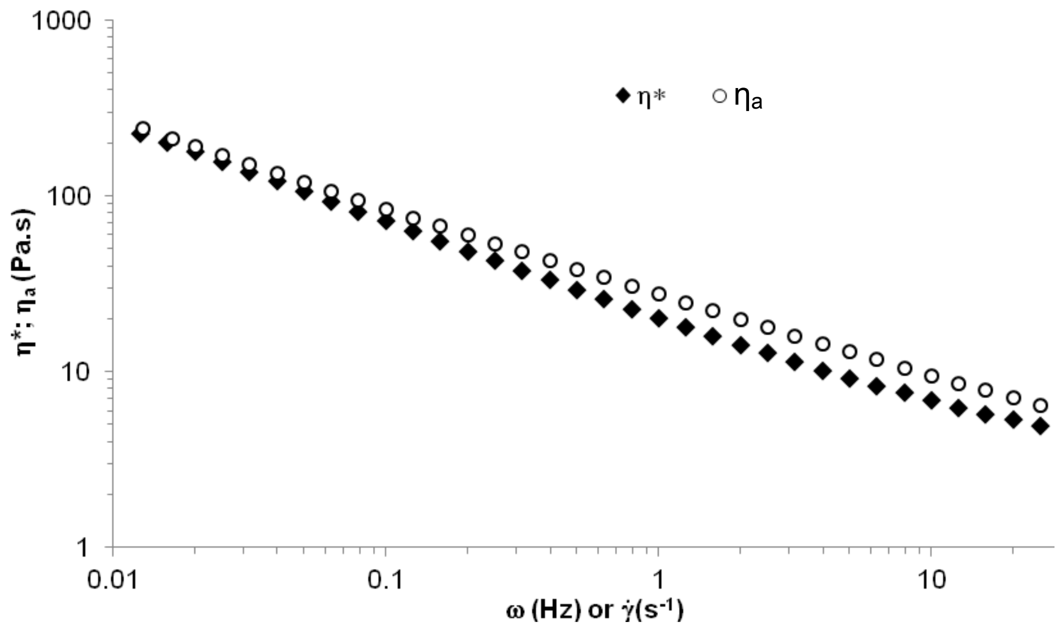

Figure 3.2: Complex viscosity (η^*) and apparent viscosity (η_a) of FCOJ at -10°C (dots are the mean values).

As expected the complex viscosity (η^*) decreased with increase in oscillatory frequency, as also the apparent viscosity (η_a) with increase in shear rate. Food products commonly show magnitudes for η^* higher than those for η_a. This behaviour is due to structural decay during shearing, as well as the presence of high-density entanglements, structure development and/or intermolecular aggregation (Ahmed and Ramaswamy, 2006; Da Silva and Rao, 1992). However, as can be observed in *Figure 3.2*, in the present experiment both viscosities showed similar values throughout the whole experimental interval, with the apparent viscosity values higher than the complex viscosity ones. Therefore, a simple structure, with minor interactions among its constituents, is expected for the FCOJ. In fact, this result is in accordance with those obtained usingthe steady-state shear (flow properties) and time-dependent (thixotropic) evaluations Chapter 2), as well as with the G’’>G’ results.

Although the complex viscosity and apparent viscosity are very similar at low shear rates and oscillatory frequency (*Figure 3.2*), they became different at higher shear rates and oscillatory frequency. So, in spite of having directly applied the Cox-Merz rule (Equation 3), linear modifications can be used, according to the *Equation 3.8* and *Equation 3.9*. Since the use of both equations has been reported in the literature, they were evaluated here to allow for comparisons with other vegetable products (*Table 3.1*).

$$\eta^*(\alpha \cdot \omega) = \eta_a(\dot{\gamma})\Big|_{\omega=\dot{\gamma}} \qquad \text{(\textit{Equation 3.8})}$$

$$\beta \cdot \eta^*(\omega) = \eta_a(\dot{\gamma})\Big|_{\omega=\dot{\gamma}} \qquad \text{(\textit{Equation 3.9})}$$

Both modifications of the Cox-Merz rule could be used in order to correlate the steady-state and viscoelastic properties of the FCOJ with the R^2 values obtained in the regression, which were always higher than 0.99. Therefore the values of both modified Cox-Merz rule parameters were higher than those described for other food products, as described in Table 1. This was to be expected due to the similar elastic (G’) and viscous (G’’) behaviours of the FCOJ.

The microstructure of a food directly impacts on its rheological behaviour, such as, for example, the size and shape of the suspended particles and ice crystals. In addition, the product stability design and its prediction for a food product can be correlated with its viscoelastic characteristics. The Modified Cox-Merz rule can be used to estimate both rheological behaviours (steady state and dynamic rheology) using only one experimental procedure.

3.2. Effect of HPH on the Viscoelasticy of FCOJ

Figure 3.3 shows the storage (G') and loss (G") moduli of FCOJ at -10 °C after HPH processing at various homogenization pressures. *Figure 3.4* shows the effect of the HPH process on the complex viscosity of FCOJ at -10 °C.

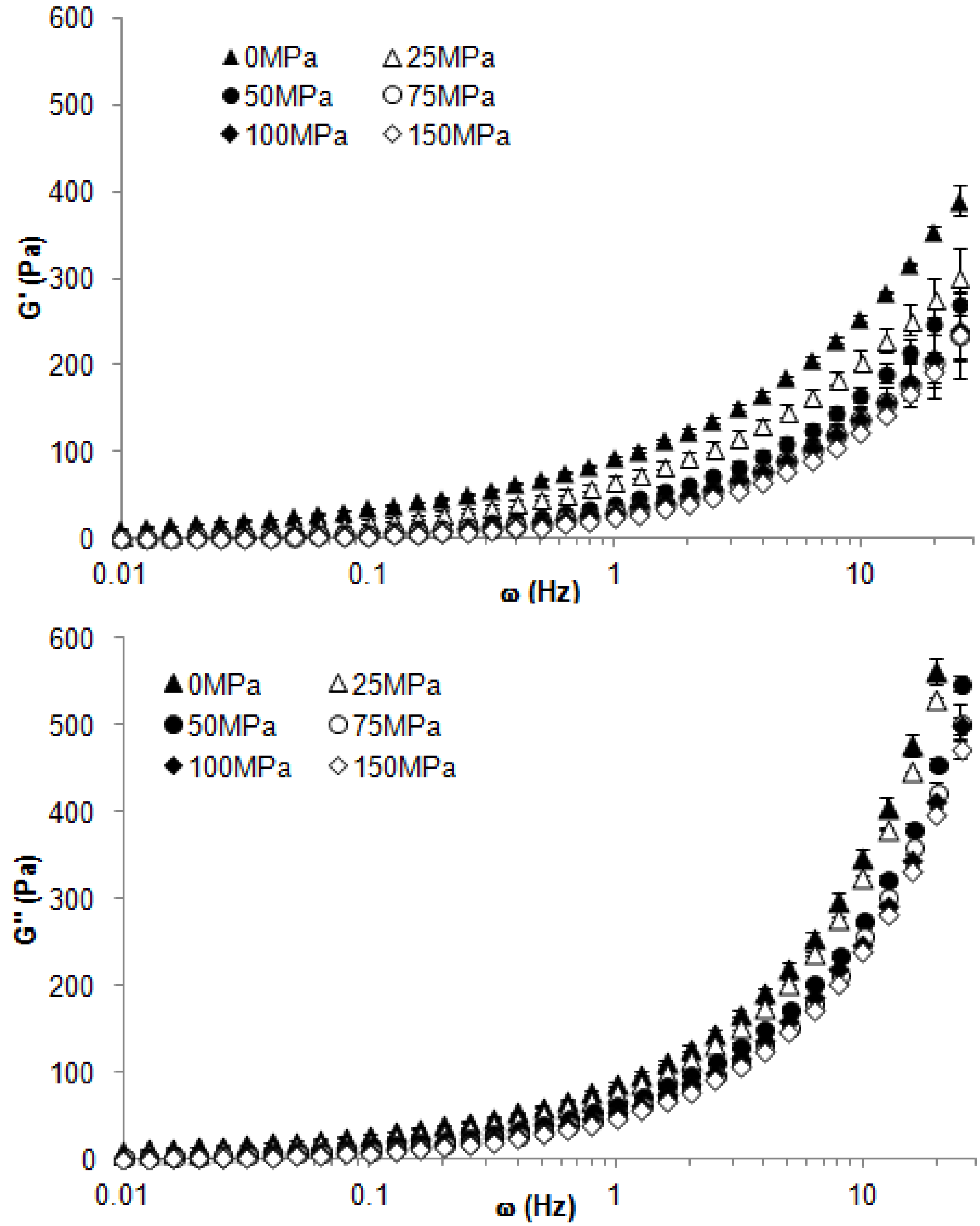

Figure 3.3: Viscoelastic behaviour of FCOJ at -10°C: effect of HPH processing (dots are the mean values, and vertical bars are the standard deviation).

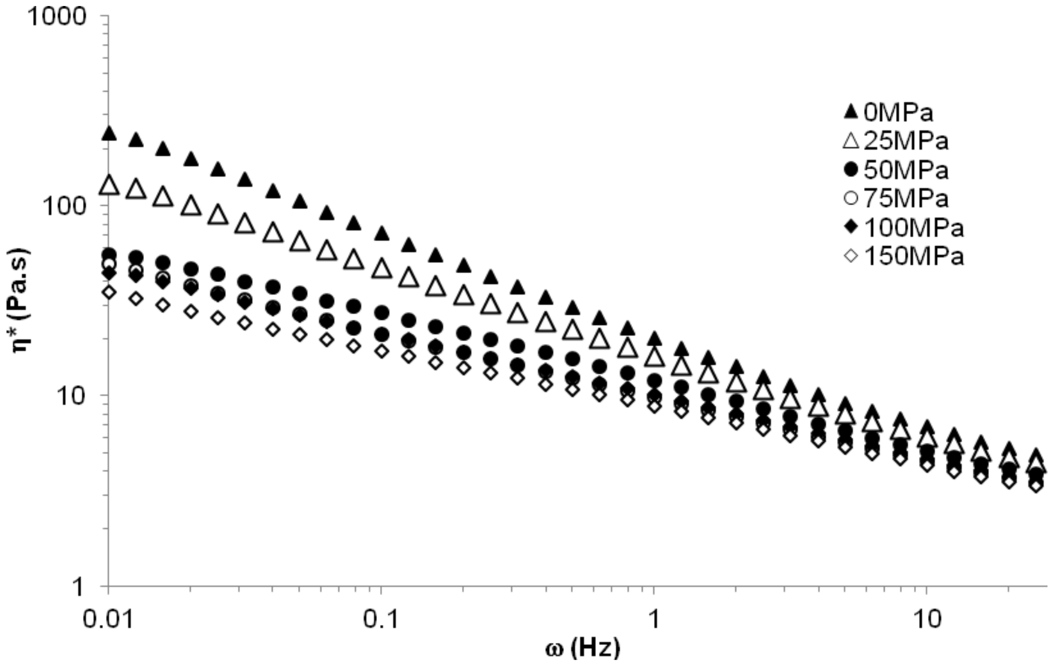

Figure 3.4: FCOJ Complex Viscosity at -10°C: effect of HPH processing (dots are the mean values, and the vertical bars are the standard deviation).

Figures 3.3 and *Figure 3.4* show that the HPH process had an impact on the rheology of FCOJ. A reduction in the complex viscosity can be noticed as the homogenization pressure increased and the same effect was observed for both the storage (G') and loss (G") moduli. Both figures also show asymptotic behaviour in relation to the effect of the HPH process. Thus the differences between higher homogenization pressures were smaller when compared to the differences between smaller homogenization pressures, which is in agreement with several other studies on both steady state shear (Silva et al., 2010, Augusto, et al., 2012b) and viscoelastic properties (Augusto et al., 2013b).

Thus HPH processing reduces both the viscous (G'') and elastic (G') behaviours of FCOJ, although the values for G" were always higher than the values for G'. This information can be better visualized in *Figure 3.5*, which presents the values for the loss tangent (δ) at different values of P_H. It can be clearly seen that the values for tanδ increased with HPH processing, indicating that

HPH also affects the ratio between G'' and G'. The processed FCOJ showed predominantly viscous (fluid) behaviour and reduced elastic (solid) behaviour.

All the previous results could be correlated with the flow behaviour of FCOJ, (Chapter). The reduction in elastic behaviour (solid component) could be correlated with the reduction in initial stress (σ_i; thixotropic behaviour) and with the consistency index (k; flow behaviour). Also, the viscous behaviour (liquid component) could be correlated with the reduction in the consistency index (k; flow behaviour) and flow behaviour index (n), and consequently with the apparent viscosity (η_a).

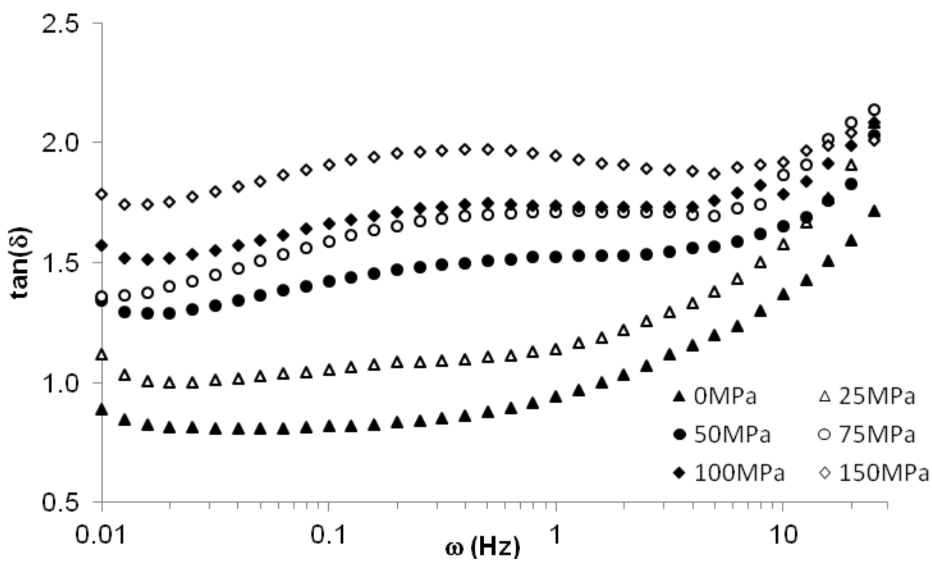

Figure 3.5: Loss tangent of FCOJ at -10°C: effect of HPH processing (dots are the mean values).

