UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO TECNOLÓGICO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DE ALIMENTOS

AVALIAÇÃO DO EMPREGO DA CRIOCONCENTRAÇÃO E DA NANOFILTRAÇÃO NAS PROPRIEDADES DE COMPOSTOS FUNCIONAIS DO SORO DE TOFU E APLICAÇÃO DO CONCENTRADO NA OBTENÇÃO DE BEBIDA LÁCTEA FERMENTADA

SILVIA BENEDETTI
Engenheira de Alimentos
M.Sc. Engenharia de Alimentos

Orientador: Prof. Dr. José Carlos Cunha Petrus
Coorientadora: Profª. Dra. Elane Schwinden Prudêncio

FLORIANÓPOLIS-SC
2014

UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO TECNOLÓGICO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DE ALIMENTOS

AVALIAÇÃO DO EMPREGO DA CRIOCONCENTRAÇÃO E DA NANOFILTRAÇÃO NAS PROPRIEDADES DE COMPOSTOS FUNCIONAIS DO SORO DE TOFU E APLICAÇÃO DO CONCENTRADO NA OBTENÇÃO DE BEBIDA LÁCTEA FERMENTADA

Tese apresentada ao Programa de Pós-Graduação em Engenharia de Alimentos do Centro Tecnológico da Universidade Federal de Santa Catarina, como requisito final à obtenção do título de Doutor em Engenharia de Alimentos.

Orientador: Prof. Dr. José Carlos Cunha Petrus
Coorientadora: Profª. Dra. Elane S. Prudêncio

SILVIA BENEDETTI
Engenheira de Alimentos
M.Sc. Engenharia de Alimentos

Florianópolis- SC
2014

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Benedetti, Silvia

Avaliação do Emprego da Crioconcentração e da Nanofiltração nas Propriedades de Compostos Funcionais do Soro de Tofu e Aplicação do Concentrado na Obtenção de Bebida Láctea Fermentada / Silvia Benedetti / orientador, José Carlos Cunha Petrus, coorientadora, Elane S. Prudêncio - Florianópolis, SC, 2014.

189 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro Tecnológico. Programade Pós-Graduação em Engenharia de Alimentos.

Inclui referências

1. Engenharia de Alimentos. 2. crioconcentração. 3. nanofiltração. 4. soro de tofu. 5. isoflavonas. Petrus, José C.C. Prudêncio, Elaine S. Universidade Federal de Santa Catarina. II. Programa de Pós-Graduação em Engenharia de Alimentos. III.Título.

Dedico este trabalho aos meus pais,
Leila e Araujo, pelo amor incondicional
e pelo apoio em todos os momentos.

AGRADECIMENTOS

Agradeço a Deus, pela presença constante em minha vida, me orientando e guiando meus passos para sempre trilhar o melhor caminho.

À Universidade Federal de Santa Catarina e ao Programa de Pós-graduação em Engenharia de Alimentos, pela oportunidade de realização do doutorado, e aos professores do programa pelo bom exemplo dado e pelo conhecimento adquirido o longo desses seis anos.

Ao meu orientador Prof. Dr. José Carlos Cunha Petrus pela orientação ao longo desses seis anos de mestrado e doutorado, pela confiança em mim depositada e pelo apoio para realização deste trabalho.

À minha coorientadora Dra. Elane Schwinden Prudêncio pela amizade, apoio, orientação e pelas valiosas contribuições para realização deste trabalho e para meu crescimento profissional.

À Embrapa Soja, por todo suporte na realização dos ensaios ao longo desses anos, especialmente ao pesquisador José Marcos Gontijo Mandarino pelas valiosas sugestões para o trabalho; e ao Rodrigo Santos Leite, pela amizade, pelo apoio e por dispor de um coração enorme, sempre disposto a ajudar.

A mi tutora en la Universitat Politêcnica de Cataluña, Dra. Mercè Raventós y todo su equipo de trabajo por la receptibilidad, por el conocimiento compartido y por recibirme tan amablemente durante el periodo que estuve en Barcelona.

À empresa Tofutura (Campo Largo-PR), por ceder a matéria-prima para realização deste trabalho.

Aos meus pais, Leila e Araujo, e meu irmão Bruno pelo apoio, compreensão, carinho e por estarem sempre ao meu lado em todos os momentos. A vocês, minha eterna gratidão.

Aos meus colegas do Laboratório de Processos de Separação com Membranas (LABSEM), pela amizade, pelo apoio, pela troca de conhecimentos, pelo suporte nos experimentos, pelos momentos descontraídos que tornaram nossa rotina muito mais leve e proveitosa.

Todo meu carinho por esse grupo de trabalho maravilhoso do qual sentirei muita saudade.

Às meninas do Laboratório de Leite, da CAL-UFSC, pela amizade, carinho, parceira de trabalho e por todo suporte e ajuda na realização dos experimentos. Vocês foram fundamentais nesse trabalho!

A todos meus amigos queridos, por sempre acreditarem em mim, pelo apoio e incentivo ao longo de todos esses anos; aos que estão próximos, pelo carinho e bons momentos; e aos que estão distantes, por compreenderem a minha distância e ausência em muitos momentos importantes de suas vidas e mesmo assim estarem sempre presentes na minha vida.

Ao CERMAT, da Engenharia de Materiais da UFSC, principalmente à Priscila e à Verônica, pelo auxílio na realização dos ensaios de reologia.

Ao Laboratório de Propriedades Físicas de Alimentos (PROFI-EQA/UFSC), especialmente ao Giustino, pelo empréstimo do equipamento e auxílio na liofilização das amostras.

Ao CNPq e à CAPES, pela concessão da bolsa de estudos e pelo apoio financeiro.

E a todos que de alguma forma contribuíram para a realização e evolução desta tese. Muito obrigada!

RESUMO

Este trabalho teve como objetivo a caracterização e o aproveitamento do soro de tofu, visando à aplicação de compostos bioativos oriundos da soja, resultando em um concentrado com maiores teores de isoflavonas e oligossacarídeos e, que possa ser aplicado na elaboração de uma bebida láctea. Inicialmente avaliaram-se os processos de crioconcentração(CF) em sistema de filme descendente e nanofiltração (NF) para recuperação e concentração das isoflavonas, presentes no soro de tofu. A quantificação de isoflavonas foi realizada nos concentrados obtidos em ambos os processos, além do permeado da NF e do gelo remanescente da CF. Além disso, realizou-se um estudo do potencial antioxidante do soro de tofu concentrado através da crioconcentração em blocos e da NF. A atividade antioxidante foi avaliada pelos métodos FRAP (baseado na capacidade de redução do ferro) e $ABTS^{+}$ (habilidade dos antioxidantes em capturar o cátion radical $ABTS^{+}$). Posteriormente, o soro de tofu concentrado por NF foi aplicado na elaboração des bebidas lácteas fermentadas, denominadas como Bebida 1 (10 % de soro de tofu concentrado + 90 % de leite) e Bebida 2 (20 % de soro de tofu concentrado + 80 % de leite), além do controle (apenas leite). As bebidas foram avaliadas quanto à contagem total de bactérias ácido-láticas, propriedades físico-químicas, cor, índice de sinerese, propriedades reológicas e quantificação dos compostos bioativos, tais como isoflavonas e oligossacarídeos. A partir dos resultados obtidos, pôde-se observar que ambos os processos de CF em sistema de filme descendente e NF resultaram na concentração das isoflavonas. Com relação à atividade antioxidante, observou-se que seus valores foram significativamente mais elevados nos concentrados obtidos do que no soro de tofu inicial, sendo correlacionados positivamente com o teor de isoflavonas. A utilização do soro de tofu concentrado por NF na elaboração de bebida láctea fermentada não interferiu na sobrevivência das bactérias ácido-láticas, cujas contagens foram maiores que 8 log UFC mL^{-1} ao longo dos 30 dias de armazenamento das bebidas. Observou-se que a adição de maior quantidade de soro de tofu concentrado na bebida 2 promoveu redução do teor de sólidos totais e proteínas em comparação ao controle. Todas as amostras apresentaram perfil similiar quanto ao comportamento do pH, acidez e índice de sinerese ao longo do tempo de armazenamento. Tanto o controle quanto as bebidas lácteas fermentadas adicionadas do soro de tofu concentrado apresentaram coloração amarelo-esverdeada. Além disso, as bebidas bem como controle apresentaram comportamento reológico de fluido

pseudoplástico com propriedades tixotrópicas. Na bebida 2, observou-se a manutenção das isoflavonas totais ao longo do tempo de armazenamento da bebida, enquanto na bebida 1 ocorreu uma pequena redução nesses teores. Os oligossacarídeos avaliados na bebida 2 apresentaram uma redução na sua concentração ao longo do tempo de armazenamento. Dessa forma, os resultados obtidos neste trabalho sugerem que o soro de tofu concentrado pelo processo de nanofiltração pode ser utilizado para obtenção de uma bebida láctea fermentada com atividade biológica especial, conferidas pelas isoflavonas e oligossacarídeos da soja.

***Palavras-chave**:* crioconcentração, nanofiltração, soro de tofu, isoflavonas, oligossacarídeos, bebida láctea fermentada.

ABSTRACT

This study aimed at the characterization and use of tofu whey, for the application of bioactive compounds derived from soybeans, resulting in a concentrate with higher concentrations of isoflavones and oligosaccharides, and that can be applied in the preparation of a fermented lactic beverage. Primarily it was evaluated the processes of freeze concentration (CF) in falling film system and nanofiltration (NF) for the recovery and concentration of isoflavones, present in the tofu whey. The isoflavone quantification was performed in the concentrates obtained in both processes, besides of the NF permeate and the remaining ice of freeze concentration. Furthermore, it was carried out a study of the antioxidant potential of tofu whey concentrated by block freeze concentration and NF. The antioxidant activity was evaluated by FRAP (based on the ability of iron reduction) and ABTS + (ability of antioxidants to capture the radical cation $ABTS^{+}$) methods . Posteriorly, the tofu whey concentrated by NF was applied in the preparation des functional fermented milk beverages, denominated as beverage 1 (10 % concentrate tofu whey + 90 % milk) and beverage 2 (20 % concentrate tofu whey + 80 % milk), and control (only milk). The beverages were evaluated for total count of lactic acid bacteria, physicochemical properties, color, syneresis index, rheological properties and quantification of functional compounds, isoflavones and oligosaccharides. From the results obtained, it was observed that both falling-film freeze concentration and NF processes resulted in the concentration of isoflavones. With respect to the antioxidant activity, it was found that the values were significantly higher in the concentrate tofu whey than initial tofu whey, being positively correlated with the isoflavones . The use of tofu whey concentrated by NF in the preparation of fermented lactic beverages did not affect the survival of lactic acid bacteria, whose counts were higher than 8 log CFU mL^{-1} over the 30 days of storage of beverages. It was observed that the addition of a larger amount of concentrate tofu whey in beverage 2 produced a greater reduction in total solids and protein compared to the control. All samples showed similiar profile as the behavior of pH, acidity and syneresis index throughout the storage time. Both control and fermented lactic beverages with concentrate tofu whey showed greenish-yellow. Moreover, the beverages and control presented rheological behavior of pseudoplastic fluid with thixotropic properties. In the beverage 2 it was observed the maintenance of the total isoflavones over time storage, the beverage 1 as a slight decrease in these levels. The oligosaccharides

evaluated in the beverage 2 presented a decrease in their concentration throughout the storage time. Thus, the results obtained in this study suggest that the tofu whey concentrated by nanofiltration process can be used to obtain a fermented lactic beverage with functional properties conferred by the isoflavones and oligosaccharides soy of soybean.

***Keywords**:* freeze concentration, nanofiltration, tofu whey, isoflavones, oligosaccharides, fermented lactic beverage.

LISTA DE TABELAS

Capítulo 2

Capítulo 3

Capítulo 4

LISTA DE FIGURAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

SUMÁRIO

1. INTRODUÇÃO

O mercado de alimentos funcionais vem crescendo nos últimos anos, o que aumenta a necessidade de encontrar novas fontes de compostos bioativos, bem como avaliar novas técnicas para sua extração e concentração, objetivando a produção em larga escala. A recuperação de compostos bioativos a partir de produtos naturais é muito importante, principalmente devido ao seu amplo espectro de aplicação na indústria de alimentos e farmacêutica. Dentre estes se tem as isoflavonas e os oligossacarídeos, que apresentam importantes propriedades biológicas e tecnológicas. Desta forma, a investigação de alternativas tecnológicas visando agregar valor a resíduos agroindustriais, poderá contribuir para reduzir seu volume e/ou os custos do seu apropriado descarte.

O tofu é um alimento muito consumido nos países orientais e sua produção geralmente ocorre em pequenas indústrias. É um produto fabricado a partir da coagulação do extrato hidrossolúvel de soja, sendo que uma de suas etapas de produção é a prensagem, na qual é eliminada grande quantidade de resíduo líquido, denominado soro de tofu. O soro de tofu é caracterizado por elevados valores de demanda química de oxigênio (DQO) e demanda bioquímica de oxigênio (DBO), elevado teor de proteínas (CHAI et al., 1999), concentrações substanciais de açúcares e minerais (coagulantes) (BAZINET; IPPERSIEL; LAMARCHE, 1999). Além disso, este resíduo contém moléculas de baixa massa molar, como peptídios, lipídios, e alguns compostos bioativos, tais como isoflavonas e oligossacarídeos (KIM; KIM; YOO, 2005). As isoflavonas são compostos fenólicos pertencentes à classe dos fitoestrógenos, que apresentam estrutura química semelhante ao estrogênio humano e estão presentes na soja nas seguintes formas químicas: β-glicosídeos, malonil glicosídeos e acetil glicosídeos e na forma não-conjugada aglicona, sendo que cada forma química possui três isômeros que são a genistina, a daidzina e a glicitina (CHUN; KIM; KIM, 2008). Já os oligossacarídeos são carboidratos não-digeríveis, que apresentam importantes propriedades físico-químicas e fisiológicas benéficas à saúde, muito utilizados como ingrediente alimentício, por comportar-se como fibra dietética e prebiótico. Os principais oligossacarídeos da soja são a rafinose, a estaquiose e a verbascose, sendo que suas propriedades biológicas, intermediárias entre os açúcares simples e os polissacarídeos, são capazes de promover o equilíbrio da microbiota intestinal (QIANG; YONGLIE; QIANBING, 2009).

Dentre os métodos tradicionalmente utilizados para extração e concentração das isoflavonas e oligossacarídeos a partir da soja estão as extrações com solventes, a purificação aliada a técnicas cromatográficas e a evaporação. Embora esses produtos apresentem pureza relativamente alta, o uso de solventes orgânicos tem algumas restrições como a contaminação ambiental, a co-extração de compostos indesejáveis, aumento dos custos para remoção do solvente e purificação dos compostos, preocupações com a segurança devido à toxicidade dos solventes, e podem ainda afetar a qualidade dos componentes. Com a finalidade de superar essas limitações dos processos convencionais de extração e concentração e também recuperar compostos de interesse comercial a partir de um resíduo industrial altamente poluente, os processos de separação por membranas e a crioconcentração surgem como processos alternativos para obtenção de compostos bioativos com alta pureza, além de preservarem as características físico-químicas devido ao uso de temperaturas amenas no processo (BELÉN et al., 2013; MACHADO; MELLO; HUBINGER, 2013). Neste contexto, os processos de separação por membranas e a crioconcentração se destacam, devido ao uso de baixas temperaturas, preservando as propriedades dos compostos de interesse e por não necessitarem o uso de solventes.