The storage and loss moduli were then evaluated using the power law model (*equation 3.1* and *equation 3.2*), whose parameters are shown in *Figure 3.6* as a function of the homogenization pressure (P_H).

It can be observed that the n' values for FCOJ were always smaller than the values for n'', showing that the elastic behaviour of the product (G' = f(ω)) was more affected by the oscillatory frequency than the viscous behaviour (G'' = f(ω)). On the other hand, the non-processed FCOJ showed a k' value that was higher than the k’’ value, the opposite behaviour of the HPH processed samples. Thus the magnitude of the elastic (k’) and viscous (k’’) behaviours, as well as their ratio, were changed by HPH processing.

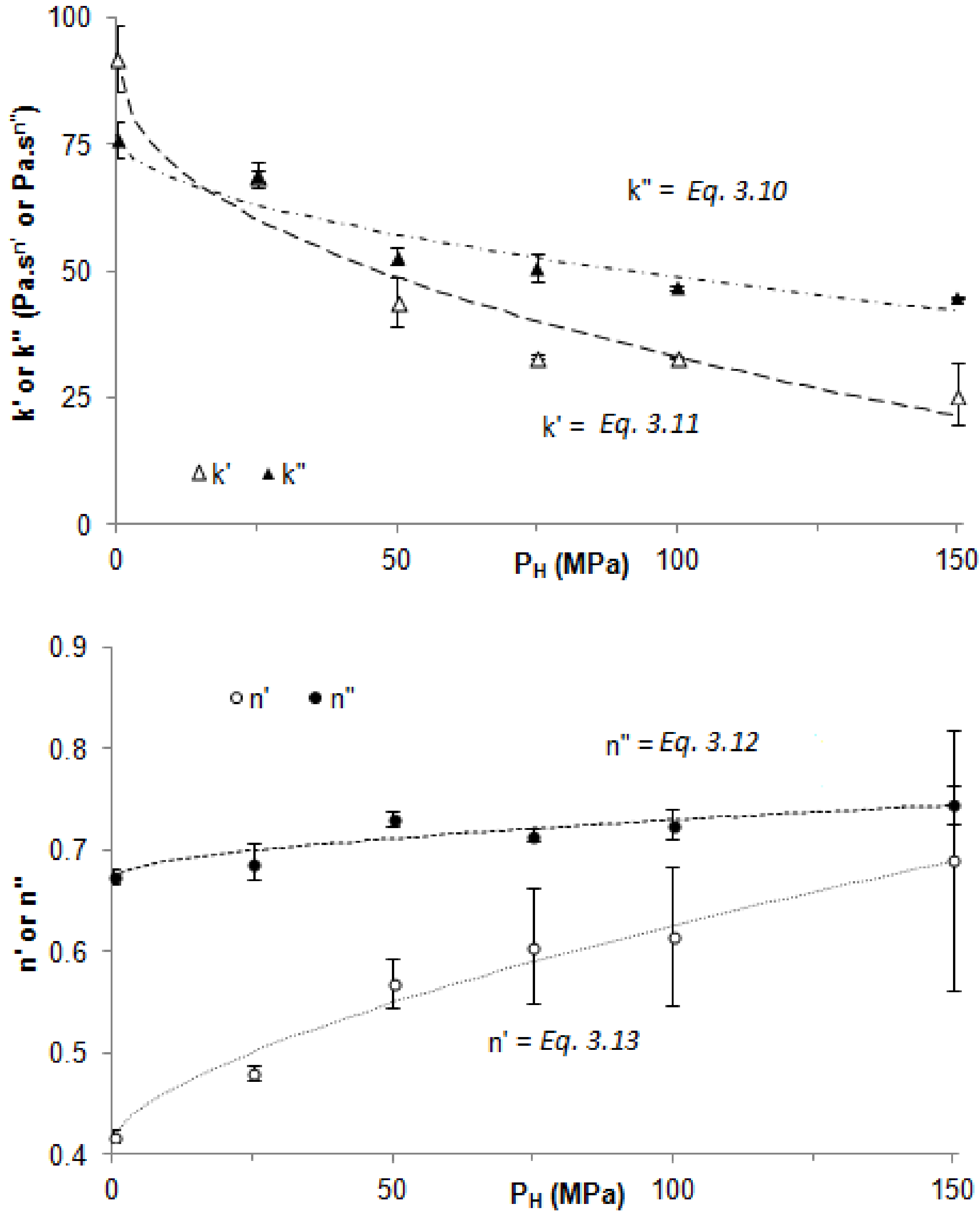

Figure 3.6: Viscoelastic Power Law model (Equation 3.1 and Equation 3.2) parameters for FCOJ at -10°C: effect of HPH processing (dots are the mean values, vertical bars are the standard deviation, curves are the models).

Although the HPH technology reduced both G' and G", its effect had different magnitudes in each case. From 0 MPa to 150 MPa, the value for k' dropped from 91.87 to 25.65 ($Pa.s^{n'}$), or reduced to ~28% of its original value, while the value for k" only dropped from 76.12 to 44.87 ($Pa.s^{n''}$), which represents a reduction to ~59% of its original value. The same trend can be observed with n', which increased by ~65%, as against a smaller increase for n'' (~10%).

Thus these parameters were modelled as a function of the homogenization pressure. Due to the behaviour observed (Figure 3.6), a power-type function with the initial value (that of the original properties of the FCOJ, i.e., those processed at 0 MPa) was used to model the parameters k', k'', n' and n' and the equations obtained can be seen in Figure 3.6 and Equation 3.10, 3.11, 3.12 and 3.13.

$$k' = 76.90 - 2.16 {P_H}^{0..572} \quad R^2=0.951 \qquad (\textit{Equation 3.10})$$

$$k'' = 92.76 - 7.86 {P_H}^{0..4403} \quad R^2=0.961 \qquad (\text{Equation 3.11})$$

$$n' = 0.674 + 0.004 {P_H}^{0..572} \quad R^2=0.919 \qquad (\text{Equation 3.12})$$

$$n'' = 0.414 + 0.0108 {P_H}^{0..646} \quad R^2=0.919 \qquad (\text{Equation 3.13})$$

These results show that the elastic behaviour was more affected by HPH technology than the viscous one. This is similar to the results of Bengsson and Tornberg (2011) with apple, tomato and carrot fibre suspensions, where G' was more affected by HPH processing than G" (with the exception of just one specific apple fibre suspension). Wang et al. (2012) studied the effect of HPH on the viscoelasticity of high amylose maize starch. After HPH processing (up to 100 MPa) both G' and G" decreased, which was related to the breakdown of the molecular chain.

On the other hand, Augusto et al (2013b) studied the effect of HPH on tomato juice viscoelasticity (work pressures up to 150 MPa). In this study, both k' and k'' increased as the homogenization pressure (P_H) increased. Lopez-Sanchez, et al. (2012a) studied the impact of HPH processing on tomato, carrot and broccoli dispersions. The HPH process decreased both the storage (G') and loss (G") moduli of the carrot and broccoli suspensions, but increased those of tomato.

Vegetable products are complex dispersions composed of two phases: the pulp (dispersed phase) and the serum (dispersant phase). It is known that HPH disrupts suspended particles and ruptures some polysaccharides present in the vegetable serum phase (Augusto et al., 2012a, Corredig and Wicker, 2001, Floury et al., 2002, Wang et al., 2011, Lagoueyte and Paquin, 1998 and Harte and Venegas, 2010). Thus the increase or decrease of G' and G'' will be a function of the interactions amongst the particles and between the particles and the serum.

Since each cell wall has its own composition and structure, each will show a unique resistance to shear during the HPH process. This has a major impact on the changes to the rheological properties of a product due to the HPH process, since each fragmented cell will expose and release unique internal and wall constituents, such as pectin and protein (Lopez-Sanchez et al. 2011b). The final interaction between the serum and the suspended particles is a function of this composition, justifying the differences found between vegetables.

In Chapter 2, the impact of HPH on the FCOJ particle size distribution (PSD) was studied, with the same range of homogenization pressures used in this study. The mean particle diameter of the product decreased to ~12% of its original value when processed at 150 MPa. Furthermore, the shape of the particle distribution curve changed. While the non-processed sample showed a monomodal distribution, the processed samples showed a plateau of small particles. Thus the ratio of smaller to larger particles increased, as also with other fruit products (Silva et al, 2010; Augusto et al, 2012b; Moelants et al, 2012).

This information is important to explain the changes in the dynamic rheology of FCOJ. Smaller particles can occupy the spaces between larger particles. When a strain is applied, they can easily change their position, decreasing the product resistance to flow (viscous behaviour), which is mainly related to a decreased serum drag. The smaller particles can also act as a lubricant for the larger ones. Since the ratio of the smaller to the larger particles increased, there was less friction to flow. This effect was more evident at higher frequencies. Furthermore, the smaller particles have a minor alignment effect, which can be observed by the increase in n' and n" (i.e., tending to unity).

The molecules in the serum phase can interfere with the rheological behaviour of the product. HPH can reduce the molecular size of soluble polysaccharides, as studied by many authors with several products (Floury et al., 2002; Lagoueyte and Paquin, 1998; Augusto, et al 2012a). Thus HPH reduces the consistency of the FCOJ serum phase.

The steady-state shear properties of FCOJ showed shear thinning ($n < 1$) behaviour with no yield stress (σ_0), and the consistency decreased with increase in homogenization pressure. This result is described in Leite et al., (under review), and was confirmed in order to evaluate the Cox-Merz rule. It was concluded that the liquid component, mainly responsible for the viscous response, was related to the friction due to the drag through the serum, and smaller particles show less serum drag, leading to a reduction in G" (Loss Module).

The elastic response, or the solid component of the FCOJ viscoelasticity, can be related to the capacity for energy storage of the product. This is mainly caused by the particle-particle interactions, acting like a hookean spring. HPH decreased the suspended particle size (Chapter 2). It is known that small particles have a higher specific surface area. When the attractive forces between particles are high, this can lead to greater particle-particle interaction. This greater interaction is responsible for the formation of a small network and/or of increasing particle aggregation and is of an attractive nature. Repulsive interaction can result in elastic behaviour but forms no structures. These effects were observed for tomato juice (Augusto et al., 2013b), and in this case resulted in greater solid behaviour (an elastic response) and an increase in G'. This was also evidenced by an increase in the yield stress (σ_0).

However, FCOJ does not show this characteristic (σ_0), as observed by Leite et al., (under review). This was evident from the reduction in FCOJ thixotropy when processed by HPH (Leite et al., under review), suggesting that the forces of interaction between the suspended particles were negligible during flow. However these interactions only affect the viscoelasticity of FCOJ under low strain conditions (very low frequencies) and are responsible for the elastic behaviour (G’). Since HPH decreased the particle size, the distance between the particles increased, and with a greater distance between the particles, there were more free spaces to configure reorganization during the strain, resulting in a decrease in interaction and hence in G’. Such reorganization without particle interaction is also a viscous response.

This is in accordance with the results obtained in the present study, i.e. a reduction in PSD affects the viscoelasticy, with a greater effect on elastic behaviour as compared with viscous behaviour. These results corroborate with the idea that a reduction in the consistency of FCOJ is mainly affected by the reduction in particle size, but also by the reduction in molecular size of the soluble content.

Finally the correlation between the apparent (η_a, Equation 3.7) and the complex (η^*, Equation 3.6) viscosities was evaluated.

The behaviours of the apparent (η_a, Equation 3.7) and the complex (η^*, Equation 3.6) viscosities were similar to that described for the non processed juice. The applicability of the modified Cox-Merz rule (Equations 8, 9) is shown in Table 1, with good agreement (R^2 above 0.99).

Table 3.1: Linear modifications of the Cox-Merz rule for vegetable products.

Product		α	β	Reference
FCOJ (66 °Brix,-10 °C)	0 MPa	1.61	1.26	Present work
	25 MPa	1.54	1.21	
	50 MPa	1.05	1.01	
	75 MPa	0.82	0.93	
	100 MPa	0.71	0.88	
	150 MPa	0.56	0.85	
Tomato juice (5.4 °Brix, 25 °C)		0.12	0.18	Augusto et al., (2013)
Tomato paste (26-30 °Brix, 40 °C)		0.0029-0.029	-	Rao and Cookey (1992)
Spanish Honey (80.4-82.0 °Brix, 25°-30°C)		1.100-1.250	-	Oroian et al., (2013)
Potato puree (18.5-20.5% solids, 25° C)		0.008-0.020	-	Canet et al., (2005); Alvarez et al., (2004)
Rice starch-xanthan gum mixtures (25 °C)		0.307-0.478	-	Kin and Yoo (2006)
Sweet potato baby food (11.2 °Brix, 20 °C)		-	0.14	Ahmed and Ramaswamy (2006)

It can be seen that HPH decreased the modified Cox-Merz parameters (α and β). In addition, more important than the absolute values, the FOCJ processed up to 50 MPa showed values for α and β higher than 1.0, with the opposite behaviour when processed at P_H ≥75 MPa. This means that the non-processed sample, as well as those processed at P_H ≤50 MPa had higher apparent (η_a) than complex (η^*) viscosities. However, for the FCOJ processed at P_H ≥75 MPa, when both α and β were below 1.0, the apparent viscosity (η_a) was smaller than the complex viscosity (η^*). This was probably due to structure decay, due to the expressive reduction in the particle and serum components. In addition the modified Cox-Merz rule parameters were higher than the values commonly described for other food products, as can be seen in Table 1. This suggests that

FCOJ does not show a high density of entanglement or even strong particle interaction. Thus once again, the results are in accordance with previous results.

The results obtained here indicated that the rheological properties of FCOJ can be obtained by either oscillatory or steady-state shear experiments, even after high pressure homogenization. By using these linear correlations, it is possible to convert the results obtained in one experimental procedure into the other, which is interesting due to the experimental limitations.

The results of this study highlight the potential for the industrial application of HPH to promote changes in the rheological properties of FCOJ, leading to energy saving in many unit operations, since a decrease in both the elastic and viscous responses will lead to less energy being stored or dissipated and effectively transferred to the FCOJ to make it flow, making the flow more efficient.

3.4 Conclusion

The present work first evaluated the viscoelasticity of FCOJ, which showed similar values for the elastic (solid) and viscous (fluid) behaviours. The effect of high pressure homogenization (HPH) on these properties was then evaluated.

HPH decreased the complex viscosity (G*) of FCOJ by reducing both the loss (G'') and storage (G') moduli, although the elastic behaviour (G') was more affected by the technology. This reduction is mainly related to the reduction in particle size and a change in the molecular size of the serum phase.

These results show that HPH has great potential for industrial application in the production of FCOJ, leading to a reduction in energy consumption during pumping and other unit operations.

3.5 Acknowledgments

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Chapter 4. Multi-Pass High Pressure Homogenization (MP-HPH) of Frozen Concentrated Orange Juice (FCOJ)

Abstract

In this study multiple cycles of high pressure homogenization (HPH) were used to promote rheological changes on frozen concentrated orange juice (FCOJ). This non-thermal technology, initially studied for microbiological inactivation, can also be used in several other applications, such as intentionally changing the structure of fluid foods. It was recently shown that HPH could be used to reduce the consistency of FCOJ, minimizing the amount of energy to flow during processing and distribution. The use of multiple passages (MP-HPH) has already been studied for several applications, including changes in fluid consistency. However, FCOJ has not yet been studied, although it could reduce the process costs. The effect of MP-HPH on FCOJ (66º Brix) was evaluated up to 150 MPa and three cycles, with respectto the rheological proprieties (steady-state shear and time dependent properties), particle size distribution (PSD) and instrumental colour. The MP-HPH process showed a decreasing asymptotic effect on the FCOJ consistency index, since both the homogenization pressure and number of cycles increased. The PSD decreased as the pressure and number of cycles increased, but the processes showed no impact on product colour. MP-HPH could be an interesting alternative in the production of FCOJ, as it could provide the same effects as a single pass through the HPH but with lower costs and higher production rates.

Keywords: food properties, high pressure homogenization, homogenization in consecutive cycles, rheology.

Resumo

Neste estudo a homogeneização a alta pressão (HAP) foi consecutivamente usada múltiplas vezes para promover mudanças reológicas no suco de laranja concentrado e congelado (FCOJ). Esta tecnologia não térmica, inicialmente foi estudada para o controle microbiológico, pode também ser usada em várias outras aplicações, como por exemplo, para promover intencionalmente mudanças na microestrutura de alimentos fluidos. O uso de múltiplos processos consecutivos de HAP (MP-HAP) já foi estudado em diversas aplicações, incluindo mudanças na consistência de fluidos. Entretanto, não foi estudada para o FCOJ, apresentando um grande potencial para reduzir os custos de processo. O efeito do MP-HAP foi avaliado em FCOJ (66°Brix), até a pressão de 150 MPa, e três ciclos, considerando as propriedades reológicas (estado estacionário e dependentes do tempo), distribuição do tamanho de partículas e cor instrumental. As MP-HPH apresentaram um efeito assintótico decrescente no índice de consistência do FCOH, em relação ao aumento da pressão e número de ciclos. O processo não apresentou efeito na cor do produto. O uso de MP-HPH demonstra ser uma relevante alternativa para a produção do FCOJ, podendo alcançar os mesmos resultados de um único processo de HAP, porém com um menor custo e com maior produtividade.

Palavras chave: Propriedades dos Alimentos, Homogeneização a Alta Pressão, Ciclos consecutivos de Homogeneização. Reologia

4.1 Introduction

High pressure homogenization (HPH) is a technology originally studied with the objective of replacing thermal food processes. In this technology, a fluid is continuously pumped through a narrow gap and according to the mass and energy conservation laws, the speed of the fluid increases drastically, resulting in a great pressure drop to atmospheric pressure. This results in high shear stress, high turbulence and cavitation, which are the main causes of product change (Floury, et al., 2004, Pinho et al., 2011).

HPH can be used to promote several desirable changes in food products, such as the improvement of the rheological properties of fruit juices (Augusto et al., 2012); a reducing in sedimentation during storage (Kubo et al., 2013; Poliseli-Scopel et al., 2012; Silva et al., 2010); an improvement in the activity of enzymes applied in food processing (Tribst et al., 2013); a reducing in proteolysis of fermented milk (Oliveira et al., 2013; and an improvement in the emulsifying properties of polysaccharides (Porto et al., 2012; T. Wang et al., 2012, B. Wang et al., 2012) and in the functional properties of proteins (Dong et al., 2011).

HPH can be carried out using consecutive cycles, which is called Multi-Pass High Pressure Homogenization (MP-HPH). It has been shown that MP-HPH processing at lower homogenization pressures (P_H) was able to produce the same changes as one-pass processing at higher P_H, when considering enzyme activity (Tribst et al., 2013; Caligaris et al., 2012; Welti-Chanes et al., 2009) and microbial inactivation (Maresca et al., 2011; Donsi et al., 2009; Patrignani et al., 2009). This could be interesting from an industrial point of view, because lower pressures reduce equipment and maintenance costs.

Orange juice is one of the most popular juices in the world, and its production is often derived from frozen concentrated orange juice (FCOJ). HPH technology has been shown to be a powerful tool in the promotion of a reduction in the consistency of FCOJ, has both its flow and viscoelastic properties (Chapters 2 and 3). A reduction in the consistency of FCOJ reduce the energy consumption during the unit operations, promoting the use of smaller and cheaper equipment, with less energy consumption and more efficient FCOJ processing. However, the effect of MP-HPH on the rheological properties of FCOJ is still unknown, although a lower P_H could result in even smaller processing costs (equipment and operation).

The aim of this work was to evaluate the use of multiple cycles of high pressure homogenization (MP-HPH) on FCOJ, aiming to optimize its processing by maximizing the HPH effect with lower costs.

4.2 Materials and Methods

4.2.1. Frozen Concentrated Orange Juice

The frozen concentrated orange juice was obtained directly from a local producer (Citrosuco, Limeira, São Paulo, Brazil). It was a commercial blend of juices from the Natal and Valencia varieties, concentrated to 66°Brix, pH 3.8, 3,24% citric acid and 11.7% of pulp, and stored in a conventional freezer at -18°C before processing.

4.2.2. High Pressure Homogenization (HPH)

The process was carried out within the range from 0 MPa (control) to 150 MPa (gauge, homogenization pressures – P_H) in a high pressure homogenizer (Panda Plus, GEA Niro Soavi, Italy). The juice was first thawed at 5 °C and then processed. The inlet temperature was 25ºC, simulating the conditions just after the concentration of the juice during industrial processing.

The FCOJ was introduced into the equipment by suction and quickly cooled using an ice bath just after the homogenization valve. After the first processing and cooling to 25ºC, the samples were re-introduced into the homogenizer in order to evaluate the effect of multi-pass processing.

The pressure of 150 MPa is the most drastic processing condition, with a greater impact on the reduction in product consistency. Thus the FCOJ was processed by multiple passes at homogenization pressures below the upper limit, in order to evaluate if a number of processes under mild conditions could be compared with one at the highest one. Thus, the 0 and 150 MPa samples were processed with 1 pass, and the 50 MPa and 100 MPa samples were processed with 1, 2 and 3 passes. All processes were carried out in triplicate.

4.2.3. Particle Size Distribution (PSD) Analysis

The particle size distribution was evaluated by light scattering (Malvern Mastersizer 2000 with Hydro 2000s, Malvern instruments Ltd, UK). The mean diameter was also evaluated based on the particle volume (D[4,3]; *Equation 4.1*), and the mean diameter base on the particle surface area (D[3,2]; *Equation 4.2*). This is useful once the particles are not ideal spheres, and the D[3,2] is more influenced by small particles while the D[4,3] is more influenced by the larger ones (Lopez-sanchez et al., 2011a; Bengtsson and Tornberg, 2011). These analyses were performed immediately after processing, in triplicate.

$$D[4,3] = \frac{\sum_i n_i d_i^{\,4}}{\sum_i n_i d_i^{\,3}} \qquad \text{(\textit{Equation 4.1})}$$

$$D[3,2] = \frac{\sum_i n_i d_i^{\,3}}{\sum_i n_i d_i^{\,2}} \qquad \text{(\textit{Equation 4.2})}$$

4.2.4. Instrumental Colour

The instrumental colour was obtained using a ULTRA PRO colorimeter (Hunter Associates Laboratory, USA), with illuminant D65 and angle of 10°, previously calibrated with a RSIN white reference (L*=92.03, a*=−0.88, b*=0.63) as described by Sánchez-Moreno et al. (2006). The samples were placed in glass cuvettes and three readings were obtained for each replicate.

The CIELab technique was used for the evaluation, where the values for L* (lightness), a* (redness: green to red) and b* (yellowness: blue to yellow) were first obtained, and then used to express the colour changes.

4.2.5. Rheological Analyses

Rheological analyses were carried out using a controlled stress (σ) rheometer (AR2000ex, TA Instruments, USA) with the temperature maintained constant at -10°C using a Peltier system, and a cross hatched plate-plate geometry (40 mm of diameter). The samples were analyzed immediately after the HPH process.

Prior to this, a gap-independency procedure was carried out, as described by Tonon et al. (2009), which consists of varying the distance between the plates until the flow curve reaches a steady state. The gap obtained gap was 1000 μm.

The rheological evaluation was carried out with new samples, which were first placed in the rheometer and maintained at rest for 5 min before shearing. After resting, the samples were sheared at a constant shear rate (300 s^{-1}) for 300 s, while the shear stress was measured for the time-dependent (thixotropic) behaviour evaluation. After the time-dependent shear period, a stepwise linear decreasing protocol (300 s^{-1} to 0.1 s^{-1}) was used to guarantee steady-state shear conditions for the flow behaviour evaluation.

The FCOJ time-dependent rheological properties were evaluated using the first part of the protocol. The shear stress decay was evaluated using the Figoni and Shoemaker model (Figoni and Shoemaker, 1983; *Equation 4.3*).

The steady-state shear rheological properties were evaluated using the second part of the protocol, and the product flow behaviour was modelled using the Ostwald-de-Waele model (*Equation 4.4*), according to the results presented in Chapter 2.

Both the time-dependent and steady-state shear procedures were carried out at -10ºC in order to evaluate the rheological properties of the product under at the conditions of freezing and pumping during storage and transportation conditions.

$$\sigma = \sigma_e + \left(\sigma_i - \sigma_e\right) \cdot \exp\left(-k_{FS} \cdot t\right) \qquad (\textit{Equation 4.3})$$

$$\sigma = k \cdot \dot{\gamma}^n \qquad (\textit{Equation 4.4})$$

The parameters for each model (*Equation 4.3* and *Equation 4.4*), were obtained by linear or nonlinear regression using the CurveExpert Professional software (v.1.6.3, http://www.curveexpert.net/, USA) with a significant probability level of 95%. Moreover, the effects of the homogenization pressure (P_H) and of the number of cycles on the properties of the FCOJ were evaluated using the analysis of variance (ANOVA) and the Tukey test at a 95% confidence level. The STATISTICA 5.5 (StatSoft, Inc., USA) software was used for this purpose.

4.3 Results e Discussion

4.3.1. Particle Size Distribution (PSD)

Figure 4.1 shows the effect of the HPH process and of the number of HPH cycles on the PSD of FCOJ. This result is similar to that obtained for a single HPH processed FCOJ (Chapter 2). It is also similar to that observed by other authors for other vegetables matrices (Augusto et al, 2012b, Silva et al, 2010, and Moelants et al, 2012). Regarding the number of processes, two and three processes at 50 MPa showed a distribution close to that obtained with the single process at 100 MPa. Furthermore, two and three processes at 100 MPa showed a larger amount of smaller particles than the single 150 MPa process.

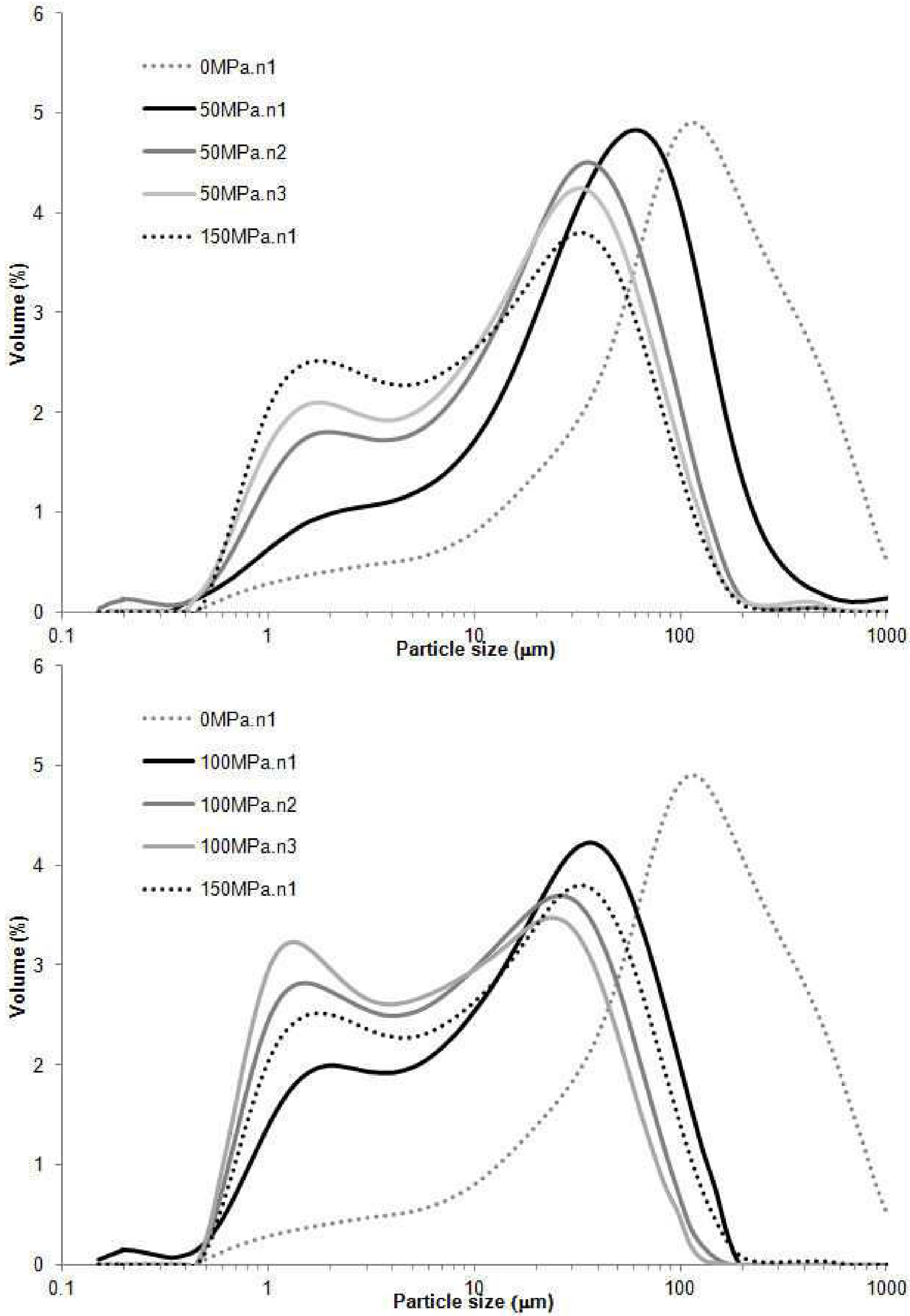

Figure 4.1: Effect of MP-HPH on the particle size distribution (PSD) of FCOJ (figure caption: P_H.number of cycles [MPa.n])

Analysing the shapes of the PSD curves, an increase in P_H resulted in narrower distributions, with an asymptotic behaviour. The number of cycles presented an asymptotic effect, i.e., the subsequent process had a smaller impact on PSD if compared with the previous process using the same homogenization pressure.