Dentre os métodos de separação por membranas, destacam-se a microfitração e a nanofiltração. A microfiltração constitui um processo alternativo aos métodos convencionais e vem sendo muito utilizada para clarificação de bebidas e sucos (ALMANDOZ et al., 2010; RAZI; AROUJALIAN; FATHIZADEH, 2012; DOMINGUES et al., 2014) e para remoção de micro-organismos (pasteurização a frio) (DEBON; PRUDÊNCIO; PETRUS, 2010; CISSÉ et al., 2011; DEBON et al., 2012; LAORKO; TONGCHITPAKDEE; YOURAVONG, 2013; REZZADORI et al., 2013). Já a nanofiltração, é destinada à concentração de micro e macromoléculas, cujas características peculiares são a capacidade de fracionamento parcial de íons mono, bi e trivalentes, bem como a alta rejeição de compostos orgânicos com massa molar entre 100 e 500 g mol^{-1} (HE et al., 2008), justamente na faixa onde se situa a maioria dos compostos funcionais presentes nos alimentos.

Outro processo promissor para concentração de compostos termossensíveis em alimentos é a crioconcentração, que se baseia na separação de fases sólido-líquido a baixas temperaturas e pode ser uma alternativa promissora às técnicas de concentração convencionais utilizadas no processamento de alimentos. Este processo já foi utilizado

para concentração de compostos presentes em soro lácteo (SÁNCHEZ et al., 2011), mosto (HERNÁNDEZ et al., 2010), soro de tofu (BELÉN et al., 2012; BELÉN et al., 2013), café (MORENO et al., 2013; MORENO et al., 2014a,b), extrato aquoso de erva-mate (BOAVENTURA et al., 2013) e no tratamento de águas residuárias industriais (YEE et al., 2003).

Devido à presença de compostos bioativos, tais como as isoflavonas e os oligossacarídeos da soja, o soro de tofu torna-se um resíduo industrial de grande interesse tecnológico. Além disso, sua reutilização reduz o impacto ambiental causado pelo seu descate. Entretanto, são escassos os estudos existentes na literatura sobre a recuperação de compostos bioativos do soro de tofu visando ao reaproveitamento industrial. Assim, tendo em vista que esse resíduo também se apresenta como um grave problema ambiental devido à sua elevada carga orgânica torna-se necessário o estudo da viabilidade técnica da recuperação de seus compostos de interesse, utilizando processos de concentração tais como nanofiltração e crioconcentração, e sua posterior utilização na elaboração de um alimento com atividade biológica.

Este trabalho teve como objetivo a caracterização e o aproveitamento do soro de tofu, visando à aplicação de compostos bioativos oriundos da soja, resultando em um concentrado com maiores teores de isoflavonas e oligossacarídeos e, que possa ser aplicado na elaboração de uma bebida láctea. Já os objetivos específicos foram os seguintes:

a) concentrar o soro de tofu através da crioconcentração e nanofiltração e avaliar o desempenho desses processos através da determinação dos teores de isoflavonas nos concentrados obtidos;

b) avaliar o potencial dos processos de crioconcentração e nanofiltração na concentração das isoflavonas e na melhoria da atividade antioxidante do soro de tofu concentrado;

c) avaliar a utilização do concentrado de soro de tofu na elaboração de uma bebida láctea fermentada;

d) caracterizar a bebida láctea fermentada quanto às características físico-químicas, microbiológicas e reológicas, durante o armazenamento; e

e) avaliar a estabilidade das isoflavonas e oligossacarídeos durante o armazenamento da bebida láctea fermentada.

Por fim, este trabalho encontra-se organizado da seguinte forma:

a) Capítulo 1: **Revisão bibliográfica** abordando os temas envolvidos no trabalho, ou seja, a soja, a produção de tofu e a obtenção da matéria prima utilizada neste trabalho; seus compostos de interesse, as isoflavonas e os oligossacarídeos; os processos de concentração utilizados para recuperação dos compostos bioativos; definições e características e as análises mais utilizadas para caracterização dos leites fermentados.

b) Capítulos 2: **Potencial dos processos de concentração na recuperação de isoflavonas presentes no soro de tofu**, cujo objetivo foi avaliar o potencial de aplicação dos processos de concentração, tais como nanofiltração e crioconcentração, para a recuperação das isoflavonas presentes no soro de tofu.

c) Capítulo 3: **Propriedades antioxidantes do soro de tofu concentrado através dos processos de crioconcentração e nanofiltração**, cujo objetivo foi avaliar a atividade antioxidante do soro de tofu concentrado por crioconcentração e nanofiltração.

d) Capítulo 4: **Utilização do soro de tofu concentrado na formulação de bebida láctea fermentada**, cujo objetivo foi investigar a utilização do soro de tofu concentrado na produção de bebidas lácteas, pela avaliação do crescimento e sobrevivências dos micro-organismos *Streptococcus salivarius* ssp. *thermophilus* e *Lactobacillus delbrueckii* ssp. *Bulgaricus* durante a fermentação e armazenamento, caracterização desses produtos e comportamento dos compostos bioativos durante o período de armazenamento dos leites fermentados.

e) **Considerações finais e sugestões de trabalhos futuros**

Ao final deste trabalho estão também apresentadas as publicações já realizadas de acordo com os resultados obtidos neste trabalho. O Anexo A apresenta o artigo publicado em parceria com a *Universistat Politècnica da Cataluña*, desenvolvido durante o período de doutorado sanduíche. O Anexo B apresenta dos trabalhos publicados em eventos científicos das áreas de Engenharia, Ciência e Tecnologia de Alimentos. Os Anexos C e D apresentam as comprovações de submissão dos artigos para publicação em revistas indexadas internacionais.

Referências

ALMANDOZ, C.; PAGLIERO, C.; OCHO, A.; MARCHESE, J. Corn syrup clarification by microfiltration with ceramic membranes. **Journal of Membrane Science**, v.363, p.87-95, 2010.

BAZINET, L.; IPPERSIEL, D.; LAMARCHE, F. Recovery of magnesium and protein from soy tofu whey by electrodialytic configurations. **Journal of Chemical Technology and Biotechnology**, v.74, p.663-668, 1999.

BELÉN, F.; SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. One option for the management of wastewater from tofu production: freeze concentration in a falling-film system. **Journal of Food Engineering**, v.110, p.364-373, 2012.

BELÉN, F.; BENEDETTI, S.; SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; PRUDÊNCIO, E.S.; PETRUS, J.C.C.; RAVENTÓS, M. Behavior of functional compounds during freeze concentration of tofu whey. **Journal of Food Engineering**, v.116, p. 681-688, 2013.

BOAVENTURA, B.C.B.; MURAKAMI, A.N.N.; PRUDÊNCIO, E.S.; MARASCHIN, M.; MURAKAMI, F.S.; AMANTE, E.R.; AMBONI, R.D.M.C. Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (*Ilex paraguariensis* A. St. Hil.) through freeze concentration technology. **Food Research International**, v. 53, 686–692, 2013.

CHAI, X.; MI, Y.; YUE, P-L.; CHEN, G. Bean curd wastewater treatment by membrane separation. **Separation and Purification Technology**, v.15, p.175–180, 1999.

CISSÉ, M.; VAILLANT, F.; SORO, D.; REYNES, M.; DORNIER, M. Crossflow microfiltration for the cold stabilization of roselle (*Hibiscus sabdariffa* L.) extract. **Journal of Food Engineering**, v. 106, p.20–27, 2011.

CHUN, J.; KIM, J.S.; KIM, J.H. Enrichment of isoflavone aglycones in soymilk by fermentation with single and mixed cultures of

Streptococcus infantarius 12 and *Weissella sp*. **Food Chemistry**, v.109, p.278-284, 2008.

DEBON, J.; SCHWINDEN PRUDÊNCIO, E.; CUNHAS PETRUS, J. C. Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. **Journal of Food Engineering** , v. 99, p. 128-135, 2010.

DEBON, J.; PRUDÊNCIO, E.S.; PETRUS, J.C.C.; FRITZEN-FREIRE, C.B.; MÜLLER, C.M.O.; AMBONI, R.M.C.; VIEIRA, C.R.W. Storage stability of prebiotic fermented milk obtained from permeate resulting of the microfiltration process. **LWT - Food Science and Technology,** v.47, p.96-102, 2012.

DOMINGUES, R.C.C.; RAMOS, A.A.; CARDOSO, V.L.; REIS, M.H.M. Microfiltration of passion fruit juice using hollow fibre membranes and evaluation of fouling mechanisms. **Journal of Food Engineering**, v.121, p.73-79, 2014.

HE, T.; FRANK, M.; MULDER, M.H.V.; WESSLING, M. Preparation and characterization of nanofiltration membranes by coating polyethersulfone hollow fibers with sulfonated poly(ether ether ketone) (SPEEK). **Journal of Membrane Science**, v.307, p.62-72, 2008.

HERNÁNDEZ, E.; RAVENTÓS, M.; AULEDA, J.M.; IBARZ, A. Freeze concentration of must in a pilot plant falling film cryoconcentrator**. Innovative Food Science and Emerging Technologies**, v.11, p.130-136, 2010.

KIM, W.J.; KIM, H.H.; YOO, S.H. Ultra- and- nano-filtration process optimization of isoflavones and oligosaccharides from sunmul. **Food Science and Biotechnology**, v.14, p.380-386, 2005.

LAORKO, A.; TONGCHITPAKDEE, S; YOURAVONG, W. Storage quality of pineapple juice non-thermally pasteurized and clarified by microfiltration. **Journal of Food Engineering**, v.116, p.554-561, 2013.

MACHADO, M.T.C.; MELLO, B.C.B.S.; HUBINGER, M.D. Study of alcoholic and aqueous extraction of pequi (*Caryocar brasiliense* Camb.) natural antioxidants and extracts concentration by nanofiltration. **Journal of Food Engineering**, v.117, p.450-457, 2013.

MORENO, F.L.; ROBLES, C.M.; SARMIENTO, Z.; RUIZ, Y.; PARDO, J. M. Effect of separation and thawing mode on blockfreeze-concentration of coffee brews. **Food and Bioproducts Processing**, v.91, p.396-402, 2013.

MORENO, F.L.; RAVENTÓS, M.; HERNÁNDEZ, E.; RUIZ, Y. Block freeze-concentration of coffee extract: Effect of freezing and thawing stages on solute recovery and bioactive compounds. **Journal of Food Engineering**, v.120, p.158-166, 2014.

MORENO, F.L.; HERNÁNDEZ, E.; RAVENTÓS, M.;ROBLES, C.M.; RUIZ, Y. A process to concentrate coffee extract by the integration of falling film and block freeze-concentration. **Journal of Food Engineering**, v.128, p.88-95, 2014.

RAZI, B.; AROUJALIAN, A.; FATHIZADEH, M. Modeling of fouling layer deposition in cross-flow microfiltration during tomato juice clarification**. Food and Bioproducts Processing**, v.90, p.841-848, 2012.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Freeze concentration of whey in a falling-film based pilot plant: process and characterization. **Journal of Food Engineering**, v.103, p.147-155, 2011.

QIANG, X., YONGLIE, C., QIANBING, W. Health benefit application of functional oligosaccharides. **Carbohydrate Polymers**, v.77, p.435-441, 2009.

YEE, P.L.; WAKISAKA, M.; SHIRAI, Y.; HASSAN, M.A. Effects of single food components on freeze concentration by freezing and thawing technique. **Japan Journal of Food Engineering,** v.4, p.77-83, 2003.

CAPÍTULO 1
REVISÃO BIBLIOGRÁFICA

1 REVISÃO BIBLIOGRÁFICA

1.1 A soja (*Glycine max*) e produtos da soja

A soja (*Glycine max*) é uma leguminosa de origem asiática, que foi introduzida no Ocidente em meados do Século XIX (BARNES et al., 2006). Atualmente, o Brasil é o segundo maior produtor mundial, cuja produção ultrapassou 81 milhões de toneladas na safra 2012/2013. A maior produção está concentrada nos estados de Mato Grosso, Paraná e Rio Grande do Sul (CONAB, 2014).

A composição da soja depende de vários fatores, como cultivar, localização geográfica, condições ambientais, época de cultivo, dentre outros. Em matéria seca, a composição aproximada é de 40 % de proteínas, 20 % de lipídios, 35 % de carboidratos e 5 % de cinzas (LIU, 1997; MOREIRA, 1999). Além disso, destaca-se como fonte de, potássio, fósforo, ferro, magnésio, zinco e cálcio, e de vitaminas, como a tiamina, riboflavina, niacina, ácido nicotínico e ácido ascórbico, e por possuir baixos teores de sódio (MANDARINO, 2002; SOUZA, 2006).

A soja foi primariamente utilizada para produção de óleo vegetal, devido à sua elevada concentração de lipídios. No entanto, nos últimos anos, tem sido pesquisada também como fonte de substâncias fitoquímicas, entre as quais se destacam os flavonóides, que apresentam atividade biológica, relacionada à prevenção de doenças crônicas não-infecciosas como as doenças cardiovasculares e osteoporose (SILVA et al., 2006), além de atuar sobre os sintomas climatéricos e sobre o perfil lipídico em mulheres durante a menopausa (NAHÁS et al., 2003). A soja destaca-se também por suas proteínas, que são de excelente qualidade nutricional, pois contém aminoácidos essenciais necessários para uma dieta equilibrada. Com relação às isoflavonas, a concentração pode vaiar entre 100 e 300 mg 100 g^{-1}, dependendo da cultivar e das condições de processamento da soja (LIU, 1997; RIAZ, 1999).

Apesar da alta produtividade e de seus benefícios à nutrição e à saúde, a soja é ainda pouco consumida no Brasil. As razões para o baixo consumo são atribuídas principalmente ao seu sabor e odor característicos, além do *beany flavor* (sabor de feijão cru), atribuído à ação das enzimas lipoxigenases formando hidroperóxidos a partir dos ácidos graxos poli-insaturados (MORAIS; SILVA, 2000; SILVA et al., 2006). Porém, nos últimos anos, a soja e seus produtos têm se destacado devido aos seus potenciais benefícios à saúde, o que vem estimulando também a busca de novas técnicas de processamento para melhorar as

características sensoriais e preservar a composição dos alimentos (PENHA et al., 2007).

A soja pode ser consumida de diversas formas, destacando-se o extrato hidrossolúvel de soja, o tofu e os produtos fermentados, como por exemplo, o *miso*, *tempeh*, molho de soja e o *sufu* (LI-JUN et al., 2004). O extrato hidrossolúvel de soja, que contém boa parte das proteínas do grão, pode ser convertido para uma forma semi-sólida, por coagulação, obtendo-se o tofu (BARNES et al., 2006).