When both P_H and the number of cycles increased, there was an accumulation of small particles, evidenced by the formation of a plateau. This can be attributed to the disruption of suspended particles. In fact, the distribution changed from a monomodal to a bimodal distribution, as can be seen in *Figure 4.1*. There is a minimum particle size that the equipment can produce, which is related to the peak of small particles (~1-2μm). Its position practically does not change with P_H or the number of cycles. However, the amount of larger particles decreases and, consequently, the mean particle size, as evidenced by the change in position of the larger particles peak.

Figure 4.2 shows the mean diameter of FCOJ as a function of the homogenization pressure (P_H) and number of cycles. The value of D[4,3] showed an almost tenfold higher mean diameter value than the D[3,2], for all the samples. D[4,3] is more influenced by larger particles, and its values showed no statistical difference ($p<0.05$) between processing at 50 MPa/2 cycles and the others. On the other hand, D[3,2], which is more influenced by smaller particles, showed statistical differences with multiple cycles. This corroborates with the PSD results (*Figure 4.1*), since the multiple HPH cycles increased the number of small particles.

Therefore, the proportions of small and large particles were changed with increases in homogenization pressure (P_H) and the number of cycles, also, suggesting changes in product rheology.

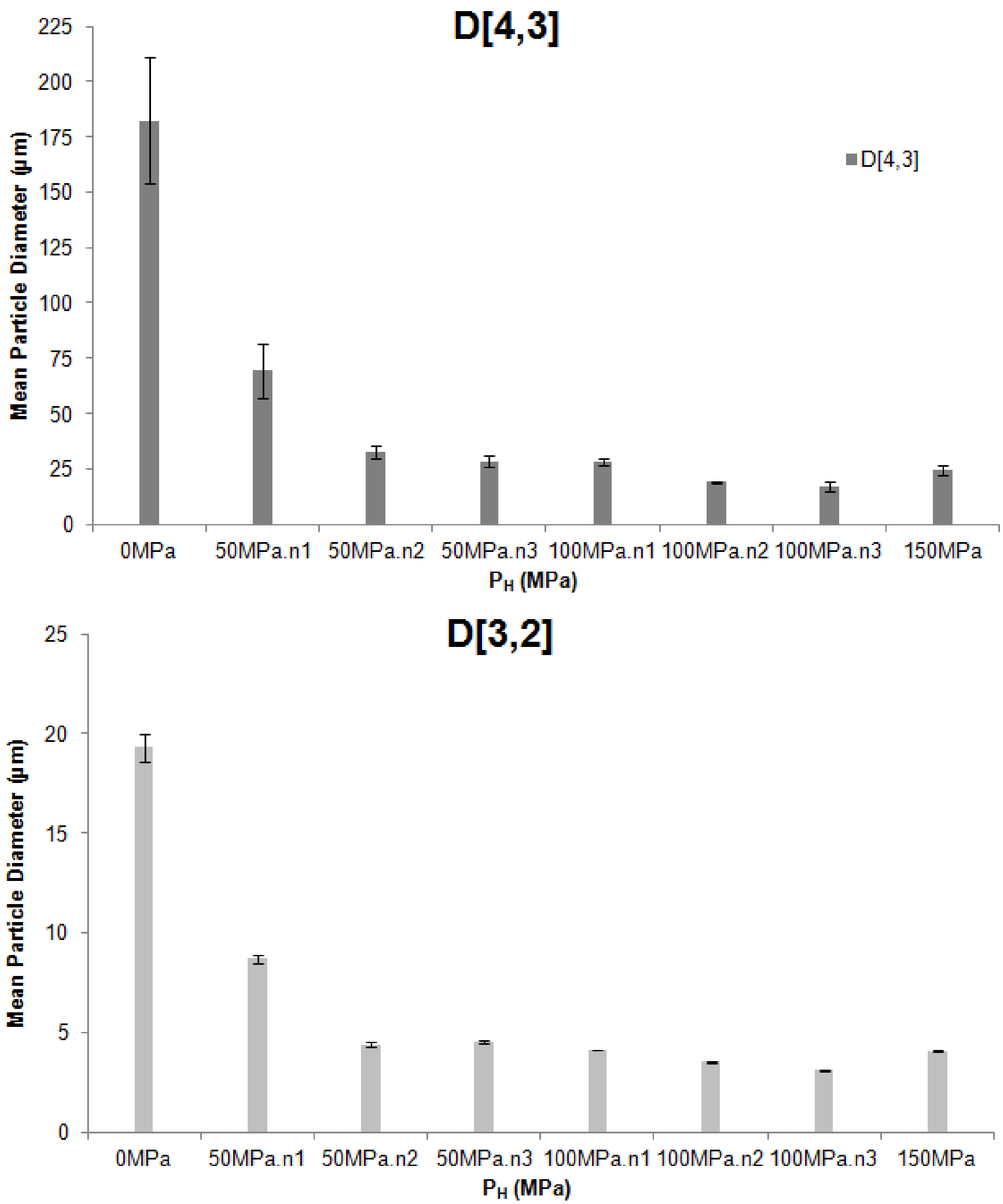

Figure 4.2: Effect of MP-HPH on the mean particle diameters (D[4,3] and D[3,2]) for FCOJ (Vertical bars are standard deviation; different letters represent significantly different values ($p < 0.05$).)

4.3.2. Instrumental Colour

The effects of both homogenization pressure (P_H) and the number of cycles on the instrumental colour parameters of FCOJ (CieLAB method; L*, a*, b*) are presented in *Figure 4.3*.

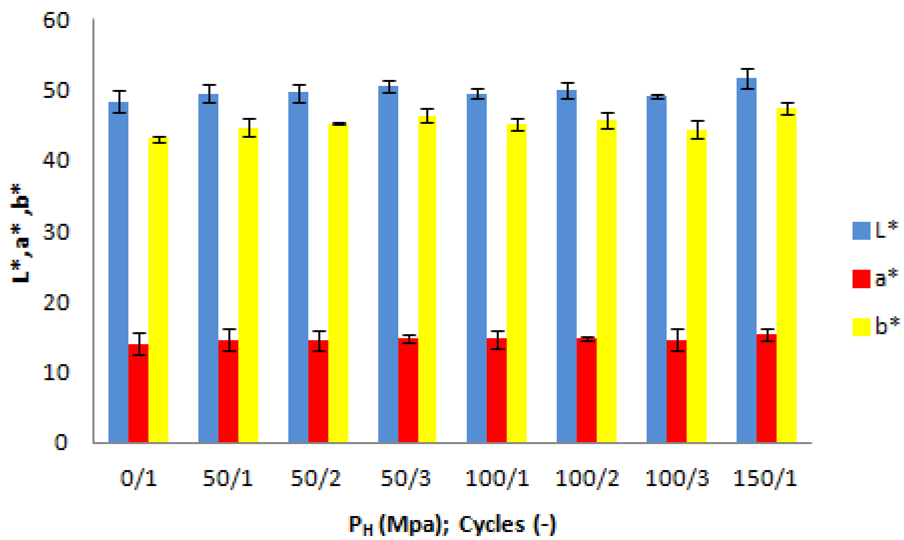

Figure 4.3: FCOJ primary parameters for instrumental colour (CieLAB method) as a function of the MP-HPH process (vertical bars are the standard deviation, the values for each parameter show no statistical difference in relation to the homogenization pressure ($p<0.05$))

As expected, none of the primary parameters for instrumental colour was influenced by the HPH processing, as well the derived hue and chroma (data not shown), neither by single nor by multiple processing ($p<0.05$).

This result is compatible with those obtained in Chapter 2 with single HPH processed FCOJ. FCOJ showed major changes in its structure during its production (for example during the unit operations of extraction, pulping, thermal process, concentration and freezing). Consequently, the pigments were already well dispersed in the fluid medium and HPH promoted no additional pigment liberation. The opposite occurs with to products that still contain whole cells, for example tomato juice, where Kubo et al, (2013) showed that HPH was able to rupture cells containing pigments, which were then oxidized during storage showing a change in colour .

It is interesting to highlight that this result is highly desirable, since the use of HPH in the production of FCOJ would not be noticed by the consumers.

4.3.3. Rheological Behaviour

*Figure 4.*4 shows the effect of multiple cycles on flow behaviour of FCOJ. As expected, FCOJ showed shear thinning behaviour without yield stress (Chapter 2) which was described by the Ostwald-de-Waele model (*Equation 4.4*; $R^2>0.99$). *Figure 4.5* shows the parameters for the Ostwald-de-Waele model obtained for each sample, considering both the homogenization pressure (P_H) and the number of cycles. *Table 4.1* shows the flow behaviour equations for each sample for the pressure of homogenization and the number of cycles.

Table 4.1: Effect of single and multiple HPH processing on flow properties of FCOJ: Ostwald-de-Waele model at -10°C.

P_H (MPa)	Cycles		
	1	2	3
0	$17.93\dot{\gamma}^{0.67}$		
50	$11.07\dot{\gamma}^{0.72}$	$8.87\dot{\gamma}^{0.73}$	$7.42\dot{\gamma}^{0.74}$
100	$8.08\dot{\gamma}^{0.74}$	$7.19\dot{\gamma}^{0.75}$	$6.35\dot{\gamma}^{0.77}$
150	$6.60\dot{\gamma}^{0.75}$		

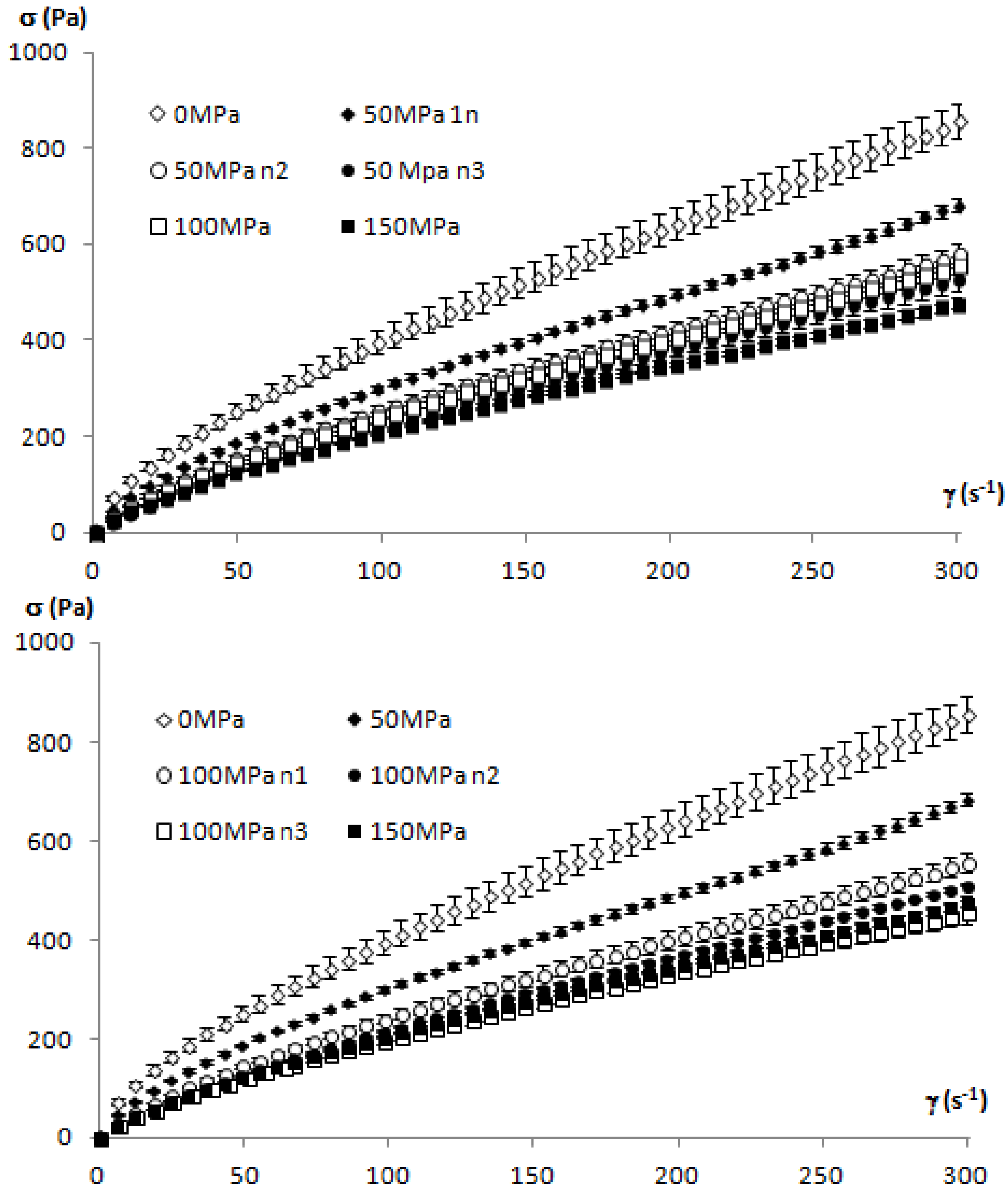

Figure 4.4: Flow behaviour of FCOJ at -10°C: effect of MP-HPH (dots are the mean values and vertical bars are the standard deviations).