O tofu é um alimento muito consumido nos países orientais e no leste asiático, apresentando variações na sua fabricação, de acordo com a finalidade, sendo classificado como firme ou macio (CHAI et al., 1999). Em base úmida, a composição típica do tofu é de aproximadamente 85 % de umidade, 7,8 % de proteína, 4,2 % de lipídeos e 2 mg g^{-1} de cálcio (LIU, 1997). Devido às suas características sensoriais e de textura, o tofu pode ser consumido como substituto da carne ou de queijos. As técnicas de processamento do tofu podem variar, mas consistem basicamente na maceração e moagem da soja, filtração e aquecimento do extrato de soja, e adição de um ou mais coagulantes. A formação do gel protéico envolve duas etapas: primeiramente, ocorre a desnaturação da proteína pelo calor e, em seguida, a coagulação hidrofóbica promovida por um coagulante (KOHYAMA et al., 1995; NOH, 1995). O coágulo formado é rico em proteínas, e o soro é constituído basicamente de água e compostos minoritários (VILLARES et al., 2011).

Os coagulantes utilizados para o preparo do tofu podem ser sais inorgânicos ou ácidos orgânicos, como, como glucono-δ-lactona. A natureza e a concentração do coagulante não afeta apenas o produto final, mas também a concentração de proteínas, isoflavonas e oligossacarídeos retidos no tofu e/ou eliminadas no soro (VILLARES et al., 2011). Dentre os coagulantes, o sulfato de cálcio ($CaSO_4$) demonstra ser eficiente no processo de coagulação, pois retém mais isoflavonas no coágulo do que o cloreto de cálcio ($CaCl_2$). Isso pode ser explicado pela coagulação mais lenta com $CaSO_4$ (KAO et al., 2004). Com relação às suas propriedades físicas, também se observa que o tofu apresenta maior firmeza e maior retenção de isoflavonas quando utilizados sais de cálcio como coagulante, pois a coagulação é mais completa do que quando utilizado sais de magnésio (PRABHAKARAN; PERERA, 2006). Com relação à concentração de coagulante utilizada, Kao et al. (2004) mostraram que a perda de isoflavonas é maior quando se utilizam baixas concentrações de $CaSO_4$.

Os ácidos orgânicos, como o ácido cítrico, ácido acético e o glucono-δ-lactona, também podem ser utilizados como coagulantes. Eles atuam enfraquecendo as interações eletrostáticas repulsivas entre as proteínas. O emprego de soluções fracas de ácido permite a retenção de isoflavonas semelhantes ao tofu coagulado com cloreto de cálcio ou magnésio. Entretanto, podem ocorrer alterações sensoriais no produto como, por exemplo, gosto ácido (PRABHAKARAN; PERERA, 2006).

O *okara* é o resíduo sólido obtido durante o processamento do extrato hidrossolúvel de soja e do tofu. Aproximadamente 1,1 kg de *okara* fresco é produzido a partir de cada kg de grãos de soja processados para fabricação de extrato de soja. Contém 27 % de proteína, lipídios e fibras solúveis e insolúveis. Embora possua elevada concentração de proteínas de alta qualidade, aminoácidos essenciais e isoflavonas, é destinado basicamente como ração animal ou segue para o tratamento de efluentes líquidos. Devido a sua composição, o okara já vem sendo utilizado para extração de proteínas e também como ingredente de alguns produtos alimentícios (VISHWANATHAN et al., 2011).

Além do okara, o soro de tofu, tradicionalmente conhecido como *sunmul* nos países orientais, é um subproduto da produção do tofu e, por apresentar elevada carga orgânica, constitui um sério problema ambiental, caso não receba tratamento e destinação adequada (CHUNG; EOM; KIM, 2006). Este soro geralmente é utilizado como alimento para animais ou então encaminhado para a estação de tratamento de efluentes, apresentando elevados valores de DBO (Demanda Bioquímica de Oxigênio) e DQO (Demanda Química de Oxigênio). Possui de 2 a 3 % de sólidos, principalmente proteínas, peptídeos, lipídeos, oligossacarídeos e isoflavonas, de média e pequena massa molar (CHAI et al., 1999; KIM; KIM, YOO, 2005). Levando-se em consideração a presença de compostos boativos no soro residual da fabricação do tofu, novos estudos visando a sua separação e concentração tem sido conduzidos, como a aplicação de processos conjugados de filtração por membranas para separação e concentração de isoflavonas e oligossacarídeos (GOULAS; GRANDISON; RASTALL, 2003; KIM; KIM, YOO, 2005).

1.2 Isoflavonas

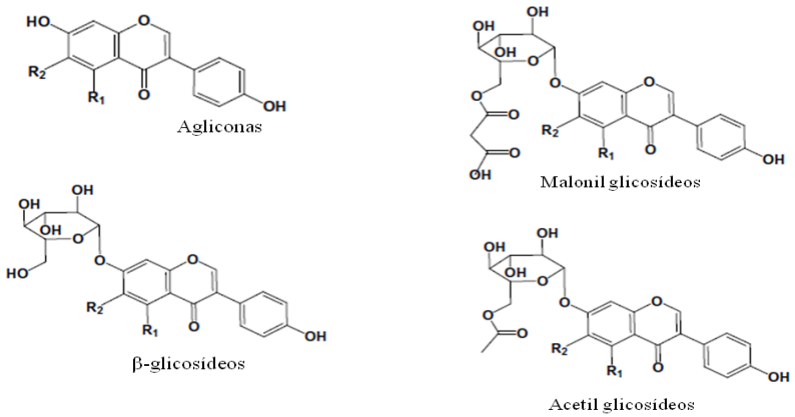
As isoflavonas, juntamente com as flavononas, flavonóis, catequinas e antocianinas, constituem um grupo de produtos naturais

denominados flavonóides. As isoflavonas são metabólitos secundários de plantas, chamados polifenóis, que se destacam pelo grande número de efeitos fisiológicos que causam em seres humanos e outros mamíferos (VACEK et al., 2008), atuando na prevenção de doenças crônicas (SILVA; CELEGHINI; CHANG, 2011), além de apresentarem atividade estrogênica, amenizando os sintomas da menopausa (KONAR et al., 2012). Estes compostos aparecem principalmente em plantas da família *Leguminosae*, como a soja (BONIGLIA et al., 2009).

A soja, uma das principais fontes desses compostos, contêm isoflavonas em doze formas químicas, incluindo as três agliconas genisteína, daidzeína e gliciteína e seus respectivos 7-*O*-β-D-glicosídeos (genistina, daidzina e glicitina), 6”-*O*-malonil- 7-*O*-β-D- glicosídeos (malonil genistina, malonil daidzina e malonil glicitina) e 6”-*O*-acetil-7-*O*-β-D- glicosídeos (acetil genistina, acetil daidzina e acetil glicitina) (SHAO et al., 2009). A estrutura química desses compostos consiste basicamente de dois anéis benzênicos unidos por uma ligação de três carbonos, que podem ou não ser fechados por um anel pirânico (LIU, 1997) (Figura 1). Dentre os doze isômeros, predominam a 6”-*O*-malonilgenistina, a genistina, a 6”-*O*-malonildaidzina e a daizina.

O processamento da soja pode também afetar a retenção e a distribuição das isoflavonas, em função de alterações químicas e enzimáticas promovidas pelo cozimento, maceração e fermentação (WANG; MURPHY, 1996). As isoflavonas não estão associadas aos lipídios, por isso não estão presentes no óleo vegetal, ficando retidas na torta durante a extração do óleo (MATSUURA; OBATA; HUKUSHIMA 1989). Em produtos como a farinha desengordurada, há predominância dos conjugados malonil glicosídeos, enquanto outros produtos que sofrem tratamento térmico com temperaturas superiores a 100 °C apresentam predominância de β-glicosídeos. As farinhas torradas e proteína isolada de soja apresentam uma distribuição homogênea de todas as isoflavonas conjugadas. Os malonil glicosídeos são termicamente instáveis e convertidos em seus respectivos β-glicosídeos em altas temperaturas devido às reações de de-esterificação. Essa reação consiste na transesterificação da ligação éster entre o grupo carboxil acetato ou malonato do grupo hidroxila da molécula de glicose, liberando metil malonato ou metil acetato e uma isoflavona glicosídica (BARNES; KIRK; COWARD, 1994).

Figura 1 - Estrutura química das isoflavonas.



	Grupos funcionais	
ISOFLAVONAS	R1	R2
Genisteína e conjugados	OH	H
Daidzeína e conjugados	H	H
Gliciteína e conjugados	H	OCH3

Fonte: Shao et al. (2009).

As isoflavonas provavelmente estabelecem ligações com as proteínas da soja, devido à sua polaridade e hidrofobicidade, bem como à sua capacidade de formar ligações de hidrogênio (RICKERT; JOHNSON; MURPHY, 2004). Essa ligação entre as proteínas e isoflavonas pode estar relacionada ao grau de associação entre os anéis de polifenóis e as porções alifáticas dos resíduos de prolina das proteínas. Neste caso, o grupo hidroxila fenólico é um excelente doador de íons H^+ para formação de ligações de hidrogênio com a carbonila da amida da estrutura peptídica das proteínas (SIEBERT, 1999). Dessa forma, o conteúdo final de isoflavonas de um determinado produto depende de sua maior ou menor associação com as proteínas durante cada etapa do processamento (SPERONI; MILESI; AÑÓN, 2010). Essa ligação também é comprovada pela elaboração do tofu, no qual não há perda significativa de isoflavonas no resíduo sólido, denominado *okara*, sugerindo que esses compostos estão associados com as proteínas solúveis e não com os carboidratos; as isoflavonas do extratto aquoso de

soja ficam retidas no coágulo ou são liberadas no soro após a prensagem (LIU, 1997).

1.3 Oligossacarídeos

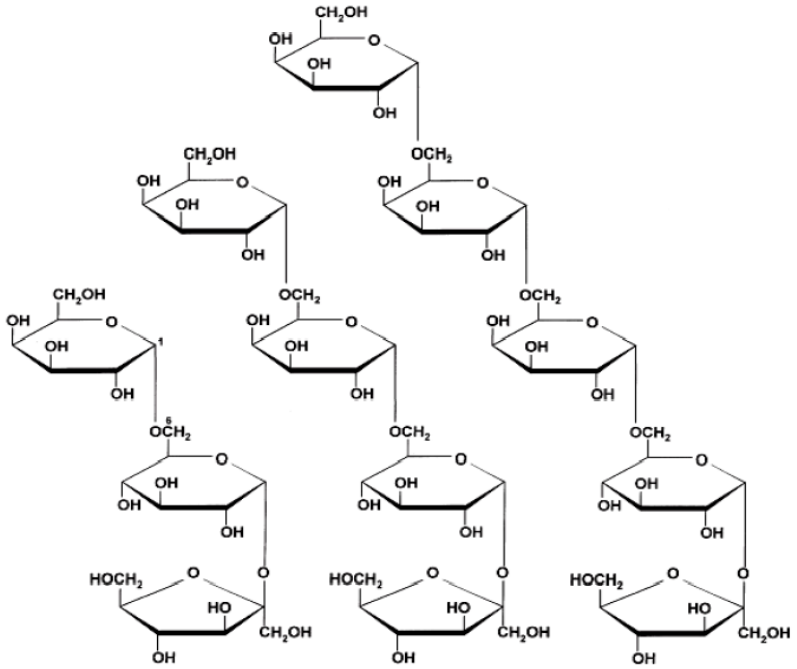
Os oligossacarídeos da soja são carboidratos não digeríveis de baixa massa molar, cuja estrutura é formada por 3 a 10 moléculas de açúcares (MUSSATTO; MANCILHA, 2007). Sua estrutura química consiste em uma molécula de sacarose ligada a diferentes quantidades de moléculas de D-galacatose, através de ligações α(1-6) (OTT, 2005). Esses compostos apresentam importantes propriedades físico-químicas e fisiológicas, benéficas ao homem, o que tem estimulado seu uso como ingrediente alimentício (KUNZ; RUDLOFF, 2006). Esses açúcares são reconhecidos por promover o crescimento de bactérias benéficas no intestino humano, principalmente as bifidobactérias, reconhecidos como prebióticas (QIANG; YONGLIE; QIANBING, 2009).

Na soja, os oligossarídeos representam aproximadamente 5 % da sua composição em base seca, englobando a rafinose (0,1 - 0,9 %), a estaquiose (1,4 - 4,1 %) e a verbascose, em menores concentrações (LIU, 1997; KARR-LILIENTHAL et al., 2005) (Figura 2), que consistem de 1, 2 ou 3 α-1-6 ligações de unidades de galactose ligadas por ligações α-1-3 à sacarose terminal. Esses compostos são solúveis em água e possuem aproximadamente a metade do poder adoçante da sacarose, dependendo da estrutura química, do grau de polimerização presente e das proporções de mono e dissacarídeos na molécula. Já a estabilidade varia de acordo com o resíduo de açúcar presente, seu formato de anel e configuração anomérica e os tipos de ligações (MUSSATTO; MANCILHA, 2007).

Devido à sua estrutura química, os oligossacarídeos podem servir de substrato para apenas alguns grupos de bactérias, como as bifidobactérias e os lactobacilos (QIANG; YONGLIE; QIANBING, 2009). Dessa forma, a ingestão regular de produtos que contenham oligossacarídeos pode resultar em importantes alterações na microbiota intestinal (redução de pH) através do estímulo ao crescimento de bactérias anaeróbias, inibindo o crescimento de outros micro-organismos patogênicos. Alguns autores indicam que a ingestão diária de 10 a 15 mg é suficiente para causar efeito bifidogênico (CRITTINDEN; PLAYNE, 1996), auxiliando na formação do bolo fecal, reduzindo a incidência de diarréias e infecções gastrintestinais e

constipação intestinal (FERNÁNDEZ-BAÑARES, 2006). A extração dos oligossacarídeos da soja é feita diretamente dos grãos e não requer processos enzimáticos (MUSSATTO; MANCILHA, 2007).

Figura 2 - Estrutura química dos oligossacarídeos da soja (a) estaquiose, (b) rafinose e (c) verbascose.



Fonte: Ott (2005).

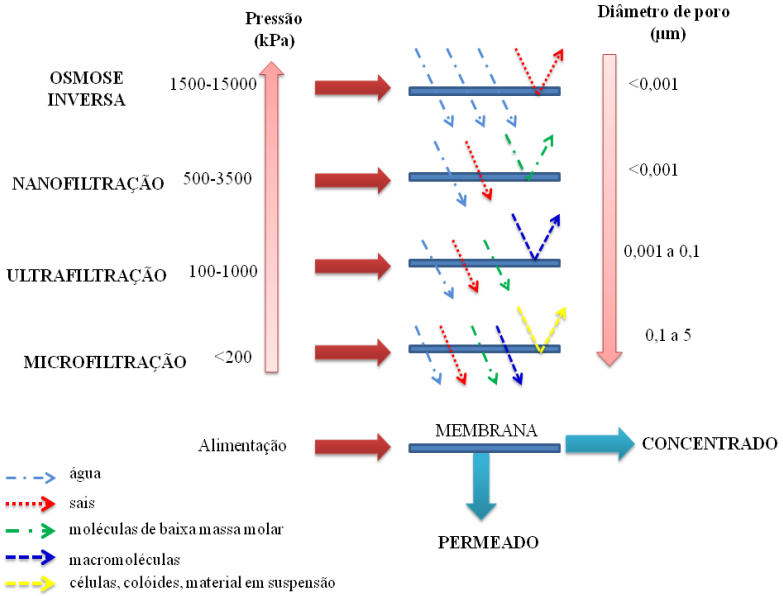
1.4 Processos de separação por membranas (PSM)

Nos últimos anos, a utilização dos processos de separação e concentração por membranas na indústria de alimentos vem aumentando. A principal aplicação está relacionada ao tratamento de

águas (PEINEMANN; NUNES; GIORNO, 2010), seguido da indústria de laticínios, de bebidas (vinho, cerveja, sucos), produtos de ovos e da soja, além da remoção ou recuperação de compostos de águas residuárias e subprodutos industriais (DAUFIN et al., 2001).