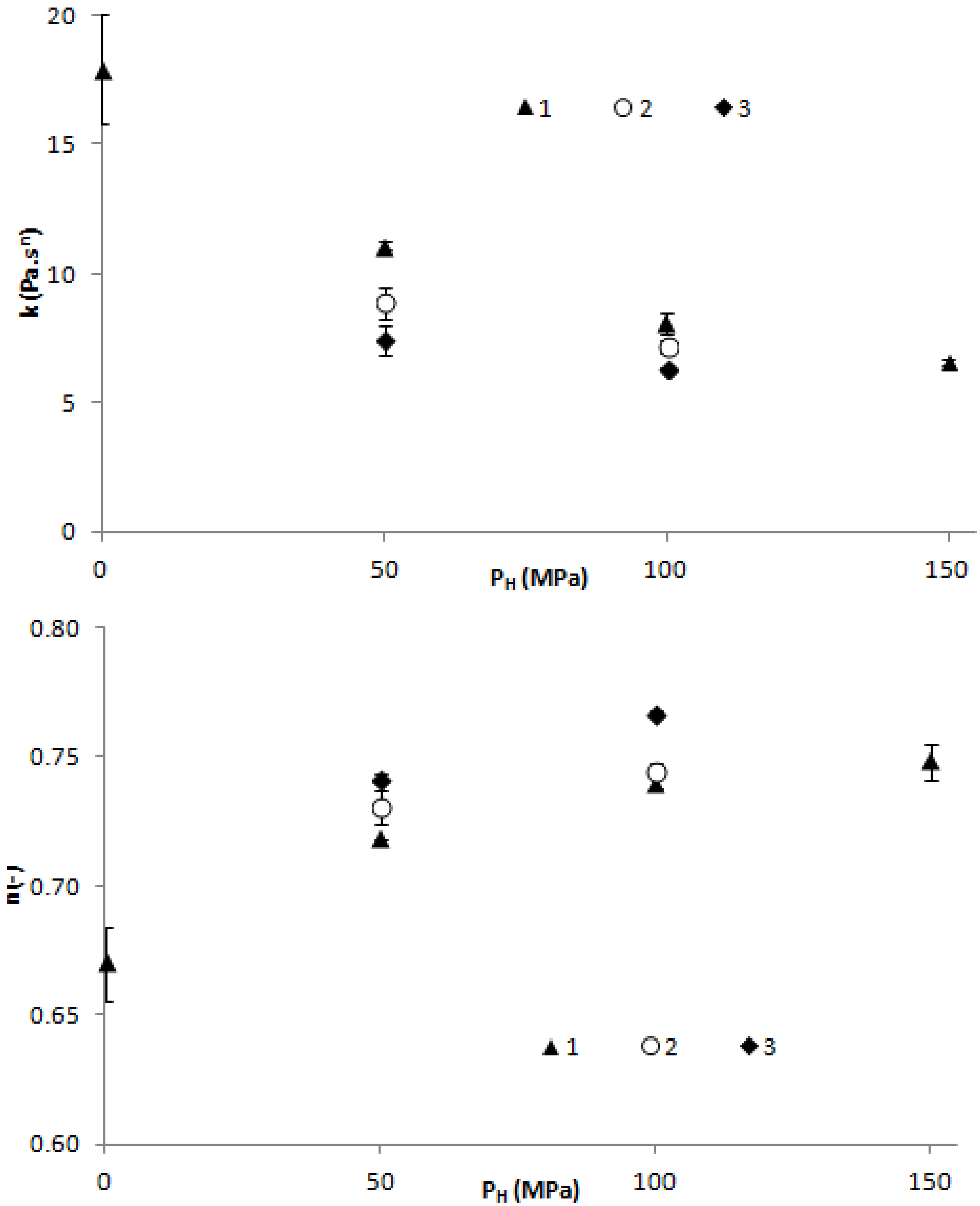

Figure 4.5: Ostwald-de-Waele parameters for FCOJ at -10°C: effect of MP-HPH (dots are the mean values and the vertical bars are standard deviations).

As expected, HPH reduced the juice consistency as described by the consistency index (k). Asymptotic behaviour was also noticeable for both the homogenization pressure (P_H) and the number of cycles. The effect of processing with multiple cycles was greater with lower homogenization pressures. The juice processed at 50 MPa showed greater changes if than those processed at 100 MPa. Two cycles at 50 MPa had almost the same effect as one passage at 100 MPa, and three cycles at 50 MPa showed a reduction in consistency between those obtained with a single process at 100 and one at 150 MPa, being close to that obtained with cycles at 100 MPa. Three cycles at 100 MPa showed a greater reduction than at 150 MPa. Nevertheless, it must be highlighted that MP-HPH is not a practical processing, and would be more interesting if a small number of cycles are effective. Therefore, only three cycles were evaluated.

The flow behaviour index (n) increased with both the number of cycles and homogenization pressure. Two cycles at 50 MPa had almost the same effect as one passage at 100 MPa, and three cycles at 50 MPa showed a flow behaviour index equal to that obtained with one single process at 100 MPa, and close to that obtained with two cycles at 100 MPa. Three cycles at 100 MPa showed a greater increase in the flow behaviour index when compared with the 150 MPa sample.

The asymptotic behaviour with the number of cycles was expected and has been shown in other studies, for example in microbial inactivation (Maresca et al., 2011). Therefore, the effect of MP-HPH was not an additive effect but was related to a physical limitation of the equipment (the gap dimension and pressure) which limits the maximum shear stress and mechanical energy delivered to the product. Larger particles are more affected by HPH, being broken into small particles, whose resistance to disruption is greater. Consequently, the smaller particles pass through the gap with little or no change. This was confirmed by the increase in the amount of small particles, and concomitant to the decrease in the larger ones, a shown on *Figure 4.1*. Thus the importance of multi-passage processing is highlighted when using few cycles at small homogenization pressures.

As described in Chapter 2, the reduction in consistency of FCOJ was due to changes in both the suspended particles and the serum phase. The HPH process promoted a reduction in the molecular size of some macromolecules in the serum phase, as described in several studies (Augusto et al., 2012a, Corredig and Wicker, 2001, Floury et al., 2002, Wang et al., 2011, Lagoueyte and Paquin, 1998 and Harte and Venegas, 2010). The disruption of polysaccharides promotes a reduction in serum consistency, which changes the drag of the particle and molecules through the serum.

Also, HPH disrupted the suspended particles, resulting in smaller particles and a different ratio between the small and larger ones. Smaller particles show less resistance to flow, leading to a lower consistency index (k). Also, the smaller particles can occupy the spaces between the larger particles, creating a lubricant effect, and leading to less friction between particles (Servais et al, 2002). Therefore, both phenomena relates to the effect of HPH on FCOJ rheology.

The reduction in PSD also impacts the rheological properties of FCOJ due to the particle alignment factor, which can be measured from the flow behaviour index (n). Smaller particles align to the flow direction easily when compared with larger ones. Newtonian fluids have no alignment factor, showing a flow behaviour index equal to 1.0. The “n” value for FCOJ increased towards 1.0 with the increase in both homogenization pressure and the number or cycles. This indicates that the disrupted suspended particles have a lower alignment factor. This also contributes to a less complex flow and less friction during the flowing.

Figure 4.6 shows the thixotropic behaviour of FCOJ as a function of MP-HPH processing. The results showed that the effect of MP-HPH on the thixotropy of FCOJ was similar to the tendency observed for the flow behaviour, with asymptotic behaviour for both homogenization pressure (P_H) and number of cycles.

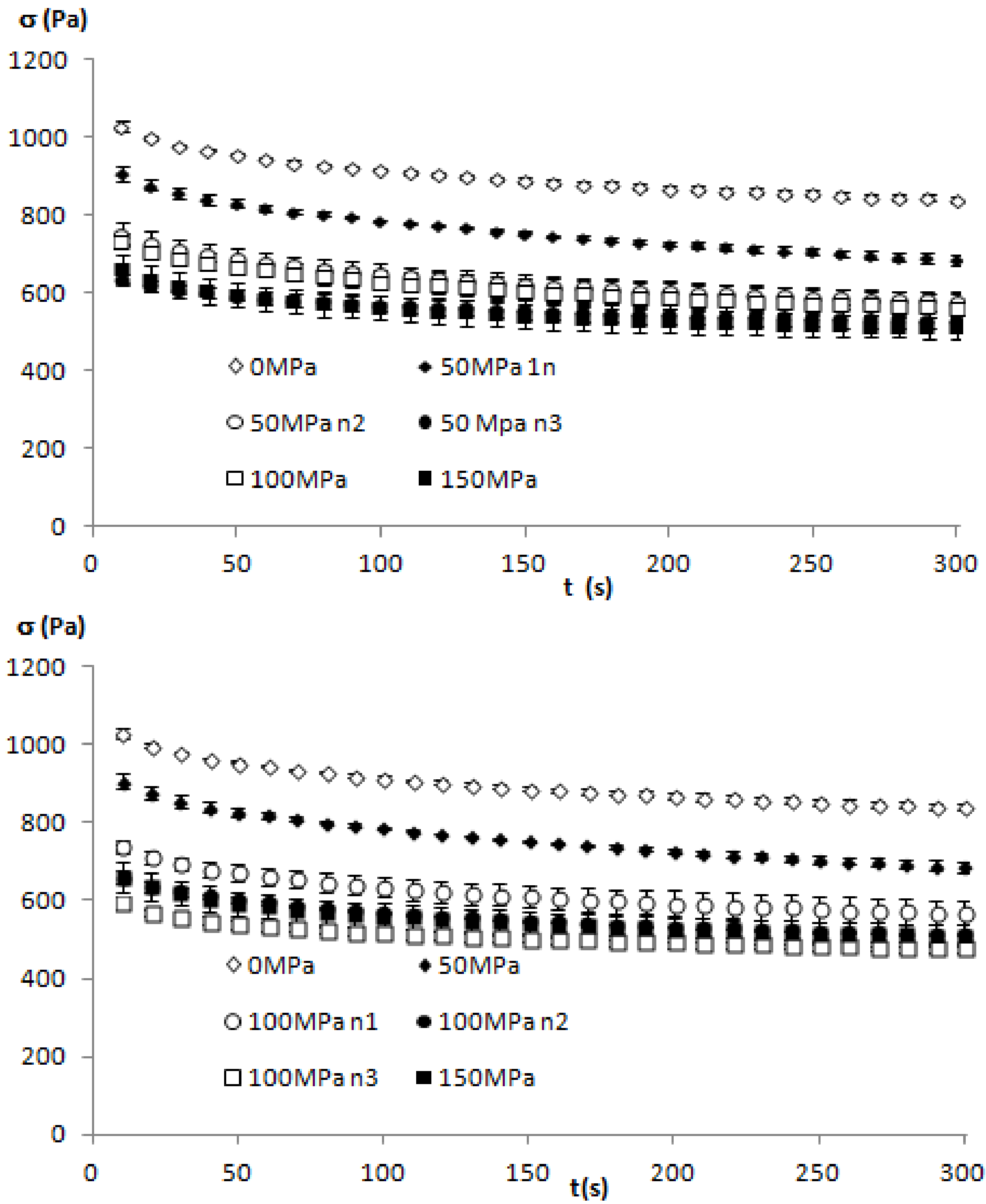

Figure 4.6: Shear stress against time (thixogram) for FCOJ during shearing at 300 s^{-1} at -10°C: effect of MP-HPH (dots are the mean values, vertical bars are the standard deviations).

The thixotropy of FCOJ was well described by the Figoni-Shoemaker model (*Equation 4.3*), whose parameters are shown in *Table 4.2*, and *Figure 4.7* (R^2>0.97). Similar to the flow characterization, multiple cycles at 50 MPa caused reductions in both the initial (σ_i) and equilibrium stresses (σ_e) that were equal or even greater than those caused by single processing at 100 MPa. Similarly multiple cycles at 100 MPa caused the same or even greater reductions than single processing at 150 MPa.

Table 4.2: Effect of single and multiple HPH processing on the thixotropic properties of FCOJ: Figoni-Shoemaker model at -10°C.

P_H	Single Cycle	
0	$829.8 + (1030.5 - 829.8) * e^{0.0084t}$	
50	$648.6 + (906.5 - 648.5) * e^{0.0065t}$	
100	$558.7 + (740.8 - 558.7) * e^{0.0087t}$	
150	$526.0 + (638.1 - 526.0) * e^{0.0049t}$	
P_H	Cycles	
	2	3
50	$654.6 + (758.5 - 645.6) * e^{0.0112t}$	$507.9 + (667.7 - 507.9) * e^{0.0078t}$
100	$504.3 + (664.9 - 504.3) * e^{0.0090t}$	$478.3 + (549.0 - 478.3) * e^{0.0107t}$

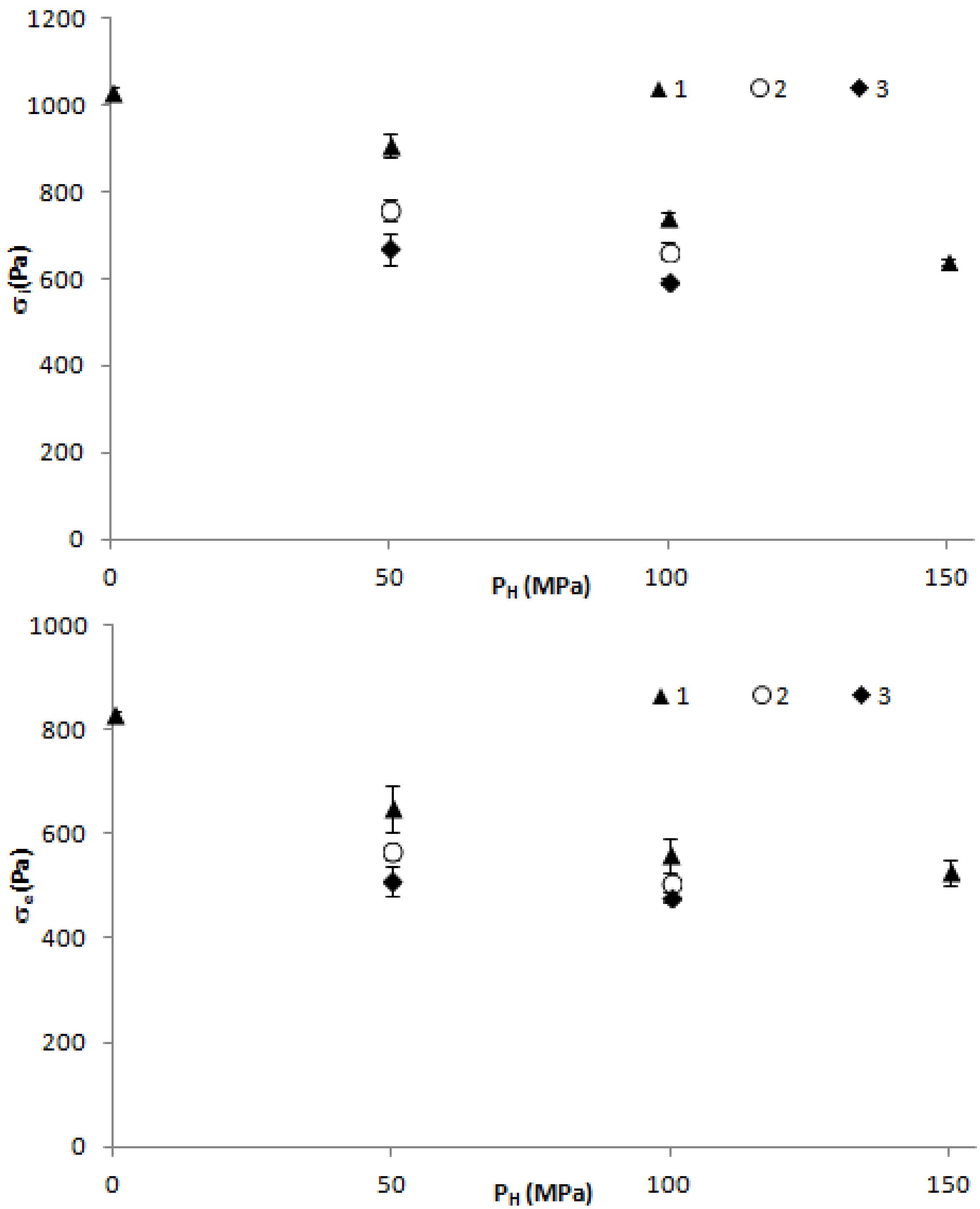

Figure 4.5: Figoni-Shoemaker parameters for FCOJ at -10°C: effect of MP-HPH (dots are the mean values and the vertical bars are the standard deviations).

As the PSD decreased (*Figure 4.1 and Figure 4.2*), the initial resistance to shear also decreased, explaining the reduction on the initial stress parameter (σ_i).

Also, the bimodal PSD resulted in a smaller friction during flow, less drag by the small particles through the serum and a smaller alignment factor. This explains the decrease in equilibrium stress (σ_e).

Thixotropy is related to the destruction or disruption of the internal structure due to flow (Ramos and Ibarz 1998; Cepeda et al. 1999). This internal structure can be formed by strong attractive interactions, but this is not the case for FCOJ, a fluid with no yield stress (σ_o). However, even the random non-alignment distribution and entanglement of the constituents could show an initial resistance to flow. Another factor that could result in thixotropic behaviour is related to the initial particle inertia to flow, especially due to the high consistency of FCOJ. These factors contribute to an initial resistance to flow, gradually changing during the flow to an optimal alignment, until reaching equilibrium stress.

Similar to the previous results (Chapter 2), it was not possible to observe a tendency for the kinetic factor (k_{FS}) in relation to either the homogenization pressure (P_H) or the number of cycles.

Similar to flow behavior, both the initial and equilibrium stresses of the FCOJ processed multiple times at 50 MPa showed similar results when compared with one or two cycles at 100 MPa. Three cycles at 100 MPa caused a greater reduction when compared with a single processing at 150 MPa. Also the physical limitation of the gap is responsible for the non additive effect of multiple process. Since the initial stress(σ_i) is relevant and high (together with the high consistency), the thixotropic behavior must be taken into consideration during the industrial process. A reduction in the FCOJ initial and equilibrium stresses of the FCOJ is highly desirable from an energy saving point of view.

Fluid consistency has a major role in the process development of FCOJ, since it has a great impact on energy consumption during processing. A reduction in the consistency of FCOJ is thus highly desirable for various unit operations, especially considering the amount of FCOJ produced. The reduction in consistency at 150 MPa represents approximately 58% when compared to the original value for apparent viscosity of the FCOJ (*Table 4.1*). However, the use of such high pressures (150 MPa) increases the cost of the equipment and resulting high energy consumption, also reducing process capacity. The use of multiple processing is a good alternative to obtain results similar to the single pass but using cheaper equipment and a smaller amount of energy. The maintenance costs are also lower due to less wear and tear of valves and gaskets. Multi-pass high pressure homogenization (MP-HPH) in the production of FCOJ is a good alternative and use lower pressures to reduce product consistency.

4.4 Conclusion

Multi-pass high pressure homogenization (MP-HPH) affected the microstructure of FCOJ and consequently its rheological behaviour. The PSD of the FCOJ changed from a monomodal distribution to a bimodal distribution, the ratio between small and large particles also changing.

The consistency of FCOJ decreased with both homogenization pressure (P_H) and number of cycles. It was concluded that similar results could be obtained with 2 or 3 cycles using a lower homogenization pressure, when compared to single processes at higher pressures. The results obtained could be industrially advantageous, since a small reduction in pressure decreases the need for highly specific equipment and also reduces energy consumption, enabling the use of more robust equipment with greater capacity, leading to a smaller fixed and maintenance costs.

4.5 Acknowledgment

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Chapter 5: FCOJ Processed by HPH technology: Effect on the ready-to-drink orange juice

Abstract

The high pressure homogenization (HPH) process can be used to reduce the consistency of frozen concentrated orange juice (FCOJ), which is highly desirable in industrial processing due to the reduction in energy costs. The production of FCOJ is almost entirely destined to obtaining the reconstituted (ready-to-drink - RTD) juice. This study aimed to evaluate the effect of the HPH processing of the on the ready-to-drink juice. FCOJ (66 °Brix) was processed by HPH up to 150 MPa, and then diluted to 11 °Brix for evaluation. The RTD-juice was evaluated by any of pulp sedimentation, instrumental colour and turbidity (serum cloudiness). The HPH process decreased the absorbance of the serum phase, which was related to the disruption of the suspended particles. However HPH showed no effect on product colour and pulp sedimentation behaviour. Therefore it was concluded that the HPH process promoted desirable effects on FCOJ and did not affect the RTD-juice properties.

Keywords: fruit juices, high pressure homogenization, physical stability,

Resumo

A homogeneização a alta pressão (HAP) pode ser usada para a redução na consistência de suco de laranja concentrado e congelado (FCOJ), sendo altamente desejável em um processo industrial devido à redução do gasto energético. A produção de FCOJ é quase toda destinada à obtenção do suco reconstituído (pronto para beber - *ready-to-drink* - RTD). Portanto, se faz necessário o entendimento do efeito do processamento HAP no suco de laranja RTD. Neste trabalho o FCOJ (66 °Brix) foi processado por HAP até a pressão de 150MPa, sendo diluído para 11 °Brix para avaliação. O suco RTD foi avaliado através da sedimentação de polpa, cor, e opalescência (turbidez da fase soro). A HAP decresceu a absorbância da fase soro, que pode ser relacionada ao rompimento das partículas suspensas. Porém a HAP não apresentou efeitos sobre a cor do produto ou no comportamento de sedimentação de polpa. Portanto, foi concluído que o processo HAP promove alterações desejadas no FCOJ, como redução da viscosidade aparente, mas não afeta as características físicas do suco RTD, o que é altamente desejável.

Palavras chave: Suco de Frutas, Homogeneização a alta pressão, Estabilidade física.

5.1 Introduction

High pressure homogenization (HPH) is a non thermal technology that can be used for fluid foods. As a consequence of the mass and energy conservation laws, HPH promotes several changes due to high shear, cavitation and turbulence. These phenomena are caused by the reduction in pressure as a result of the sudden increase in fluid velocity when it is forced to flow through a narrow gap. (Floury, et al., 2004; Paquin, 1999; Pinho et al., 2011). HPH was initially studied to guarantee the necessary microbial and enzyme inactivation in foods, but was recently proposed as a useful unit operation to promote desirable changes in food products. Thus this technology has been studied as a means for changing the rheological properties of fruit juices, reduce phase separation, improve enzyme activity, reduce proteolysis fermented milk and improve the properties of polysaccharides and proteins (Augusto et al., 2012b, Augusto et al., 2013; Kubo et al., 2013; Poliseli-Scopel et al., 2012; Silva et al., 2010; Tribst et al., 2013; Oliveira et al., 2013; Porto et al., 2012; T. Wang et al., 2012, B. Wang et al., 2012; Dong et al., 2011).

Orange juice is one of the most popular juices in the world. The ready-to-drink (RTD) juice at 10-12ºBrix is often obtained from the dilution of the frozen concentrated orange juice (FCOJ) at 65-66ºBrix

The effect of HPH on orange juice, processing the single-strength juice, has been studied by several authorswith respect to microbial and enzyme inactivation, cloud stability, and the improvement some of the orange juice quality properties (Lacroix et al., 2005; Croak & Correig, 2006; Campos & Cristianini, 2007; Betoret, et al, 2009; Sentandreu, et al, 2011; Betoret, et al, 2012; Carbonell, et al, 2013; Cerdán-Calero, et al, 2013). In previous chapters, the HPH process was first studied using the concentrated product (FCOJ). It was shown that HPH change the rheological behaviour of FCOJ, reducing both the viscous and elastic properties and promoting an important reduction in its consistency. Therefore HPH reduces the amount of energy used in its production, especially during some unit operations such as pumping. However, it is necessary to understand the effect of HPH processing of the FCOJ on the RTD-juice.

The objective of this work was to evaluate the instrumental colour, pulp sedimentation and serum cloudiness (turbidity) and the rheology of a ready-to-drink orange juice (11 °Brix) obtained from HPH processed FCOJ (66 °Brix).

5.2 Materials and Methods

5.2.1. Frozen Concentrated Orange Juice (FCOJ)

The frozen concentrated orange juice was obtained directly from a local producer (Citrosuco, Limeira, São Paulo, Brazil). It was a commercial blend of juices from the Natal and Valencia varieties, concentrated to 66°Brix, pH 3.8, 3,24% citric acid and 11.7% of pulp, was stored in a conventional freezer at -18°C before and after processing.

5.2.2. High Pressure Homogenization (HPH) Processing of FCOJ and dilution to the Ready-to-drink Product

The process was carried out within the range from 0 MPa (control) to 150 MPa (gauge, homogenization pressures – P_H) in a high pressure homogenizer (Panda Plus, GEA Niro Soavi, Italy). The juice was first thawed to 5 °C and then processed The inlet temperature was 25ºC, simulating the conditions just after juice concentration during industrial processing. After the procedure, the samples were stored at -18ºC until all the analyses had been carried out. All processes were carried out in triplicate, and the homogenization pressures were 0, 25, 50, 75, 100 and 150 MPa.

After the procedure, the FCOJ samples were used in the production of RTD-juice by direct dilution with deionised water to 11°Brix. Both the processing and the analyses were carried out in triplicate.

5.2.3. Ready-to-drink (RTD) juice evaluation

The analyses were chosen to evaluate important orange juice quality factors, such as the colour (using the CIELAB instrumental methodology), pulp stability in the dispersion (through its sedimentation during storage) and juice cloudiness (by the absorbance of the serum, correlated with the turbidity).

Juice colour is the first element in the evaluation by the consumers, being mandatory for its acceptance. Pulp sedimentation and loss of cloudiness are important drawbacks. A decrease in cloud stability can negatively affect consumer acceptability, since cloudiness improve the characteristic colour, flavour and mouthfeel of orange juice.

5.2.3.1. Instrumental colour

The instrumental colour evaluation, using the parameters L*, a* and b*, is a good technique to objectively quantify the colour of both liquid and solid samples, without the subjectivity of human testers. With this technique, it is possible to evaluate the differences in colour of the processed and non-processed samples. It was expected that the samples would not present any differences, as verified for FCOJ in Chapter 2.

The instrumental colour was obtained using a ULTRA PRO colorimeter (Hunter Associates Laboratory, USA), with illuminant D65, angle of 10° previously calibrated with a RSIN white reference (L*=92.03, a*=−0.88, b*=0.63), as described by Sánchez-Moreno et al. (2006) and Rodrigo et al. (2007). The RTD-juice samples were placed in glass cuvettes, and three readings were obtained for each replicate. The evaluation was carried out immediately after HPH processing of the FCOJ and dilution to the RTD-juice.

The CIELab technique was used for the evaluation, where the values for L* (lightness), a* (redness: green to red) and b* (yellowness: blue to yellow) were obtained and could then be used to express the colour changes.

5.2.3.2. Turbidity (serum cloudiness)

For the turbidity evaluation, a 50 mL aliquot of the RTD-sample was centrifuged at 10000 g for 10 minutes (ALEGRA 25 TR, Beckman Coulter, Corona CA). The absorbance of the supernadant (serum) was read at 660 nm in a spectrophotometer (Beckman DU800; Beckman Coulter, Corona, CA, USA), using distilled water as the blank. The results for absorbance were correlated with the turbidity as described by Cameron et al., (1997), Silva et al., (2010) and Kubo et al., (2013). The evaluation was carried out immediately right after the HPH processing of the FCOJ and dilution to the RTD-juice.

The serum cloudiness (turbidity) is related to the suspended particles, which hinder the passage of a light beam. The juice is first centrifuged in order to precipitate the larger particles. The technique is based on passing a light beam through the sample, and then evaluating the optical density of the sample, which is related to its turbidity.

5.2.3.3. Pulp Sedimentation

The suspended pulp stability is related to particle size, density and charge balance, as well as to the serum phase properties, such as density and viscosity. The loss of juice cloudiness due to pulp sedimentation, is a sensory defect that the industry always tries to eliminate or at least control. Since the HPH decreased particle size of the FCOJ (Chapter 2), it is to be expected that the sedimentation rate would decrease.

Pulp sedimentation was evaluated throughout the 60 days of storage at 25ºC, simulating the RTD-juice shelf life. For this, 0.5% of potassium sorbate was added to the sample, in order to control microbiological growth and ensure microbial stability during the evaluation. After the HPH processing of the FCOJ and dilution, the RTD-juice was transferred to graduated cylinders and stored at 25ºC (BOD TE391, Tecnal, Brazil) for 60 days. Pulp sedimentation was measured directly from the graduated cylinders, evaluated the sedimentation index (SI - *Equation 5.1*), as described by Kubo et al. (2013) and Silva et al (2010).

$$SI = \text{sedimentation volume} / \text{total sample volume} \qquad (\textit{Equation 5.1})$$

5.2.4. Rheologycal Analyses

The rheological analysis were carried out using a controlled stress (σ) rheometer (AR2000ex, TA Instruments, USA), with the temperature maintained constant at 25°C using a Peltier system and a cone-plate geometry (60 mm of diameter, angle 2°). The samples were analyzed immediately after dilution.