Dentre os PSM, destacam-se a microfiltração, ultrafiltração, nanofiltração e osmose inversa, que utilizam pressão como força motriz de acordo com o tamanho dos poros das membranas, permitindo ou não a passagem de determinados componentes (MIERZWA et al., 2008) (Figura 3).

Figura 3 - Classificação dos processos de separação por membranas quanto à sua seletividade.



Fonte: Mierzwa et al. (2008).

As membranas são definidas como filmes poliméricos ou inorgânicos semipermeáveis que servem como uma barreira seletiva para uma filtração em escala molecular de uma solução, quando aplicada alguma força motriz (STRATHMANN, 1990). Esta barreira controla a transferência de massa através de sua superfície, permitindo a passagem de determinados componentes de uma solução e restringindo

a passagem de outros, gerando duas correntes distintas, denominadas como permeado, que é o fluido que atravessa a membrana, e o concentrado ou retentado, constituído pelos solutos que não ultrapassam a membrana por apresentarem partículas maiores do que o tamanho médio dos poros (CHERYAN, 1998; ORDÓÑEZ, 2005).

O transporte de moléculas ocorre em etapas através da membrana: sorção das moléculas na superfície da membrana, difusão através da membrana e dessorção das moléculas no lado do permeado (MULDER, 2000; HABERT; BORGES; NÓBREGA, 2006). Em função disso, a retenção é expressa como razão nominal que se refere a um diâmetro de corte (*cut off*), definido como o valor da massa molar das moléculas para o qual a membrana apresenta coeficiente de rejeição de 95 % (CHERYAN, 1998).

As membranas podem ser fabricadas a partir de diversos materiais, sendo as membranas poliméricas e as cerâmicas as mais utilizadas. As membranas poliméricas podem ser fabricadas com acetato de celulose, poliamida, polisulfona, polietersulfona, polifluoreto de vinilideno, dentre outros (HABERT; BORGES; NÓBREGA, 2006; HE et al., 2008). Essas membranas apresentam altos fluxos, boa rejeição a sais, tolerância a altas temperaturas e variações de pH, boa resistência ao cloro e à compactação, variando de acordo com o tipo de membranas que são constituídas. Com relação ao tipo de processo, a filtração tangencial (*cross-flow filtration*) é a mais utilizada industrialmente, pois o fluido escoa paralelamente à superfície da membrana evitando o acúmulo de solutos sobre a mesma, permitindo a manutenção do fluxo e a maior eficiência do processo de separação (HABERT; BORGES; NÓBREGA, 2006).

Nos PSM, ocorre uma redução do fluxo permeado com o tempo, geralmente ocasionado pelo fenômeno denominado *fouling*. O *fouling* é caracterizado pela deposição e acúmulo de solutos na superfície e dentro dos poros da membrana, por adsorção ou bloqueio físico dos poros (AL-AMOUDI; LOVITT, 2007). A intensidade do *fouling* depende do tipo da membrana, da concentração e solutos presentes na solução, bem como da temperatura, pH e tempo de operação. Como o *fouling* ocorre devido a interações físicas e químicas entre os solutos e a membrana, não pode ser minimizado apenas por modificações das condições hidrodinâmicas do sistema, sendo necessária a aplicação de processos de limpeza, com substâncias detergentes, soluções alcalinas e ácidas ou agentes oxidantes, recomendados a cada tipo de membrana ou até mesmo a substituição das membranas (PETRUS, 1997; RODRIGUES, 2002).

Os PSM, especialmente a microfiltração (MF), já vem sendo muito utilizados industrialmente em processos de pré-tratamento de água potável, recuperação de sólidos de interesse e tratamento de águas residuárias (ALMANDOZ et al., 2010). Além disso, nos últimos anos, a MF vem sendo muito utilizada em processos de clarificação de bebidas, como substituto às técnicas convencionais de centrifugação e filtração a vácuo para remoção de sólidos em suspensão; e também para estabilização microbiológica de bebidas (pasteurização a frio), apresentando vantagens em relação aos processos térmicos por usar temperaturas baixas e pressões amenas, que preservam a qualidade nutricional e os atributos sensoriais dos produtos (CARNEIRO et al., 2002; SALAZAR et al., 2007; CISSÉ et al., 2011).

A ultrafiltração (UF), que compreende membranas com tamanho de poro de 0,001 a 0,1 µm, vem sendo utilizada para separar ou concentrar componentes de uma solução ou mistura, tais como açúcares, biomoléculas, polímeros e partículas coloidais (CHEN et al., 2006). Suas aplicações na indústria de alimentos geralmente são para concentração de proteínas do leite e produção de queijos (GOVINDASAMY-LUCEY et al., 2011), recuperação de proteínas do soro do leite (BALDASSO; BARROS; TESSARO, 2011), clarificação de sucos e bebidas alcoólicas (SEVERO et al., 2007; ECHAVARRÍA et al., 2012) e tratamento e purificação de água (MIERZWA; HESPANHOL, 2005). Além disso, pode ser utilizada para remoção de compostos químicos e estabilização de bebidas e fluidos alimentícios (GONÇALVES; FERNANDES; PINHO, 2001; RAO et al., 2011; CHHAYA et al., 2012; MAKTOUF et al., 2014).

A nanofiltração (NF) abrange todo o espectro de filtração entre a ultrafiltração e osmose reversa, combinando os mecanismos de convecção da ultrafiltração com aqueles tipicamente característicos de transporte através de membranas densas de osmose reversa (OTERO et al., 2006). Sua principal característica é a capacidade de separação de íons mono e divalentes, bem como alta rejeição para os compostos orgânicos com massa molar entre 100-500 g mol $^{-1}$ (HE et al., 2008). As membranas de NF apresentam cargas e poros na ordem de 1 nm. Dessa forma, tanto os efeitos das cargas elétricas quanto os mecanismos de filtração influenciam no comportamento da rejeição dos solutos nas membranas de NF (AL-AMOUDI; LOVITT, 2007). Devido às suas características, associadas ao baixo consumo de energia e aos altos fluxos obtidos, as membranas de NF tornam-se úteis para fracionamento e remoção seletiva de solutos a partir da filtração de soluções complexas. O transporte de solutos sem carga ocorre por convecção,

devido à diferença de pressão aplicada, e por difusão, devido ao gradiente de concentração através da membrana (OTERO et al., 2006).

A NF tem um mercado crescente no tratamento de águas, representando cerca de 65 % de sua utilização. Outros 25 % correspondem à aplicação em alimentos e produtos lácteos e menos de 10 % para a indústria química (CISSÉ et al., 2011a). Na indústria de alimentos, já vem sendo utilizada para recuperação de aromas de sucos, concentração de açúcares, desmineralização de leite, concentração de isoflavonas, dentre outros, pois muitas das membranas possuem massa molar de corte inferior a 180 g mol^{-1}, ideal para separação de moléculas de baixa massa molar (KIM; KIM; YOO, 2005).

Estudos envolvendo aplicação de processos de separação através de membranas em produtos da soja iniciaram-se em 1970 (LIU, 1997). Omosaiye, Cheryan e Matthews (1978) utilizaram a ultrafiltração e a diafiltração para concentração de proteínas a partir de extrato aquoso de soja e observaram que esses processos são eficientes para produtos proteicos de soja, pois possuem alta capacidade para separar as frações proteicas de alta massa molar, de moléculas pequenas, como fitatos e oligossacarídeos. Além disso, os PSM consomem menos energia quando comparados à evaporação ou liofilização, pois não ocorre mudança de fase ou estado físico do solvente durante o processo de filtração. Outra vantagem é a versatilidade quanto ao uso de temperaturas baixas ou altas, de acordo com a natureza de aplicação dos sólidos concentrados (CASSINI et al., 2010). Acredita-se que a qualidade de produtos obtidos através de filtração com membranas é preservada, pois não empregam calor e nem são utilizados produtos químicos. Uma limitação do processo é que os solutos não podem ser concentrados até total eliminação da umidade, além de ocorrer o acúmulo de solutos sobre a membrana, o que reduz as taxas de transferência de massa através da membrana e o aumento da viscosidade da solução torna mais difícil o bombeamento do concentrado (SHALLO et al., 2001).

1.5 Crioconcentração

A crioconcentração baseia-se na separação de fases sólido-líquido a baixas temperaturas, o que preserva as propriedades sensoriais e os componentes termicamente sensíveis dos alimentos. Devido às baixas temperaturas, esta tecnologia pode ser uma alternativa atraente às técnicas de concentração convencionais utilizadas no processamento de

alimentos (SÁNCHEZ et al., 2011a,b). Este processo assegura maior qualidade do produto concentrado e os custos totais (incluindo capital, energia e limpeza) são três a quatro vezes menores do que a evaporação ou a osmose reversa (SÁNCHEZ et al., 2011a). Como a crioconcentração envolve a cristalização parcial da água em solução aquosa, e após os cristais são separados do concentrado, ela possui algumas vantagens, como a baixa deterioração química, devido à baixa atividade enzimática e microbiológica, nenhuma perda de componentes voláteis e baixa perda de produto (CHANG; HARTEL, 1997; YEE et al., 2003). A concentração máxima obtida pela crioconcentração é de cerca de 50 ºBrix para alguns produtos, como sucos de frutas e soro de leite.

Na crioconcentração, há três mecanismos básicos para a formação dos cristais de gelo na solução de interesse: cristalização em suspensão, crioconcentração em blocos e crioconcentração em camada. A cristalização em suspensão consiste inicialmente na nucleação do gelo, seguida pela fase de crescimento dos cristais de gelo na solução. Já a crioconcentração em camada consiste na cristalização da água presente na solução sob uma superfície fria, formando uma camada de gelo (AIDER; HALLEUX, 2008; AIDER; HALLEUX, 2009). A crioconcentração em blocos, ainda pouco aplicada, baseia-se no congelamento total da solução seguida pelo descongelamento parcial pelo método de descongelamento gravitacional assistido ou outras técnicas. Esse método permite a obtenção de duas frações: a primeira é a fração concentrada (solução mãe) e a segunda é a fração de gelo contendo o mínimo de matéria seca. A eficiência desse processo é dependente da taxa de impurezas no gelo (AIDER; HALLEUX; AKBACHE, 2007). O bloco de gelo atua como uma carcaça sólida através do qual a fração do fluido rico em sólidos passa. O controle da temperatura de descongelamento é importante e possibilita atingir eficiência do processo superior a 90 %, o que significa que a quantidade de sólidos retidos no gelo é minimizada (AIDER; HALLEUX, 2009).

Na crioconcentração, um parâmetro importante que influencia na eficiência de separação, é a densidade de empacotamento dos cristais no bloco de gelo formado. Quanto maior a densidade de empacotamento, melhor é a separação do concentrado (BURDO; KOVALENKO; KHARENKO, 2007). De acordo com alguns estudos, este processo pode ser representado através de balanço de massa global de matéria seca e quantidade de gelo formado. Assumindo que a solução é quase homogênea e a radiação térmica é negligenciada no sistema, a equação de equilíbrio de calor pode ser expressa pela densidade do fluxo

de calor através da parede do cristalizador (BURDO; KOVALENKO; KHARENKO, 2008).

A crioconcentração já foi utilizada para concentração de sucos de frutas (AIDER; HALLEUX, 2009; SÁNCHEZ et al., 2010; AULEDA, RAVENTÓS; HERNÁNDEZ, 2011), soro lácteo (AIDER; HALLEUX; AKBACHE, 2007; AIDER; HALLEUX; MELNIKOVA, 2009; SÁNCHEZ et al., 2011c), açúcares (RAVENTÓS et al., 2007), mosto (HERNÁNDEZ et al., 2010) e compostos bioativos (BOAVENTURA et al., 2013; BÉLEN et al., 2013). Além da aplicação na indústria de alimentos, a crioconcentração pode ser utilizada na área de tratamento de águas residuais (YEE et al, 2003).

1.6 Leites fermentados

Os leites fermentados são definidos como preparados lácteos que passam pelo processo de fermentação, ocorrendo modificações em suas propriedades físicas, químicas e sensoriais (TSUCHIYA et al., 2006). Com a evolução das pesquisas na área da saúde e o desenvolvimento de novas tecnologias, estes produtos, inicialmente desenvolvidos como meio de preservação dos nutrientes do leite, passaram a ser produzidos com o uso de micro-organismos específicos e condições controladas de processamento, a fim de obter produtos com qualidade superior (SHAHANI; CHANDAN, 1979). Nos últimos anos, busca-se a ampliação da fonte de produtos lácteos devido ao aumento crescente de consumo (BUTTRISS, 1997).

Alguns exemplos de leites fermentados são os iogurtes e as bebidas lácteas fermentadas. As bebidas lácteas vêm se destacando como substitutos do iogurte, por apresentarem características sensoriais e físico-químicas semelhantes e também por serem comercializadas por um preço menor que os iogurtes. Além disso, a utilização do soro para elaboração das bebidas lácteas reduz o seu impacto ambiental, em virtude ao seu alto potencial poluente (CUNHA et al., 2008). Na elaboração das bebidas lácteas é permitida a adição de outros produtos além do leite, como soro de queijo, sucos de frutas, extrato de soja, porém a base láctea deve representar no mínimo 51 % (m/m) do total de ingredientes do produto (BRASIL, 2005).

A elaboração dos leites fermentados, incluindo as bebidas lácteas, se dá pelo tratamento térmico do leite, com posterior inoculação da cultura iniciadora selecionada (GINOVART et al., 2002). De acordo

com Tamime e Robinson (2000), o tratamento térmico serve para: eliminar os micro-organismos naturalmente presentes no leite; diminuir a atividade enzimática; reduzir a quantidade de oxigênio disponível, favorável ao crescimento microbiano e desnaturar as proteínas do soro. Os micro-organismos adicionados promovem a acidificação e, em muitos casos, a coagulação do produto e o desenvolvimento das características sensoriais (GINOVART et al., 2002). As bactérias láticas utilizadas devem apresentar uma contagem maior do que 10^7 Unidades Formadoras de Colônia (UFC) por mL ou g de produto, durante todo o seu o prazo de validade (BRASIL, 2000).

As bactérias ácido-láticas comumente utilizadas como culturas iniciadoras para produção de leites fermentados pertencem aos gêneros *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Bifidobacterium, Propionibacterium* (SABOYA; OETTERER; OLIVEIRA, 1997). Essas bactérias promovem redução de pH dos produtos, impedindo o crescimentos de micro-organismos indesejáveis e, consequentemente, aumentando a vida de prateleira. Alimentos fermentados por essas bactérias apresentam também maiores valores nutricionais, em função da digestão parcial de proteínas, lipídios e carboidratos, além de produzirem β-D-galactosidase (DEMIATE; OETTERER; WOSIACKI, 1994). Em iogurtes e bebidas lácteas, as bactérias comumente utilizadas são o *Lactobacillus bulgaricus* e o *Streptococcus thermophilus* (OLIVEIRA et al., 2002).

Na elaboração dos leites fermentados, uma boa alternativa como fonte de compostos biologicamente ativos para ser utilizado como ingrediente em alimentos ou bebidas é o soro proveniente da produção de tofu. A utilização desse subproduto da produção de tofu oferece uma boa relação custo/benefício (KIM; KIM; YOO, 2005), uma vez que é barato e fornece proteína de alta qualidade (KRÜGER et al., 2008).

As características químicas e nutricionais do extrato da soja e seus subprodutos, principalmente a presença de sacarose e oligossacarídeos, possibilitam sua utilização como meio de crescimento para bactérias ácido-láticas, as quais são amplamente utilizadas para fabricação de iogurtes e bebidas fermentadas, melhorando o sabor e a aceitabilidade dos produtos da soja (MORAIS; SILVA, 1996; GARRO et al., 1999; SU; HENRIKSSON; MITCHELL 2007). As bactérias ácido-láticas são capazes de metabolizar a rafinose e a estaquiose presentes na soja, aumentando a concentração de frutose, glicose e galactose (MARTÍN; CUENCA, 2009). Além disso,a metabolização dos oligossacarídeos também é vantajosa para reduzir problemas de flatulência (GUIMARÃES et al., 2001).

Para avalição das bebidas lácteas, algumas análises são fundamentais, como a análise reológica e de cor.

A reologia é definida como a ciência que estuda a resposta de um material à aplicação de uma tensão ou deformação (TABILO-MUNIZAGA; BARBOSA-CÁNOVAS, 2005), ou seja, descreve o comportamento de fluxo dos materiais, sendo que o principal interesse está relacionado a materiais relevantes industrialmente, com propriedades intermediárias entre sólidos e líquidos (SCHRAMM, 2006).

A reologia tem muitas aplicações na área de alimentos, no que diz respeito à aceitabilidade do consumidor para o desenvolvimento de novos produtos. Os alimentos são materiais estruturalmente e reologicamente complexos, consistindo, muitas vezes, de misturas de sólidos e líquidos (TABILO-MUNIZAGA; BARBOSA-CÁNOVAS, 2005). O conhecimento das propriedades reológicas de produtos alimentícios, como leites fermentados, é essencial para o desenvolvimento do produto, controle de qualidade, avaliação sensorial, projeção de equipamentos (cálculo de vazão, seleção de bombas, determinação da perda de carga em tubulações, etc.) e avaliação do processo (AICHINGER et al., 2003; MASSON, 2011).

Um fluido é caracterizado por apresentar capacidade de deformação contínua quando submetido à ação de uma força tangencial, denominada tensão de cisalhamento (CAMPOS, 1989). De acordo com Bhattacharya (1997), um parâmetro muito importante em alimentos é a viscosidade, que mede a resistência do fluido ao escoamento, quando uma taxa de deformação é aplicada. A viscosidade é dada pela relação entre a tensão de cisalhamento e a taxa de deformação, o que caracteriza o comportamento de fluxo dos fluidos, classificando-os como fluidos newtonianos ou não- newtonianos (TABILO-MUNIZAGA; BARBOSA-CÁNOVAS, 2005).

Os fluidos newtonianos são caracterizados por uma relação linear entre a tensão de cisalhamento e a taxa de deformação, independente do escoamento ser em regime laminar ou permanente, dependendo apenas da temperatura e da composição do fluido (SILVA, 2000). Alguns exemplos de alimentos que apresentam comportamento newtoniano são os sucos de frutas clarificados, leite, cerveja, vinho, óleo refinado e soluções de sacarose (HOLDSWORTH, 1971; SHARMA; MULVANEY; RIZVI, 2000). Já nos fluidos não-newtonianos, a relação entre a taxa de deformação e tensão de cisalhamento não é constante e depende ainda do tempo de observação ou das forças de recuperação elástica, o que caracteriza a maioria dos alimentos líquidos. Estes podem

ser dependentes ou independentes do tempo (SCHRAMM, 2006) (Figura 4a).

Para os fluidos não-newtonianos independentes do tempo, à temperatura e composição constantes, a viscosidade aparente depende da taxa de cisalhamento ou da tensão de cisalhamento (RAO; RIZVI, 1986). Estes se dividem em: pseudoplásticos (*shear thinning*), nos quais a viscosidade aparente do fluido diminui com o aumento da taxa de deformação; e em dilatantes (*shear thickening*), quando a viscosidade aparente aumenta com o aumento da taxa de deformação (TABILO-MUNIZAGA; BARBOSA-CÁNOVAS, 2005). Já os fluidos dependentes do tempo (Figura 4b) cuja viscosidade não depende somente da taxa de deformação, mas também do tempo de escoamento, estão divididos em tixotrópicos e reopéticos (MACHADO, 2002; TABILO-MUNIZAGA; BARBOSA-CÁNOVAS, 2005).

Os fluidos podem ser submetidos a processos ascendentes e descendentes de tensão de cisalhamento, a fim de se obter o grau de tixotropia, definido como uma diminuição contínua da viscosidade aparente com o tempo, a uma determinada tensão, e a subsequente recuperação da viscosidade, quando se interrompe o fluxo (SCHRAMM, 2006).

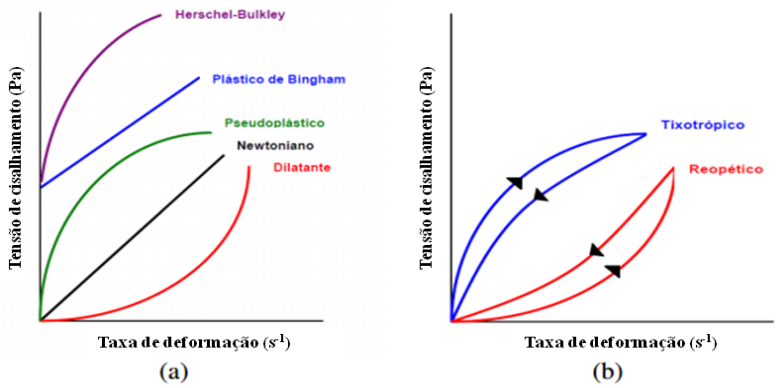
O comportamento de fluidos não-Newtonianos tem sido descrito por modelos como o da Lei da Potência, representado na Equação 1, onde σ é a tensão de cisalhamento (Pa), k é o índice de consistência ($Pa.s^n$), γ é a taxa de deformação (s^{-1}) e n é o índice de comportamento de fluxo. Neste modelo, quando $n < 1$, o fluido é chamado pseudoplástico (CAMPOS, 1989; MULLINEUX; SIMMONS, 2007).

$$\sigma = k.\gamma^n$$

Em produtos lácteos fermentados, as medidas reológicas são afetadas pelos tratamentos térmicos (ABU-JDAYIL, 2003), pela composição do leite utilizado, pela temperatura de fermentação (KRISTO; BILIADERIS; TZANETAKIS, 2003), tipo de cultura láctea utilizada, tempo de armazenamento, entre outros (SODINI et al., 2005; CHAMMAS et al., 2006). Um fator relevante na elaboração de leites fermentados é a sinerese, ou seja, o aparecimento espontâneo de soro de leite na superfície do gel, durante o armazenamento (AMATAYAKUL; SHERKAT; SHAH, 2006). A taxa e o aparecimento da sinerese desempenham um papel importante na produção de leites fermentados, pois afetam a qualidade final e a aceitabilidade desses produtos pelo consumidor (AINCHINGER et al., 2003; GENG et al., 2011). O

fenômeno de sinerese ocorre devido aos rearranjos contínuos das moléculas de caseína, levando ao estresse na rede e subsequente quebra das ligações proteicas (PEREIRA et al., 2003). Vários fatores influenciam na sinerese de bebidas lácteas, como temperatura de incubação, taxa de inoculação, baixo pH (PENNA; GURRAM; BARBOSA-CÁNOVAS, 2006) e teor de sólidos totais (AICHINGER et al., 2003).

Figura 4 - Curva de fluxo de fluidos onde se tem os (a) independentes do tempo e os (b) dependentes do tempo.



Fonte: Sharma; Mulvaney; Rizvi (2000).

Já a colorimetria refere-se à ciência e à tecnologia usada para quantificar, descrever e simular, através de modelos matemáticos, as percepções humanas da cor, sob determinados estímulos visuais (WYSZECKI; STILES, 1982). Para a física ótica, a cor é definida como um feixe de radiações luminosas com uma determinada distribuição espectral, ou seja, é a interação da luz com os materiais, percebida pelo olho humano e interpretada pelo cérebro (CALVO; DURÁN, 1997).

A percepção da cor está relacionada a quatro principais fatores: distribuição espectral da energia da luz; condições sob as quais a cor está sendo vista; características espectrais do objeto em relação à absorção, reflexão e transmissão; e sensibilidade do leitor (LEÓN et al., 2006).

O aspecto visual e a cor dos produtos alimentícios é um dos parâmetros de qualidade mais importantes, pois é a primeira sensação

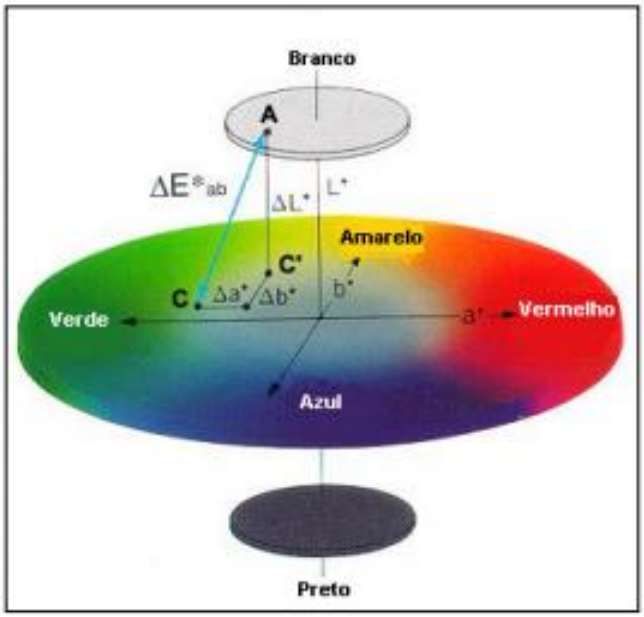
que o consumidor usa para aceitar ou rejeitar um alimento. A percepção da cor permite também a detecção de certas anormalidades ou defeitos que o alimento possa apresentar (LEÓN et al., 2006). De acordo com Macdougall (2002), a cor também serve para auxiliar no controle da matéria prima e nas alterações causadas pelo processamento e armazenamento.

A determinação da cor dos alimentos pode ser realizada através de inspeção visual ou pelo uso de um instrumento de medida de cor, que pode ser um colorímetro ou um espectrofotômetro. Embora a inspeção de cor pela observação visual seja adequada e rápida, as mudanças de iluminação podem tornar essa análise muito subjetiva e variável de observador para observador. Desta forma, para uma análise mais objetiva, sugere-se o uso de um instrumento de medida de cor, chamado colorímetro.

Os espaços de cor e os valores numéricos são utilizados para criar, representar e visualizar cores em espaços bi e tridimensionais (TRUSSEL; SABER; VRHEL, 2005). De acordo com León et al. (2006), a cor dos alimentos geralmente é medida em *L*a*b* ou sistema CIELab (Figura 5). Esse sistema é um padrão internacional de medida de cor, adotado pela *Commission Internationale d'Eclairage* (CIE) em 1976. Neste sistema, o parâmetro *L** varia de 0 a 100, o máximo valor de *L** (100) representando uma perfeita reflexão difusa, representando a coloração branca, enquanto o valor mínimo (0) representa a coloração preta. Já os eixos *a** e *b** são componentes cromáticos e não apresentam limites numéricos específicos. A coordenada a* varia do vermelho (+*a**) ao verde (-*a**), e a coordenada *b** do amarelo (+*b**) ao azul (-*b**) (YAM; PAPADAKIS, 2004).

De acordo com Debon (2009), ainda não existem padrões qualitativos para os atributos de cor em leites fermentados.

Figura 5 - Diagrama de cores para análise de cor e cálculo da diferença de cor de acordo com o diagrama CIELab.



Fonte: Minolta (1993).

REFERÊNCIAS

ABU-JDAYIL, B. Modelling the time-dependent rheological behavior of semisolid foodstuffs. **Journal of Food Engineering**, v.57, p.97-102, 2003.

AICHINGER, P.A.; MICHEL, M.; SERVAIS, C.; DILLMANN, M.L.; ROUVET, M.; D'AMICO, N.; ZINK, R.; KLOSTERMEYER, H.; HORNE, D.S. Fermentation of a skim milk concentrate with S*treptococcus thermophilus* and chymosin: structure, viscoelasticity and syneresis of gels. **Colloids and Surfaces B: Biointerfaces**, v. 3, p. 243-255, 2003.

AIDER, M.; HALLEUX, D.; AKBACHE, A. Whey cryoconcentration and impact on its composition. **Journal of Food Engineering**, v. 82, p. 92-102, 2007.

AIDER, M.; HALLEUX, D. Production of concentrated cherry and apricot juices by cryoconcentration technology. **LWT - Food Science and Technology**, v. 41, p.1768-1775, 2008.

AIDER, M.; HALLEUX, D.; MELNIKOVA, I. Skim acidic milk whey cryoconcentration and assessment of its functional properties: Impact of processing conditions. **Innovative Food Science and Emerging Technologies**, v. 10, p.334-341, 2009.

AIDER, M.; HALLEUX, D. Cryoconcentration technology in the bio-food industry: principles and applications. **LWT - Food Science and Technology**, v. 42, p. 679- 685, 2009.

AL-AMOUDI, A.; LOVITT, R.W. Review article: fouling strategies and the cleaning system of NF membranes and factors affecting cleaning efficiency. **Journal of Membrane Science**, v. 303, p. 4-28, 2007.

ALMANDOZ, C.; PAGLIERO, C.; OCHO, A.; MARCHESE, J. Corn syrup clarification by microfiltration with ceramic membranes. **Journal of Membrane Science**, v. 363, p. 87–95, 2010.

AMATAYAKUL, T.; SHERKAT, F.; SHAH, N.P. Physical characteristics of set yoghurt made with altered casein to whey protein ratios and EPS-producing starter cultures at 9 and 14% total solids. **Food Hydrocolloids**, v.20, p.314-324, 2006.

AULEDA, J.M.; RAVENTÓS, M.; HERNÁNDEZ, E. Calculation method for designing a multi-plate freeze-concentrator for concentration of fruit juices. **Journal of Food Engineering**, v.107, p. 27–35, 2011.