The rheological evaluation was carried out with recent diluted samples. A stepwise linear decreasing protocol (300 s-1 to 0.1 s-1) was used to guarantee steady-state shear conditions for the flow behaviour evaluation.

The product flow behaviour was modelled using the Herschel-Bulkley model (*Equation 5.2*), which comprises the Newton, Bingham and Ostwald-de-Waele (power law) models.

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^n \qquad (\textit{Equation 5.2})$$

5.2.6. Statistical analyses and mathematical modelling

The effect of homogenization pressure (P_H) during processing of the FCOJ on the properties of RTD-juice was evaluated using the analysis of variance (ANOVA) and the Tukey test at a 95% confidence level. The STATISTICA 5.5 (StatSoft, Inc., USA) software was used for this purpose.

When relevant, some of the properties here were evaluated modelled as a function of the homogenization pressure (P_H) using non-linear regression and the software CurveExpert Professional v.1.6.3, with a significant probability level of 95%.

5.3 Results e Discussion

5.3.1. Instrumental colour

Figure 5.1 shows the RTD-juice instrumental CieLAB colour parameters (L*, a*, b*) immediately after the HPH processing and dilution. It can be seen that HPH had no effect on any of the RTD orange juice colour parameter ($p<0.05$). This is in accordance with the results presented in Chapter 2, since the HPH did not affect the colour parameters of FCOJ.

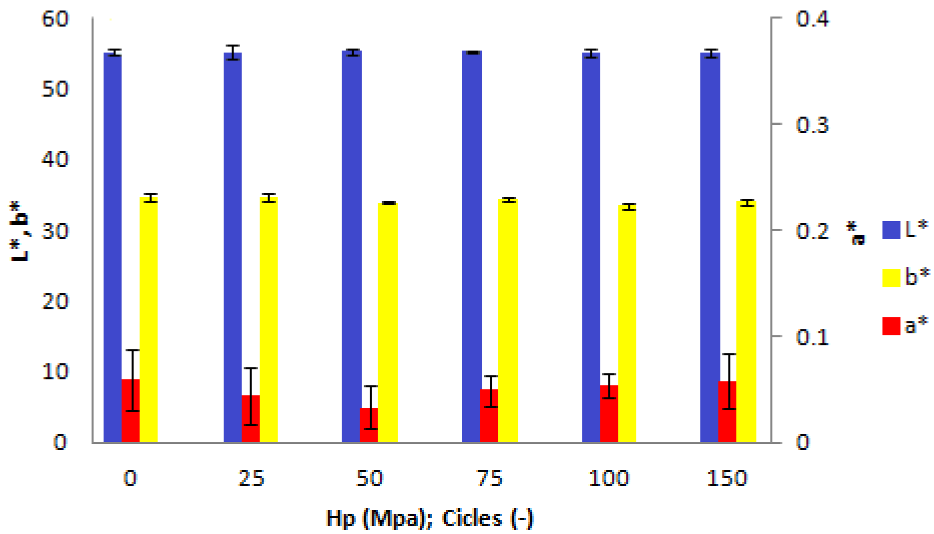

Figure 5.1: Instrumental colour parameters of the RTD Orange juice as a function of the homogenization pressure used to process the FCOJ (vertical bars are the standard deviation)

The effect of HPH on the RTD orange juice colour has been evaluated by other authors. Cerdán-Calero et al., (2013) and Betoret et al., (2009) observed that the unprocessed juice colours showed a* < L* < b*, while in the present work the juice showed a* < b* < L*. The differences between the results of the present work and those published in the literature can be attributed to several factors, such as inherent differences among varieties and the initial processes (juice extraction, blending, thermal process, concentration, freezing, storage).

The results of the effect of HPH on the colour parameters is in agreement with the studies of Betoret et al., (2009) and Sentandreu et al., (2011). Betoret et al., (2009) HPH processed orange juice of the Salustiana variety and found no difference in L* and b* after processing, and a slight increase in a*. However, for the Ortanique variety of juice, the three parameters showed a slight difference, although with no effect on the colour hue (h = arctan[b*/a*]). Sentandreu et al., (2011) found that the orange juice of a Valencia Late variety processed by conventional homogenization (20 MPa) showed a total colour difference (ΔE) equal or smaller to 2.0, when compared with the juice just before homogenization. This small value for ∆E indicates that the difference would not be perceivedby the to consumers.

It is important to highlight that the present result is highly advantageous, since the consumers would not notice any difference in product colour.

5.3.2. Turbidity (serum cloudiness)

Figure 5.2, shows the RTD-juice turbidity (absorbance at 660 nm) as a function of the homogenization pressure. The HPH process reduced the orange juice turbidity with an exponential decay in relation to the homogenization pressure (*Figure 5.2*). Therefore, the RTD-juice turbidity was modelled with an exponential function (*Equation 5.3*, R^2=0.977) as shown in the *Figure 5.2*.

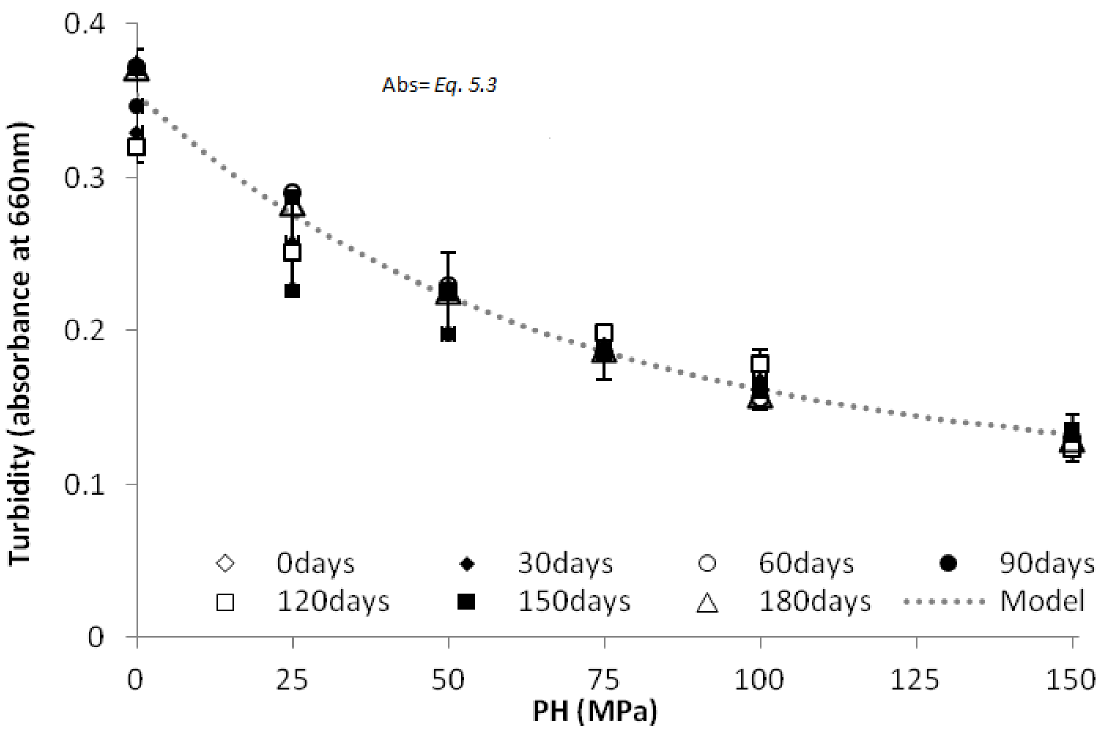

Figure 5.2: RTD orange juice turbidity as a function of the FCOJ homogenization pressure and storage time at -18ºC (dots are the mean value of experimental data, vertical bars are the standard deviation and the curve is the model).

$$\text{Abs}= 0.353+(0.107-0.353)\cdot\exp(-0.0152\cdot P_H) \quad (\textit{Equation 5.3})$$

The results obtained are in agreement with those reported for tomato juice and pineapple pulp (Silva et al., 2010 and Kubo et al., 2013), which verified the same decrease in serum absorbance. However, these results were different from those reported by Betoret et al., (2009), Sentandreu et al., (2011) and Betoret et al., (2012) for some different citrus juices. In those studies, the juices showed the opposite results after homogenization, i.e., an increase in absorbance (which is the same as a decrease in transmittance).

However, the juices used by Betoret et al., (2009), Sentandreu et al., (2011) and Betoret et al., (2012), were all freshly obtained from the fruits and then processed by HPH. On the other hand, in the present study, the RTD-juice was obtained from the dilution of a previously HPH processed FCOJ.

The juice was centrifuged before the turbidity evaluation, which promotes sedimentation of particles larger than a specific value (the cut diameter). Thus, only those particles that remains in suspension in the serum are evaluated in the turbidity assays. As the particles become smaller, more light will pass through the serum decreasing the absorbance and turbidity (Okoth et al., 2000).

The centrifugation step used before the transmittance/absorbance analyses was at a greater velocity in this work (at 10000 g) than in the studies of Betoret et al., (2009), Sentandreu et al., (2011) and Betoret et al., (2012) (at 3000 g). This difference can promote the sedimentation of particles in different size ranges. Using 3000 g, small particles remain suspended on the serum. Since HPH decreased the particle size there were suspended small particles in suspension in the serum, consequently decreasing the transmittance and increasing the turbidity. This should not occur at 10000g, which promotes sedimentation of almost all the non soluble content.

Moreover, many studies indicate that HPH reduces the molecular size of the serum polysaccharides, also changing its rheology (Augusto et al., 2012a; Corredig and Wicker, 2001; Floury et al., 2002; Wang et al., 2011; Lagoueyte and Paquin, 1998; and Harte and Venegas, 2010). Consequently, with a low serum viscosity, particle sedimentation is facilitated. Therefore, the higher the homogenization pressure, the lower the serum consistency, and consequently a smaler amount of colloidal particles remain in suspension, decreasing the optical density of the serum. In fact, the serum decrease in absorbance due to HPH was also observed in other studies that used a velocity of 10000 g during the centrifugation step (Silva et al, 2010 and Kubo et al., 2013).

Finally, the effect of the storage time at -18ºC was also plotted in *Figure 5.2*. As can be seen, the storage time did not change the turbidity of any sample (P<0.05). This suggests there were no additional changes to the suspended particles during this period, especially in particle aggregation. In fact this can be explained by the storage temperature (-18°C), which contributed to maintaining the proprieties of the FCOJ unchanged.

5.3.3. Pulp Sedimentation

*Figure 5.*3, shows the RTD-juice pulp sedimentation during storage at 25ºC. The pulp sedimentation behaviour showed almost no change due to HPH processing.

The juice SI was modelled as a function of the storage time (25ºC), using an exponential decay function (*Equation 5.4*). The mathematical model is related to the initial sedimentation index value (SI_i), the equilibrium sedimentation index value (SI_e) and the rate of pulp sedimentation (k_{SI}), as described by Kubo et al. (2013). The parameters from *Equation 5.3* are presented in *Figure 5.4*.

$$SI = SI_e + \left(SI_i - SI_e\right) \cdot \exp\left(-k_{SI} \cdot t\right) \qquad \text{(Equation 5.4)}$$

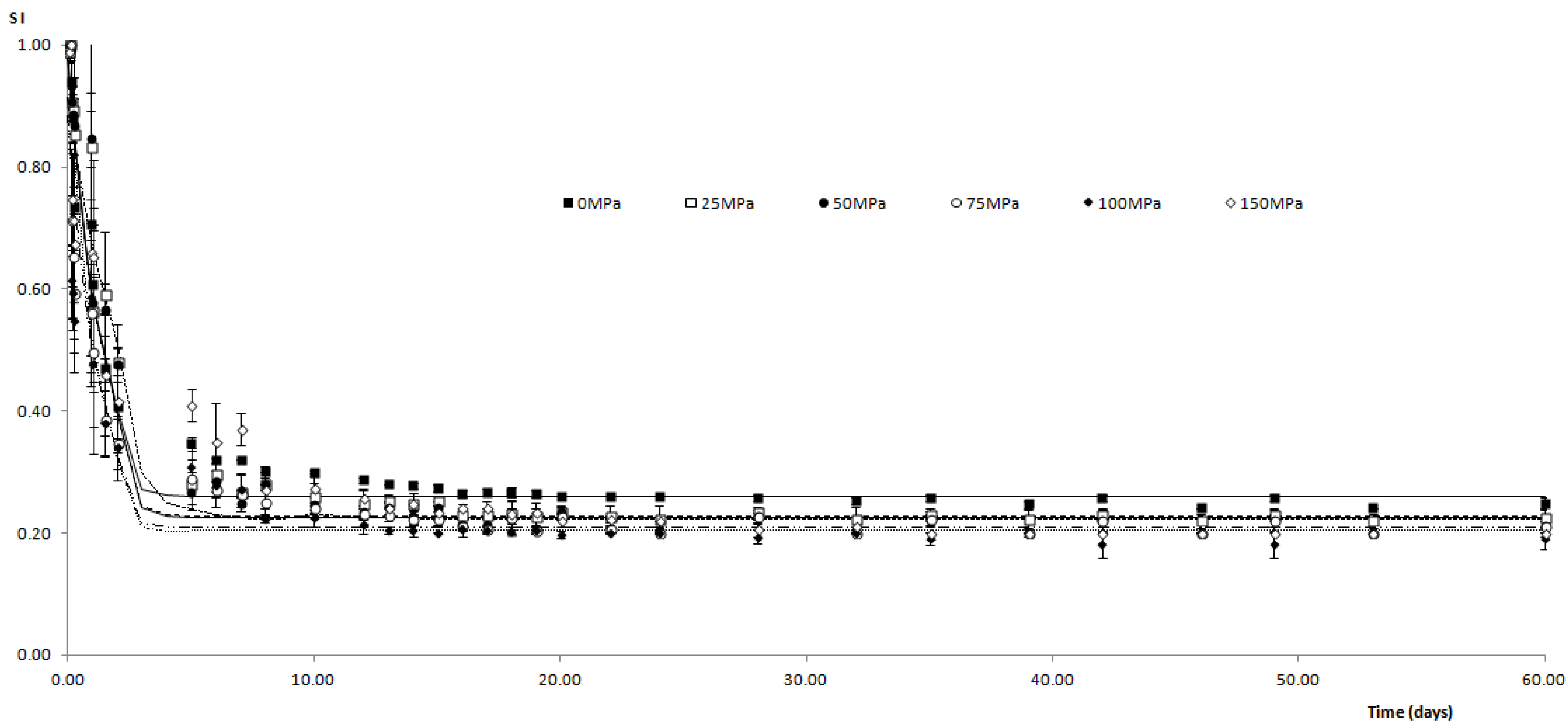

Figure 5.3: RTD orange juice sedimentation index (SI) as a function of the FCOJ homogenization pressure processing and time of storage at 25ºC (dots are the mean value of experimental data, vertical bars are the standard deviation and the curves are the models).