BARNES, S.; KIRK, M.; COWARD, L. Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC-mass spectrometry. **Journal of Agricultural and Food Chemistry**, v.42, p.2466-2474, 1994.

BARNES, S.; PRASAIN, J.; D'ALESSANDRO, T.; WANG, C.C.; ZHANG, H.; KIM, H. **Soy Isoflavones**. In: HEBER, D.; BLACKBURN, G.; GO,V.; MILNER. San Diego: Elsevier, 2006. v.2, Cap.32, p.559-571.

BELÉN, F.; BENEDETTI, S.; SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; PRUDÊNCIO, E.S.; PETRUS, J.C.C.; RAVENTÓS, M. Behavior of functional compounds during freeze concentration of tofu whey. **Journal of Food Engineering**, v.116, p. 681-688, 2013.

BHATTACHARYA, S. N. **Rheology: Fundamentals and Measurements**. Australia: Royal Melbourne Institute of Technology, 1997.

BOAVENTURA, B.C.B.; MURAKAMI, A.N.N.; PRUDÊNCIO, E.S.; MARASCHIN, M.; MURAKAMI, F.S.; AMANTE, E.R.; AMBONI, R.D.M.C. Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (*Ilex paraguariensis* A. St. Hil.) through freeze concentration technology. **Food Research International**, v. 53, 686–692, 2013.

BONIGLIA, C.; CARRATU, B.; GARGIULO, R.; GIAMMARIOLI, S.; MOSCA, M.; SANZINI, E. Content of phytoestrogens in soy-based dietary supplements. **Food Chemistry**, v.115, p.1389-1392, 2009.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Resolução n° 5, de 13 de novembro de 2000. Oficializa os Padrões de Identidade e Qualidade (PIQ) de leites fermentados. Diário Oficial da União, Brasília, DF, 27 novembro 2000, sec. 1, p. 9.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa no 16, de 23 de agosto de 2005. Regulamento Técnico de Identidade e Qualidade de Bebida Láctea. Diário Oficial da União, Brasília, DF, 24 agosto 2005, sec. 1, p. 7.

BURDO, O. G.; KOVALENKO, E. A.; KHARENKO, D. A. Development of freeze concentration device. **Communication of the Odessa State Academy of Food Technologies**, Odessa, Ukraine, 2007.

BURDO, O. G.; KOVALENKO, E. A.; KHARENKO, D. A. Intensification of the processes of low-temperature separation of food solutions. **Applied Thermal Engineering**, v.28, p.311–316, 2008.

BUTTRISS, J. Nutritional properties of fermented milk products. **International Journal of Dairy Technology**, v.50, p.21-27, 1997.

CALVO, C.; DURÁN, L. **Propiedades Físicas II – Ópticas y color**. In: 122 CITED – Instituto Politécnico Nacional. Temas en Tecnología de alimentos. Mexico: Ed: José Miguel Aguilera, v.1, 1997.

CAMPOS, S.D.S**. Reologia e Textura em Alimentos**. Campinas: ITAL, 1989. 84p.

CARNEIRO, L.; SA, I.S.; GOMES, F.S.; MATTA, V.M.; CABRAL, L.M.C. Cold sterilization and clarification of pineapple juice by tangential microfiltration. **Desalination**, v.148, p. 93-98, 2002.

CASSINI, A.S.; TESSARO, I.C.; MARCZAK, L.D.F.; PERTILE, C. Ultrafiltration of wastewater from isolated soy protein production: a comparison of three UF membranes. **Journal of Cleaner Production**, v.18, p.260-265, 2010.

CHAMMAS, G.I.; SALIBA, R.; CORRIEU, G.; BÉAL, C. Characterisation of lactic acid bacteria isolated from fermented milk "laban". **International Journal of Food Microbiology**, v.110 , p.52-61, 2006.

CHANG, Y.H., HARTEL, R.W. Flow properties of freeze-concentrated skim milk. **Journal of Food Engineering**, v.31, p.371-386, 1997.

CHAI, X.; MI, Y.; YUE, P-L.; CHEN, G. Bean curd wastewater treatment by membrane separation. **Separation and Purification Technology**, v.15, p.175-180, 1999.

CHERYAN, M. **Ultrafiltration and Microfiltration Handbook**. Lancaster: Technomic Publishing Company, 1998. 527p.

CHUNG, H-J.; EOM, K-Y.; KIM, W-J. Evaluation of physicochemical properties of muffins made with ultrafiltered sunmul powder. **Journal of Food Science and Nutrition**, v.11, p.333-338, 2006.

CISSÉ, M.; VAILLANT, F.; SORO, D.; REYNES, M.; DORNIER, M. Crossflow microfiltration for the cold stabilization of roselle (*Hibiscus sabdariffa* L.) extract. **Journal of Food Engineering**, v.106, p.20-27, 2011.

CONAB -COMPANHIA NACIONAL DE ABASTECIMENTO. Levantamento de Avaliação da safra 2012/2013. 2014. Disponível em: <http://**www.conab.gov.br/conabweb**/>. Acesso em: 12.jan.2014.

CUNHA, T.M.; CASTRO, F.P.; BARRETO, P.L.M.; BENEDET, H.D.; PRUDÊNCIO, E.S. Avaliação físico-química, microbiológica e reológica de bebida láctea e leite fermentado adicionados de probióticos. **Semina: Ciências Agrárias**, v. 29, p. 103-116, 2008.

DAUFIN, G.; ESCUDIER, J.-P.; CARREÁRE,H.; BEÂROT,S.; FILLAUDEAU, L.; DECLOUX, M. Recent and emerging applications of membrane processes in the food and dairy industry. **Food and Bioproducts Processing**, v. 79, p.89–102, 2001.

DEBON, J**. Emprego da microfiltração tangencial na obtenção de leite fermentado prebiótico**. Florianópolis: UFSC, 2009. Dissertação. (Mestrado em Engenharia de Alimentos)- Programa de Pós-graduação em Engenharia de Alimentos, UFSC, 2009.

DEMIATE, J.M.; OETTERER, M.; WOSIACKI, G. A fermentação como processo de enriquecimento nutricional. **Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos**, v.28, p.170-181, 1994.

FERNÁNDEZ-BAÑARES, F. Nutritional care of the patient with constipation. **Best Practice & Research Clinical Gastroenterology**, v. 20, p.575–587, 2006.

GARRO, M.S.; VALDEZ, G.F.; OLIVER, G.; GIORI, G.S. Starter culture activity in refrigerated fermented soymilk. **Journal of Food Protection**, v.62, p.808-810, 1999.

GENG, X.L.; VAN DEN BERG, F.W.J.; BAGER, A.N.B.; IPSEN, R. Dynamic visualization and microstructure of syneresis of cheese curd during mechanical treatment. **International Dairy Journal**, v. 21, p.711-717, 2011.

GINOVART, M.; LÓPEZ, D.; VALLS, J.; SILBERT, M. Simulation modelling of bacterial growth in yoghurt. **International Journal of Food Microbiology**, v. 73, p. 415– 425, 2002.

GOULAS, A.K.; GRANDISON, A.S.; RASTALL, R.A. Fractionation of oligosaccharides by nanofiltration. **Journal of the Science of Food and Agriculture,** v.83, p.675–680, 2003.

GUIMARÃES, V.M.; REZENDE, S.T.; MOREIRA, M.A.; BARROS, E.G.; FELIX, C.R. Characterization of α-galactosidases from germinating soybean seed and their use for hydrolysis of oligosaccharides. **Phytochemistry**, v.58, p. 67-73, 2001.

HABERT, A.; C; BORGES, C.P.; NÓBREGA, R. **Processos de Separação com Membranas**. Rio de Janeiro: E-papers Serviços Editoriais Ltda, 2006. 180p.

HE, T.; FRANK, M.; MULDER, M.H.V.; WESSLING, M. Preparation and characterization of nanofiltration membranes by coating polyethersulfone hollow fibers with sulfonated poly(ether ether ketone) (SPEEK). **Journal of Membrane Science**, v.307, p. 62-72, 2008.

HERNÁNDEZ, E.; RAVENTÓS, M.; AULEDA, J.M.; IBARZ, A. Freeze concentration of must in a pilot plant falling film cryoconcentrator**. Innovative Food Science and Emerging Technologies**, v. 11, p.130-136, 2010.

HOLDSWORTH, S. D. Applicability of rheological models to the interpretation of flow and processing behavior of fluid food products. **Journal of Texture Studies**, v.2, p. 393-418, 1971.

KAO, T.H.; LU, Y.F.; HSIEH, H.C.; CHEN, B.H. Stability of isoflavone glucosides during processing of soymilk and tofu. **Food Research International**, v.37, p.891-900, 2004.

KARR-LILIENTHAL, L.K.; KADZERE, C.T.; GRIESHOP, C.M.; FAHEY JR, G.C. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: a review. **Livestock Production Science**, v. 97, p. 1 -12, 2005.

KIM, W.J.; KIM, H.H.; YOO, S.H. Ultra- and- nano-filtration process optimization of isoflavones and oligosaccharides from sunmul. **Food Science and Biotechnology**, v.14, p.380-386, 2005.

KOHYAMA, K.; SANO, Y.; DOI, E. Rheological characteristics and gelation mechanism of tofu (soybean curd). **Journal of Agricultural and Food Chemistry**, v. 43, p. 1808-1812, 1995.

KONAR, N.; POYRAZOG, E.S.; DEMIR, K.; ARTIK, N. Determination of conjugated and free isoflavones in some legumes by LC–MS/MS. **Journal of Food Composition and Analysis**, v. 25, p. 173–178, 2012.

KRISTO, E.; BILIADERIS, C.G.; TZANETAKIS, N. Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of *Lactobacillus paracasei*. **International Dairy Journal**, v.13, p.517-528, 2003.

KRÜGER, R.; KEMPKA, A.P.; OLIVEIRA, D.; VALDUGA, E.; CANSIAN, R.L.; TREICHEL, H.; Di LUCCIO. Desenvolvimento de uma bebida láctea probiótica utilizando como substratos soro de leite e extrato hidrossolúvel de soja. **Alimentos e Nutrição**, v.19, p. 43-53, 2008.

KUNZ, C.; RUDLOFF, S. Health promoting aspects of milk oligosaccharides. I**nternational Dairy Journal**, v.16, p. 1341-1346, 2006.

LEÓN K.; MERY D.; PEDRESCHI F.; LEÓN J. Color measurement in L*a*b* units from RGB digital images. **Food Research International**, v. 39, p.1084-1091, 2006.

LI-JUN, Y.; LI-TE, L.; TATSUMI, E.; SAITO, M. Changes in isoflavone contents and composition of sufu (fermented tofu) during manufaturing. **Food Chemistry**, v.87, p.587-592, 2004.

LIU, K. Soybean Protein Products. In: LIU, K. **Soybeans: Chemistry, Technology and Utilization**. New York: Chapman e Hall, 1997. Cap. 8, p. 379-411.

MACDOUGALL D.B. **Colour in Food Improving Quality**. Woodhead Publishing Limited. 2002. 12p.

MACHADO, J.C.V. **Reologia e escoamento de fluidos: ênfase na indústria do petróleo**. Rio de Janeiro: Interciência: PETROBRÁS, 2002. 257p.

MANDARINO, J. M. G. A Soja e a Saúde Humana. In: **Encontro Franco Brasileiro de Biociência e Biotecnologia – Alimentos Funcionais e Nutracêuticos**, Brasília. Resumos das Palestras. Brasília: Embrapa, p. 9-11, 2002.

MARTÍN, M.A.B.; CUENCA, M.C.Q. Valoración de diferentes indicadores de la fermentación de bebida de soya y de leche de vaca utilizando cultivos probióticos. **Brazilian Journal of Food Technology**, VII BMCFB, 2009.

MASSON, L.M.P.; ROSENTHAL, A.; CALADO, V.M.A.; DELIZA, R.; TASHIMA, L. Effect of ultra-high pressure homogenization on viscosity and shear stress of fermented dairy beverage. **LWT - Food Science and Technology**, v. 44, p. 495-501, 2011.

MATSUURA, M.; OBATA, A.; HUKUSHIMA, D. Objectionable flavor of soy milk developed during the soaking of soybeans and its control**. Journal of Food Science**, v.54, p.602-605, 1989.

MIERZWA, J.C.; SILVA, M.C.C.; RODRIGUES, L.B.; HESPANHOL, I. Tratamento de água para abastecimento público por ultrafiltração: avaliação comparativa através dos custos diretos de implantação e operação com os sistemas convencional e convencional com carvão ativado. **Engenharia Sanitária e Ambiental,** v.78, p. 78-87, 2008.

MINOLTA. **Precise color communication: color control from feeling to instrumentation**. MINOLTA Co. Ltd., 1993.

MORAIS, A. A. C.; SILVA, A. L. **Soja: suas aplicações**. Rio de Janeiro: Editora Médica e Científica, 1996. 259p.

MOREIRA, M.A. Programa de melhoramento genético de qualidade de óleo e proteína de soja desenvolvido na UFV. In: **Congresso Brasileiro de Soja**, Paraná. Anais: Embrapa, p. 99-104, 1999.

MORAIS, A. A. C.; SILVA, A. L. Valor nutritivo e funcional da soja. **Revista Brasileira de Nutrição Clínica**, v. 15, p. 306-315, 2000.

MULDER, M. **Basic principles of membrane technology, center for membrane science and technology**, University of Twente, Enschede, The Netherlands, 2000. 564p.

MULLINEUX, G.; SIMMONS, M.J.H. Effects of processing on shear rate of yoghurt. **Journal of Food Engineering**, v. 79, p. 850-857, 2007.

MUSSATTO, S.I.; MANCILHA, I.M. Non-digestible oligosaccharides: a review. **Carbohydrate Polymers**, v. 68, p. 587–597, 2007.

NAHÁS, E. A. P.; NETO J. N.; LUCA, L. A.; TRAIMAN, P.; PONTES, A.; SALBEN, I. Efeitos da isoflavona sobre os sintomas climatéricos e o perfil lipídico na mulher em menopausa. **Revista Brasileira de Ginecologia e Obstetrícia,** v. 25, p. 337-343, 2003.

NOH, E.J.; PARK, S.Y.; PAK, J.I.; HONG, S.T.; YUN, S.E. Coagulation of soymilk and quality of tofu as affected by freeze treatment of soybeans. **Food Chemistry**, v. 91, p. 715-721, 2005.

OLIVEIRA, M. N.; SIVIER, I. K.; ALEGRO, J. H. A.; SAAD, S. M. I. Aspectos tecnológicos de alimentos funcionais contendo probióticos. **Revista Brasileira de Ciências Farmacêuticas**, v. 38, p. 1-21, 2002.

OMOSAYIE, O., CHERYAN, M., MATTHEWS, M.E. Removal of oligosaccharides from soybean water extracts by ultrafiltration. **Journal of Food Science**, v. 43, p. 354-360, 1978.

ORDÓÑEZ, J.A. **Tecnologia de Alimentos - Componentes dos Alimentos e Processos.** Porto Alegre: Artmed, v.1, 2005.

OTT, R.P. **Utilização de carboidrases em dietas para frangos de corte**. Porto Alegre: UFRGS, 2005. Dissertação (Mestrado em Zootecnia)- Programa de Pós-graduação em Zootecnia, UFRGS, 2005.