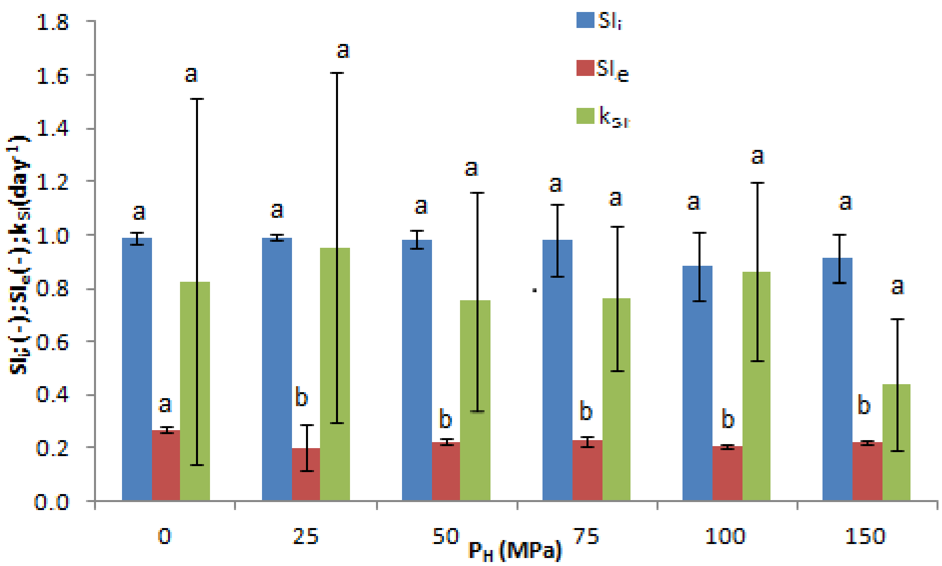

Figure 5.4: RTD orange juice sedimentation index (SI) parameters as a function of the FCOJ homogenization pressure (vertical bars are the standard deviation; for each parameter, different letters represent significantly different values (P < 0.05).

Both *Figure 5.3* and *Figure 5.4* show that HPH did not change the pulp sedimentation behaviour, the only exception being the equilibrium value between the control sample and all the processed samples. This could be related to the decrease in particle size and greater accommodation of these particles. However the standard deviations between the triplicates were high and the difference in the equilibrium value very small. It can be assumed that the HPH showed no effect on the SI during the storage time.

Initially, there was no phase separation (SI equal to 1.0) and it took approximately 6-7 days for the SI to reach values close to the equilibrium value, although some sample took up to 9-10 days and others less than 5 days. Independent of the homogenization pressure, all the processed samples reached approximately the same final equilibrium value, around 0.22 (with no difference at 5% of significance). The control sample showed an equilibrium value slightly higher, around 0.26.

Stokes Law states that the velocity of sedimentation of perfect non charged spheres in a fluid is related directly to its particle size and density, the density of the disperse phase and inversely to the disperse phase viscosity. However the RTD-juice showed a non spherical shape (Chapter 2), and HPH had no effect on the rate of sedimentation.

Different effects of HPH on the sedimentation behaviour of vegetable dispersions have been reported in the literature. For instance, the final value for SI was different for each homogenization pressure for pineapple pulp, as well as its sedimentation rate (Silva et al., 2010). For tomato juice, all the processed samples showed no sedimentation, remaining with a SI of 1.0 throughout the 60 days of the experiment (Kubo et al., 2012). In fact it is important to highlight that Lopes-Sanchez et al., (2011) reported that each vegetable matrix reacted in its own particular way to the HPH process.

Different studied have reported the effect of HPH on the sedimentation citrus juices. Sentandreu et al., (2011) reported that at high pressure could increase orange juice cloudiness right after the HPH process. Betoret et al., (2009) and Betoret et al., (2012) reported that an increase in homogenization pressure reduced orange juice pulp sedimentation, improving pulp stability after centrifugation at 3000g. Carbonell et al, (2013) observed that HPH at 150 MPa stabilized the cloudiness of orange juice with high a PME residual activity for 3 months of storage at refrigeration temperature (3 °C). Cerdán-Calero et al., (2013) carried out a study combining an initial homogenization (20 MPa) followed by centrifugation and high pressure homogenization (150 MPa). The orange juice cloud was stabilized for 15 days under refrigeration. However, all the juices used by Sentandreu et al., (2011), Betoret et al., (2009), Betoret et al., (2012), Carbonell, et al, (2013) and Cerdán-Calero et al. (2013) were freshly obtained from the fruit, and therefore had not suffered the effect of temperature, during concentration. This means that the pulp of FCOJ and fresh juices show different sizes and stabilities.

Orange juice cloudiness is related to the suspended particles in the range from 0.4 to 5 μm, those smaller than 2 μm being the most stable (Buslig and

Carter, 1974). A reduction in particle size improves cloudiness stability, since by Stokes Law, smaller particles show a smaller sedimentation velocity. This explains the results obtained by Sentandreu et al., (2011), Betoret et al., (2009), Betoret et al., (2012), Carbonell, et al, (2013) and Cerdán-Calero et al. (2013), because their initial particles were larger than 2 μm. In the present study, the particles were already smaller.

Cerdán-Calero et al. (2013) studied the effect of HPH on the stabilization of orange juice pulp. The results were similar to those obtained in the present study, where the velocity of pulp sedimentation of the samples processed by HPH was similar to that of samples not processed by HPH.

This result, suggests that HPH could be used to achieve a reduction in the consistency of FCOJ, since the process did not affect the characteristics of the RTD-juice.

5.3.4. Rheology

Figure 5.5 shows the flow behaviour of the RTD-juice at 25°C. The juice showed neither shear thinning behaviour nor yield stress, and could be well described by the Newton Law (*Equation 5.4*), which is a simplification of E*quation 2.4* (Chapter 2) without the yield stress (σ_0) or behaviour index (n). And the consistency index (k) can be used as viscosity (μ) *Table 5.1* show the mean viscosity value as a function of P_H.

$$\sigma = \mu \cdot \dot{\gamma} \qquad \text{(Equation 5.5)}$$

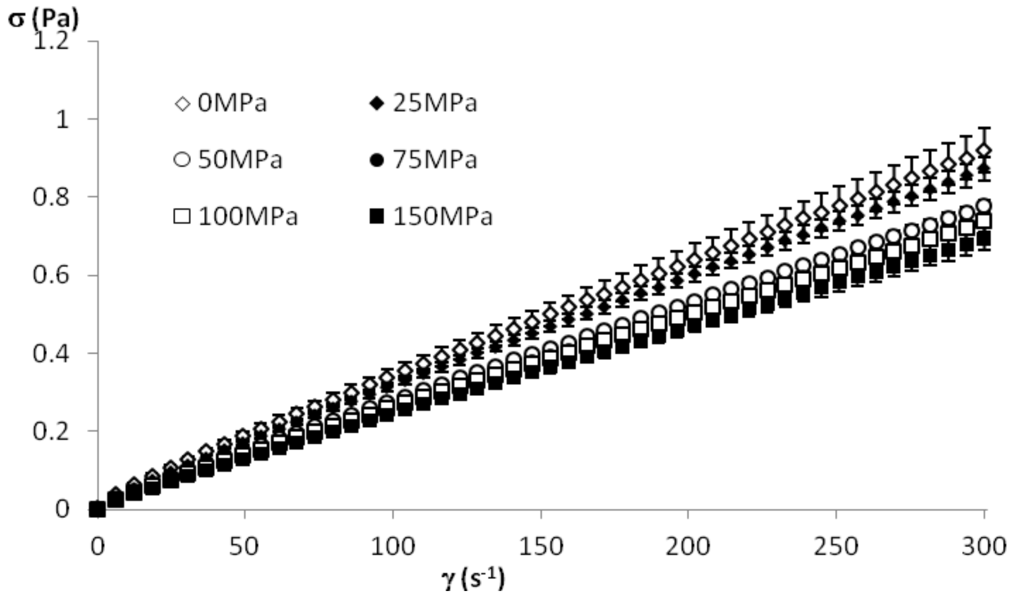

Figure 5.5: Flow curves at 25°C for RTD-juice dilueted from a FCOJ processed by HPH (dots are the mean values, and the vertical bars are the standard deviation).

Table 5.1: Viscosity (μ) of RTD-juice at 25°C as a function of P_H.

P_H (MPa)	(Pa.s)	
0	0.00300	a
25	0.00297	a
50	0.00263	ab
75	0.00257	b
100	0.00250	b
150	0.00235	b

The flow behaviour of the RTD-juice at 25°C was slightly affected by the HPH process, as shown in *Figure 5.5*, reducing the juice viscosity. This can be seen by fixing a specific shear rate ($\dot{\gamma}$) and observing the smaller values of the correspondent shear stress (σ) in the homogenized samples (*Figure 5.5*). This also can be seen by observing the angle of each curve. However the impact of HPH on the RTD-juice was minimal, since the viscosity showed close values. This can be confirmed on *Table 5.1,* since the viscosity for each sample has a low magnitude, it could not be perceived by consumers, even statistically the lower pressure samples had some difference with the higher pressure samples, that diference was minimal.

As a diluted product, the RTD-juice shows Newtonian behaviour and the impact by HPH showed similar resuts when compared with similar products: a reduction in viscosity due to polysaccharide disruption (Augusto et al., 2012a, Corredig and Wicker, 2001, Floury et al., 2002, Wang et al., 2011, Lagoueyte and Paquin, 1998 and Harte and Venegas, 2010). Augusto et al., (2012a) showed that the HPH had a slight impact on viscosity of tomato juice serum phase.

The changes in PSD can also be explained by the decrease in RTD-juice viscosity. The small particles can occupy the spaces amongst the larger ones, resulting in an important lubricant effect, decreasing the resistance to flow and leading to a reduction in the consistency index (k) (Servais et al, 2002). However in this diluted system this effect is very low, as could be seen on *Figure 5.5*.

This low diference could be imperceptible to consumers, which would be an advantage, since the HPH leads to lower energy consumption, and had no major impact on the final viscosity of the product, however only with a sensory analysys this question will be answered.

5.4 Conclusion

The effect of processing the FCOJ by HPH on the properties of the subsequently reconstituted ready-to-drink (RTD) juice was evaluated.

HPH did not change the colour parameters of the RTD-juice, and also had no major impact on pulp sedimentation, even with a reduction in particle size and juice cloudiness. Finally HPH showed a slight impact the viscosity of the RTD-juice, which could be imperceptive to consumers.

Thus, it was concluded that HPH could be used to promote desirable changes in FOCJ, without changing consumer perception of the reconstituted RTD-juice.

5.5 Acknowledgment

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Conclusões Finais

A utilização da homogeneização a alta pressão (HAP) possui grande potencial em sua aplicação no FCOJ, pois obteve-se significante redução de sua consistência, que nas condições estudas, atingiram valores de cerca da metade da viscosidade aparente. Esta redução pode ser atribuída principalmente a redução do tamanho de partículas, devido a menor resistência ao escoamento, efeito lubrificante e menor arraste da fase soro.

A distribuição do tamanho de partículas também foi afetada, apresentando um platô, ou um acumulo de partículas menores, na ordem de 1 micrometro.

Este trabalho também verificou a redução nos valores de stress inicial e de equilíbrio nas propriedades reológicas dependentes do tempo (tixotropia), apesar do parâmetro cinético não ter sido alterado por esta tecnologia. A viscoelasticidade do FCOJ também foi alterada pela HAP, ambos os módulos de estocagem e dissipação tiveram seus valores reduzidos, porém o componente elástico do produto apresentou uma redução maior se comparado ao componente viscoso. Estas mudanças também podem ser relacionadas à redução do tamanho de partículas, e a mudança na distribuição do tamanho de partículas.

A microestrutura do FCOJ foi verificada, e observa-se redução no tamanho de algumas estruturas, comprovando o resultado da análise do tamanho de partículas. A cor do FCOJ não apresentou diferenças após o processamento em nenhuma pressão, o que é desejável, uma vez que o consumidor não perceberá nenhuma diferença no produto processado. Nenhuma alteração foi constatada pelo tempo de estocagem, comprando que as alterações não são reversíveis.

Em relação ao efeito das múltiplas passagens de HAP foi possível verificar que a utilização de poucos ciclos em menores pressões possuem efeito semelhante ou até maior que apenas um processamento em uma pressão elevada. Porém o efeito dos múltiplos ciclos não é aditivo, por este motivo cada ciclo adicional ao processo apresenta uma menor redução se comparado ao ciclo anterior. Na distribuição do tamanho de partículas foi nítida a mudança da distribuição monomodal para a bimodal.

A HAP alterou o valor de turbidez da fase soro do produto reconstituído além de alterar levemente sua viscosidade, estes resultados podem ser relacionados com a redução do tamanho de partículas. A cor do suco diluído não foi alterada, bem como o comportamento da sedimentação, sendo estas características importantes para a aceitação pelo consumidor.

A redução de consistência, ou viscosidade aparente, bem como as alterações nas demais características reológicas acarreta em uma economia de energia em várias operações unitárias, como bombeamento, trazendo vantagens ao processo produtivo sem acarretar em alterações de características desejadas e esperadas pelo consumidor.

Sugestões para trabalhos futuros

Os resultados da dissertação aponta que a tecnologia de HAP tem grande potencial para a redução da consistência de Suco de Laranja concentrado, utilizando pressões de até 150 MPa ou ciclos com pressões menores. No entanto, sugere-se para estudos futuros a verificação da concentração do produto durante a homogeneização, ou mais importante o teor de polpa durante o mesmo. A aplicação em variedades que possuem consistência ainda maior que a variedade estudada. Além disso, é necessário verificar alguns outros parâmetros, não somente visuais como cor e opalescência, mas através de análise sensorial poderá ser verificada a aceitação do produto pelo consumidor. Outro ponto importante, a sugestão para se calcular, utilizando simulações computacionais, a real economia que esta redução de consistência dará ao processo, além de se realizar um balanço energético para se descobrir o quanto de energia se obtém durante a homogeneização simples ou em múltiplas passagens.