OTERO, J.A.; LENA, G.; COLINA, J.M.; PRÁDANOS, P.; TEJERINA , F.; HERNÁNDEZ, A. Characterisation of nanofiltration membranes Structural analysis by the DSP model and microscopical techniques. **Journal of Membrane Science,** v. 279, p. 410–417, 2006.

PEINEMANN, K.V., NUNES, S.P., GIORNO, L. Membrane Technology. Vol.3. **Membranes for food applications**. Weinheim. Germany: Wiley-VCH, 2010.

PENHA, L.A.O.; FONSECA, I.C.B.; MANDARINO, J.M.; BENASSI, V.T. A soja como alimento: valor nutricional, benefícios para a saúde e cultivo orgânico. **Boletim CEPPA**, v. 25, p. 91-102, 2007.

PENNA, A. L. B.; GURRAM, S.; BARBOSA-CÁNOVAS, G. V. Effect of high hydrostatic pressure processing on rheological and textural properties of probiotic low-fat yogurt fermented by different starter cultures. **Journal of Food Process Engineering**, v. 29, p. 447-461, 2006.

PEREIRA, R.B.; SINGH, H.; MUNRO, P.A.; LUCKMAN, M.S. Sensory and instrumental textural characteristics of acid milk gels. **International Dairy Journal**, v.13, p.655-667, 2003.

PETRUS, J.C.C**. Preparação, Modificação e Caracterização de Membranas Assimétricas para Clarificação de Sucos de Frutas**. Campinas: UNICAMP, 1997. Tese (Doutorado em Tecnologia de Alimentos) - Programa de Pós-graduação em Engenharia de Alimentos, UNICAMP, 1997.

PRABHAKARAN, M. P.; PERERA, C.O. Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. **Food Chemistry**, v.99, p.231-237, 2006.

QIANG, X., YONGLIE, C., QIANBING, W. Health benefit application of functional oligosaccharides. **Carbohydrate Polymers**, v. 77, p. 435–441, 2009.

RAO, M. A. Rheological properties of fluid foods. In: RAO, M.A.; RIZVI, S.S.H. **Engineering properties of foods**. New York: Marcel Dekker, Cap.5, p.1-47, 1986.

RAVENTÓS, M.; HERNÁNDEZ, E.; AULEDA, J. M.; IBARZ, A. Concentration of aqueous sugar solutions in a multi-plate cryoconcentrator. **Journal of Food Engineering**, v. 79, p.577- 585, 2007.

RIAZ, M.N. Soybeans as functional foods. **Cereal Foods World**, v.44 (2), p.88-92, 1999.

RICKERT, D.A.; JOHNSON, L.A.; MURPHY, P.A. Improved fractionation of glycinin and beta-conglycinin and partitioning of phytochemicals. **Journal of Agricultural and Food Chemistry**, v.52, p.1726-1734, 2004.

RODRIGUES, R.B. **Aplicação dos Processos de Separação por Membranas para Produção de Suco Clarificado e Concentrado de Camu Camu (***Myrciaria dubia***)**. Campinas: UNICAMP, 2002. Dissertação (Mestrado em Engenharia de Alimentos) – Programa de Pós-graduação em Engenharia de Alimentos, UNICAMP, 2002.

SABOYA, L.V.; OETTERER, M.; OLIVEIRA, A.J. Propriedades profiláticas e terapêuticas de leites fermentados - uma revisão. **Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos**, v.31(2), 1997.

SALAZAR, F.N.; BRUIJN, J.P.F.; SEMINARIO, J.; GÜEL, C.; LÓPEZ, F. Improvement of wine crossflow microfiltration by a new hybrid process. **Journal of Food Engineering**, v.79, p.1329–1336, 2007.

SÁNCHEZ, J.; RUIZ, Y.; RAVENTÓS, M.; AULEDA, J.M.; HERNÁNDEZ, E. Progressive freeze concentration of orange juice in a pilot plant falling film. **Innovative Food Science and Emerging Technologies**, v.11, p. 644-651, 2010.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Freeze concentration of whey in a falling-film based pilot plant: Process and characterization. **Journal of Food Engineering**, v. 103, p.147–155, 2011a.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Review: freeze concentration technology applied to dairy products**. Food Science and Technology International**, v.17, p.5-13, 2011b.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Freeze concentration of whey in a falling-film based pilot plant: process

and characterization. **Journal of Food Engineering**, v. 103, p.147-155, 2011c.

SCHRAMM, G. **Reologia e Reometria: Fundamentos Teóricos e Práticos**. São Paulo: Artliber, 2006. 237p.

SHAHANI, K.; CHANDAN, R. C. Nutritional and healthful aspects of cultured and culture-containing dairy foods. **Journal Dairy Science**, v. 62. p 1685-1694, 1979.

SHALLO, H.E.; RAO, A.; ERICSON, A.P.; THOMAS, R.L. Preparation of soy protein concentrate by ultrafiltration. **Journal of Food Science**, v. 66, p.242-246, 2001.

SHAO, S.; DUNCAN, A.M.; YANG, R.; MARCONE, M.F.; RAJCAN, I.; TSAO, R. Tracking isoflavones: from soybean to soy flour, soy protein isolates to functional soy bread**. Journal of Functional Foods I**, v. 1, p.119-127, 2009.

SHARMA, S.K.; MULVANEY, S.J.; RIZVI, S.S.H. **Food Processing Engineering: Theory and Laboratory Experiments**. United States of America: Wiley-Interscience. 2000. 348p.

SIEBERT, K.J. Effects of Protein-Polyphenol Interactions on Beverage Haze, Stabilization, and Analysis. **Journal of Agricultural and Food Chemistry**, v.47, p.353-362, 1999.

SILVA, F.C. da. **Reologia do suco de acerola: efeito da concentração e da temperatura.** Campinas: UNICAMO, 2000. Dissertação (Mestrado em Engenharia de Alimentos) – Programa de Pós-Graduação em Engenharia de Alimentos, UNICAMP, 2000.

SILVA, M.S.; NAVES, M.M.V.; OLIVEIRA, R.B.; LEITE, O.S.M. Composição química e valor protéico do resíduo de soja em relação ao grão de soja. **Revista Ciência e Tecnologia de Alimentos**, v. 26, p. 571-576, 2006.

SILVA, L.H.; CELEGHINI, R.M.S.; CHANG, Y.K. Effect of the fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. **Food Chemistry,** v. 128, p. 640–644, 2011.

SPERONI, F.; MILESI, V.; AÑÓN, M.C. Interactions between isoflavones and soybean proteins: Applications in soybean-proteine isolate production. **LWT - Food Science and Technology**, v. 43, p.1265-1270, 2010.

SODINI, I.; LUCAS, A.; TISSIER, J.P.; CORRIEU, G. Physical properties and microstructure of yoghurts supplemented with milk protein hydrolysates. **International Dairy Journal**, v.15, p.29-35, 2005.

SOUZA, A.S. **Efeitos da irradiação na composição e propriedades funcionais da soja.** Campinas: UNICAMP, 2006. Tese (Doutorado em Alimento e Nutrição), Programa de Pós Graduação em Engenharia de Alimentos, UNICAMP, 2006.

STRATHMANN, H. **Synthetic membranes and their preparation**. In: PORTER, M.C. Handbook of Industrial Membrane Technology. Nova Jersey: Noyes Publications, 1990. 624 p.

SU, P.; HENRIKSSON, A.; MITCHELL, H. Selected prebiotics support the growth of probiotic mono-cultures in vitro. **Anaerobe**, v. 13, p. 134-139, 2007.

TABILO-MUNIZAGA, G.; BARBOSA-CÁNOVAS, G.V. Rheology for the food industry. **Journal of Food Engineering**, v.67, p.147-156, 2005.

TAMIME, A.Y.; ROBINSON, R.K. **Yoghurt Science and technology**. 3. ed. Cambridge: Woodhead Publishing Limited, 2000.

TSUCHIYA, A.; ALMIRON-ROIG, E.; LLUCH, A.; GUYONNET, D.; DREWNOWSKI, A. higher satiety ratings following yogurt consumption relative to fruit drink or dairy fruit Drink. **Journal of the American Dietetic Association**, v. 106 (4), p. 550-557, 2006.

VACEK, J.; KLEJDUS, B.; LOJKOVÁ, L.; KUBÁN, V. Current trends in isolation, separation, determination and identification of isoflavones: A review**. Journal of Separation Science,** v.31, p.2054-2067, 2008.

VILLARES, A.; ROSTAGNO, M. A.; GARCÍA-LAFUENTE, A.; GUILLAMÓN, E.; MARTÍNEZ, J.A. Content and profile of isoflavones in soy-based foods as a function of the production process**. Food and Bioprocess Technology**, v. 4, p.27–38, 2011.

VISHWANATHAN, K.H.; GOVINDARAJU, K.; SING.; SUBRAMANIAN, R.Production of Okara and Soy Protein Concentrates Using Membrane Technology. **Journal of Food Science**, v.76, p.158-164, 2011

WANG, H-J; MURPHY, P. A. Mass balance study of isoflavones during soybean processing. **Journal of Agricultural and Food Chemistry**, v.44, p.2377-2383, 1996.

WYSZECKI, G.; STILES, W.S. **Color Science**. 2.ed. New York: John Wiley and Sons, 1982. 248p.

YAM, K.L.; PAPADAKIS, S.E. A simple digital imaging method for measuring and analyzing color of food surfaces. **Journal of Food Engineering**, v. 61, p. 137-142, 2004.

YEE, P.L.; WAKISAKA, M.; SHIRAI, Y.; HASSAN, M.A. Effec
single food components on freeze concentration by freezing and tha
technique. **Japan Journal of Food Engineering**. v.4, p.77-83, 2003.

CAPÍTULO 2
POTENCIAL DOS PROCESSOS DE CONCENTRAÇÃO NA RECUPERAÇÃO DE ISOFLAVONAS DE SORO DE TOFU

Potencial dos processos de concentração na recuperação de isoflavonas de soro de tofu

Resumo

Neste estudo, avaliou-se o potencial da nanofiltração (NF) e da crioconcentração (FC) para a recuperação de isoflavonas de soro de tofu. O soro de tofu que foi submetido à concentração por NF, passou inicialmente por uma etapa de ultrafiltração (UF), sendo o permeado resultante desse processo utilizado como alimentação da NF. O processo de NF foi conduzido até atingir os fatores de redução volumétrica (FRV) de 2,5. O soro também foi concentrado através do processo de FC operando em sistema de filme descendente, conduzido em três etapas. A quantificação das isoflavonas foi realizada no concentrado e permeado da NF, e nos concentrados e no gelo proveniente da FC. Durante a UF, observou-se que o fluxo de permeado permaneceu estável ao longo do tempo, em torno de 14 L h^{-1} m^{-2}, enquanto na nanofiltração observou-se redução gradual do fluxo com o tempo, atribuída principalmente à formação da camada gel polarizada. Com relação às isoflavonas, observou-se que a concentração de isoflavonas totais duplicou após o processo de NF, atingindo em torno de 81 mg isoflavonas por kg de soro de tofu. Dentre os isômeros, obteve-se maior concentração dos β-glicosídeos. No processo de FC, também observou-se um aumento de aproximadamente duas vezes na concentração de isoflavonas totais, atingindo em torno de 82 mg isoflavonas por kg de soro de tofu. Diferentemente do processo de NF, observou-se maior concentração de agliconas em relação aos β-glicosídeos. Ambos os processos foram eficientes para concentrar as isoflavonas presentes no soro de tofu.

Palavras-chave: Soro de tofu; Concentração; Nanofiltração; Crioconcentração; Isoflavonas.

Potential of concentration processes for the recovery of tofu whey isoflavones

ABSTRACT

In this study the potential of nanofiltration (NF) and freeze concentration (FC) for the recovery of isoflavones from tofu whey (TW) was evaluated. The tofu whey that was subjected to concentration by NF initially passed through a step of ultrafiltration (UF). The resulting permeate of UF was utilized as feed in this NF process. The NF process was conducted to achieve the volume reduction factor (VRF) of 2.5. The tofu whey was also concentrated by the FC in a falling-film system carried out by three stages. The quantification of isoflavones was determined in concentrate and permeate from NF, and in the concentrate and ice from FC. During UF, it was observed that the permeate flux remained stable over time, about 14 L h^{-1} m^{-2}, while in the NF observed gradual decrease of the flux with time, attributed primarily to the formation of polarized gel layer. With respect to isoflavones, it was found that the concentration of total isoflavones doubled after the NF process reaching around 81 mg isoflavones per kg of tofu whey. Among the isomers, yielded higher concentration of β-glucosides. In the FC process also observed an increase of approximately twice in the concentration of total isoflavones, reaching about 82 mg isoflavones per kg of tofu whey. Unlike the NF process, there was a higher concentration of aglycones in relation to β-glucosides. Both processes were effective to concentrate isoflavones present in the tofu whey.

Keywords: Tofu whey; Concentration; Nanofiltration; Freeze concentration; Isoflavones.

1 Introduction

Tofu processing generates a liquid waste material known as tofu whey (TW), which contains isoflavones. When discharged to the environment, TW causes an unpleasant odor and pollutes surface and ground water. The amount of TW generated varies with the type of coagulant used for the tofu preparation (Sudyani et al., 2007), as well as the different water holding capacities of the curd (Uzzan and Labuza, 2004). According to Wang and Murphy (1996) the loss of isoflavones in the whey during the manufacture of tofu can reach around 44%. Prabhakaran and Perera (2006) noted that this low-cost by-product is interesting to be used by food industry as a source of bioactive compounds.

Isoflavones are subclasses of flavonoids and are also described as phytoestrogen compounds because they exhibit estrogenic activity. Campos et al. (2007) reported that the isoflavones used for hormone replacement therapy are found in whole foods and commercial preparations, such as purified isoflavone supplements, fortified foods, and other preparations containing these compounds. Vacek et al. (2008) observed that isoflavones compounds are frequently used to treat symptoms associated with the menopause, osteoporosis and other estrogen-related disorders. These compounds are present in significant concentrations mainly in plants from *Leguminosae* family, as soybeans (Konar et al., 2012). Isoflavones are usually found as conjugates (acetyls, glycosides or malonyls) and are hydrolyzed in the human digestive system to aglycones that are biologically active (Alves et al., 2010). Isoflavones can form complexes with the soybean protein, which can be released into the tofu whey during the coagulation process. Because of their bioactivity, Butylina et al. (2006) indicate a potential demand for isoflavones recovered and concentrated from industrial wastewater produced during soybean processing as a concentrate. However traditional methods to recover isoflavoes require large quantities of organic solvents and various chromatographic techniques, which take longer processing time and are not cost effective (Chang, 2002). Moreover, few studies have been carried out on the concentration of TW, particularly in relation to the concentrate form.

Nanofiltration (NF) can be defined as a membrane process for the separation and concentration of substances with molar masses between 100 and 1000 Da (g mol^{-1}) (Van der Bruggen et al., 2008). This process serves a large and growing market in water treatment, which currently accounts for around 65% of NF treatments. Another 25% of

the NF market is associated with the food and dairy industries, and less than 10% with the chemical industry (Bessarabov and Twardowski, 2002). In the food industry NF is used to treat effluents and other applications have recently gain importance. According to Butylina et al. (2006), NF can be applied to the separation of isoflavones due to the suitable cut-off of their membranes, as well as electrochemical effects, which play an important role in the case of charged molecules. Other possible option is the use of freeze concentration (FC), as suggested by Auleda et al. (2011), which has been widely studied also due to its operation at low temperatures. Miyawaki (2003) reported that FC can also provide good retention of thermosensitive compounds in foods. Furthermore, this process that can be applied to aqueous systems, such as fruit juices or wastewater, allowing the food industry to obtains high quality products by preserving aromas, flavors and colors normally lost during evaporation (Aider et al., 2012). FC involves the removal of water as ice crystals by cooling the fluid to be concentrated at temperatures below the freezing point (Raventós et al.,2007; Hernández et al., 2010). Thus, the concentration of TW is of great industrial interest, enhancing its ability as a by-product and reducing environmental pollution. Both technologies (NF and FC) result in a liquid concentrate, which can be easily incorporated into food to enhance nutritional and biological quality. Thus, the objective of this study was to evaluate the potential of concentration processes (NF and FC) for the recovery and concentration of isoflavones from tofu whey, as an alternative to add value to wastewater from soybean processing plants.

2. Materials and methods

2.1 Raw material

Tofu whey (TW) was supplied by NATURSOY SL, Alimentos Naturales y Biológicos (Castellterçol, Barcelona, Spain), produced from the soybean variety BRS 258 (Embrapa Soja, Brazil).

TW sample presented the following characteristics: 1.9 °Brix, pH 5.1 and 6.26 mS cm^{-1} electrical conductivity.

Sample preparation included centrifugation (Centrifuge Rotanta 460R, Hettich Zentrifugen, Tuttlingen, Germany) at 3,000xg for 20 min

to remove suspended material and two stages of filtration in a glass fiber filter (1 and 1.2 µm) (Filter-Lab, Barcelona, Spain).

2.2 Membrane processes

Approximately 4 L of TW was submitted to the ultrafiltration (UF) process to remove large molar mass compounds, such as tofu fragments and foaming compounds, as recommended by Matsubara et al. (1996) in order to increase NF efficiency. The operation parameters used in the UF and NF processes were previously defined. The UF operation was carried out with tangential flow using a ceramic membrane with a molecular weight cut-off (MWCO) of 15,000 g mol^{-1} (Ceram Inside, Tami Industries, France) and effective filter area of 95.2 cm^2. The experimental device consisted of a reservoir tank, a pump, a closed-pipe pressure dampener to prevent pressure oscillation, pressure gauges, a filtration cell and flow meters for the concentrate and permeate (Fig. 1). The experiment was carried out at 20±2 ºC with a transmembrane pressure of 600 kPa and cross flow velocity of 1 m s^{-1}. The permeate obtained was used as a input to NF process. The NF was carried out in a SEPA CF II membrane element cell (GE Osmonics®, Philadelphia, USA) equipped with an polimeric membrane Model MPF-34 (Koch, SelRO® MPF-34, Staford, United Kingdom), with an MWCO of 200 g mol^{-1} and glucose rejection of 0.95. The experiments were carried out at 22 ± 2 °C, with a transmembrane pressure of 1000 kPa and a linear velocity of 1 m s^{-1}. The concentrate stream circulated tangentially to the membrane surface until reaching a volume reduction factor (VRF) of 2.5. VRF was calculated as the ratio between TW initial volume (L) and concentrate final volume (L).

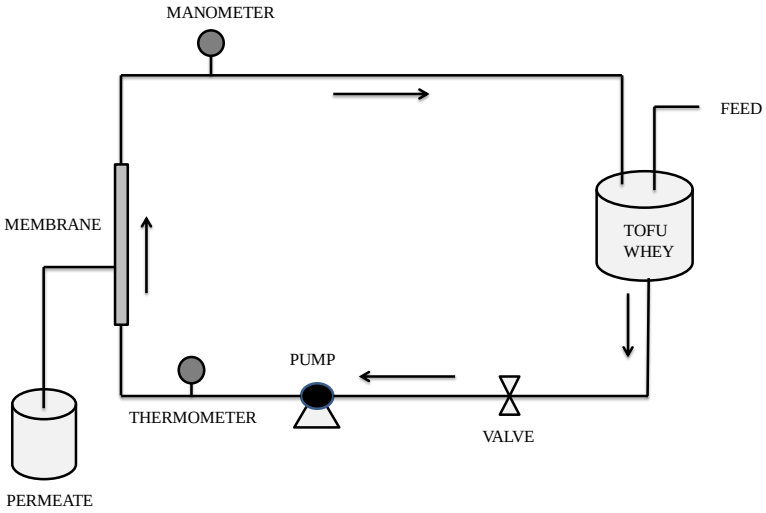
The permeate flux (J) (L h^{-1} m^{-2}) during NF was measured every 10 min and was calculated according to Eq. (1):

$$J = \frac{V_p}{t\,A_p} \quad (1)$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and A_p (m^2) is the permeation surface area of the membrane. The quality of the filtration process was assessed by on the isoflavone content, considering the highest mean J values obtained. The isoflavone contents in the TW (stage 0), and of the concentrates (CNF) and

permeates (P) for the three VRFs were denominated CNF 1.5, CNF 2, CNF 2.5, P 1.5, P 2 and P 2.5, respectively.

Figure 1- Schematic diagram of the nanofiltration unit.

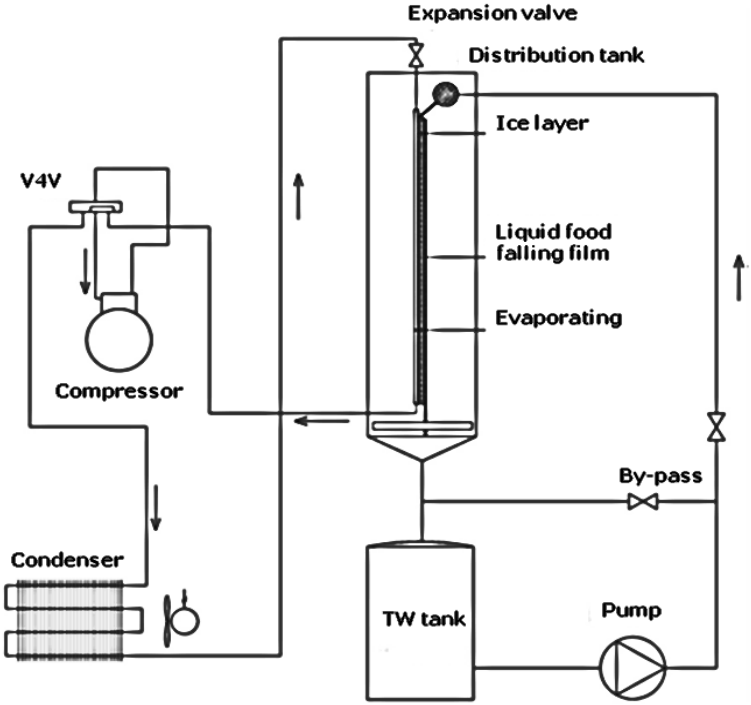


2.3 Freeze concentration (FC) process

Around 840 kg of TW, previously filtered, was submitted to freeze concentration (FC) in a pilot scale falling film plant system (Fig. 2). The process was carried out in three stages maintaining the average flow rate at 1.0 ± 0.2 L s^{-1} to ensure good contact between the evaporator plates and the fluid. TW temperature at the entry and exit of the plate, ambient temperature around the plate and temperature of the cooled plate were registered using a Testo datalogger (model 177-T4, Barcelona, Spain) with 4 K thermocouples.

Concentrate (6.0–15.5 °Brix) and ice samples were obtained according to Fig. 3, as described by Belén et al. (2012). The isoflavone contents in TW (stage 0), concentrates (CF) and ice (I) fractions, in three stages were denominated CF1, CF2, CF3, I1, I2 and I3, respectively.

Figure 2- Schematic diagram of freeze concentration equipment.



2.4 Extraction and quantification of isoflavones

The extraction and quantification of isoflavones in the samples (TW; CNF1.5, CNF2, CNF2.5 and P1.5, P2, P2.5, CF1, CF2, CF3; and I1, I2 and I3) were performed according to AOAC (2001), with modifications.

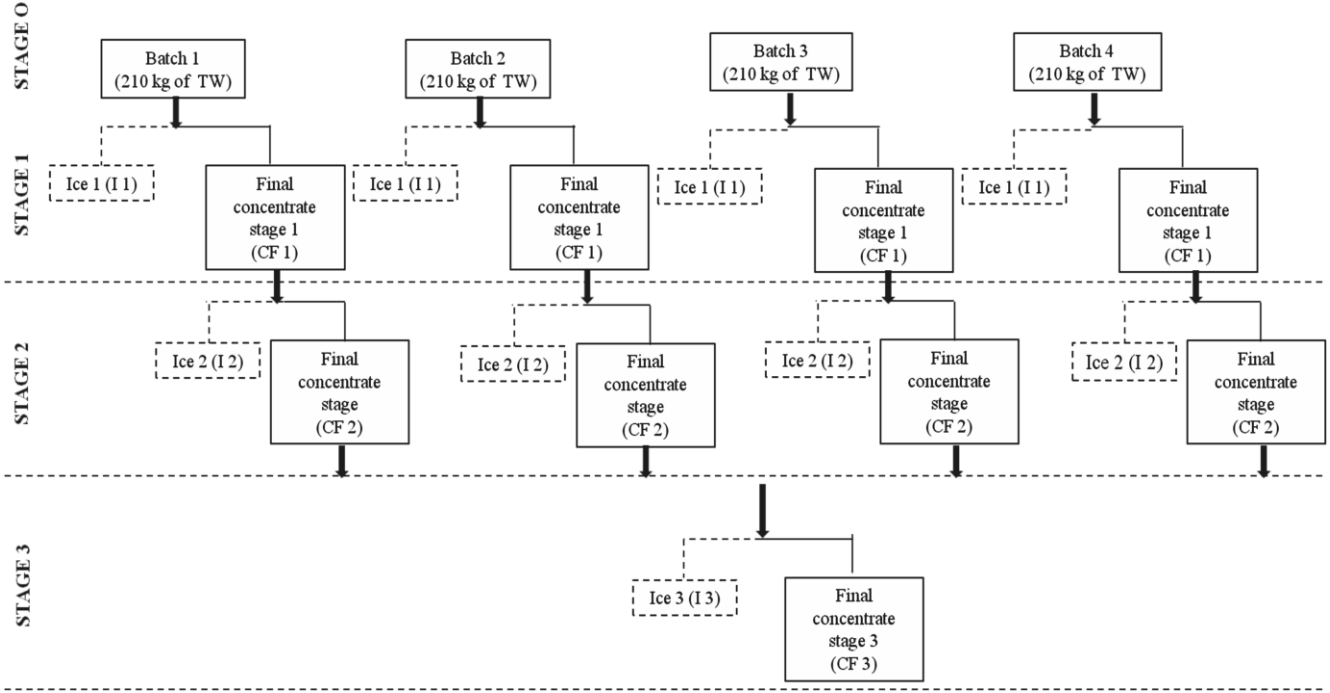
For the isoflavone extraction, 10 g of samples were mixed with 40 mL of extracting solution (80% methanol and 20% water) in test tubes, maintened for 2 h at 65 °C. The extracts were then saponified at room temperature (25 °C) with 3 mL 2M NaOH and acidified with 1 mL glacial acetic acid, then filtered through quantitative-grade filter paper and diluted with water to yield 50% water and 50% methanol. Finally, the extracts were clarified in a centrifuge (Centrifuge Rotanta38, Hettich Zentrifugen, Tuttlingen, Germany) (5 min at 7000xg) and analyzed by High Performance Liquid Chromatography (HPLC) (Agilent

Technologies, Santa Clara, CA, USA). For the separation of the isoflavones, the injection volume was 20 µL and a binary linear gradient system was used with the following mobile phases: water/methanol/acetic acid (88:10:2) and methanol/acetic acid (98:2). The mobile phase flow was 1 mL min^{-1}. Isoflavone glucosides and aglycones were separated in a C18 reverse-phase column (Nova-Pack C18150x3.9 mm4µ) with a methanol-water mobile phase and qauntified by UV detection at 260 nm in an Agilent1100 system equipped with a DAD detector set at 260 nm (Agilent Technologies, Santa Clara, CA, USA). For the identification and quantification of the peaks corresponding to each one of the isoflavones, calibration curves with linear regression based on the peak area were used. These calibration curves were constructed with external standards of daidzin, daidzein, genistin (Fluka 30408, 48756, 91955) and genistein (Sigma D7802). Glycitin and glycitein were identified through their retention times. The results were expressed in aglycone units by summing the concentrations of the aglycone isoflavones (genistein, daidzein and glycitein) and the glucosides (genistin, daidzin and glycitin) and presented as mg isoflavones per kg of liquid sample. All of these analyses were performed in triplicate.

2.4 Statistical analysis

Results were expressed as means and standard deviations. Analysis of variance (ANOVA) and Tukey's studentized range test (5% significance) were carried out to detect any significant differences, by using software Statistica 7.0 (2004) (StatSotft Inc., Tulsa, OK, USA).

Figure 3- Flowchart of the three concentration stages of TW.



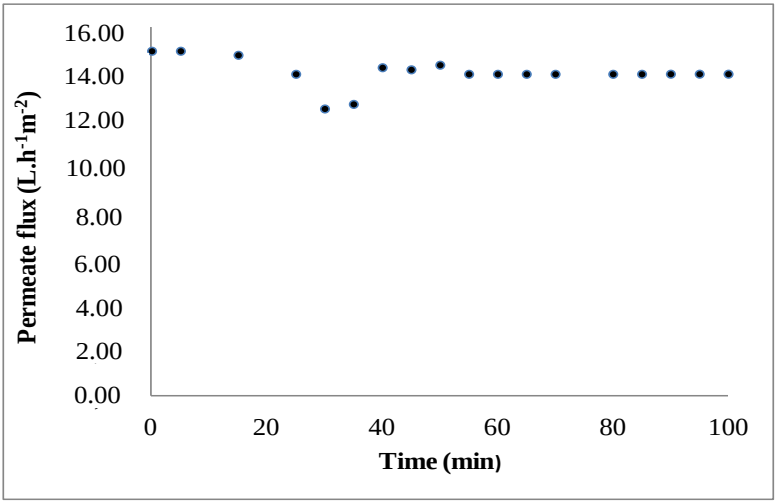
3 Results and Discussion

3.1 Membrane processes

Ultrafiltration (UF) process was used the remove macromolecules and colloidal particles, as suggested by Kim et al. (2005), to reduce the membrane fouling during the nanofiltration (NF) of the tofu whey (TW). According to Chan and Chen (2004), proteins are macromolecular species whose labile nature and complex structure can contribute to fouling, mainly by adsorption on to the membrane surface or deposition due to convection through a porous material. Therefore, fouling caused by proteins has traditionally been followed by flux decline and changes in the membrane rejection characteristics.

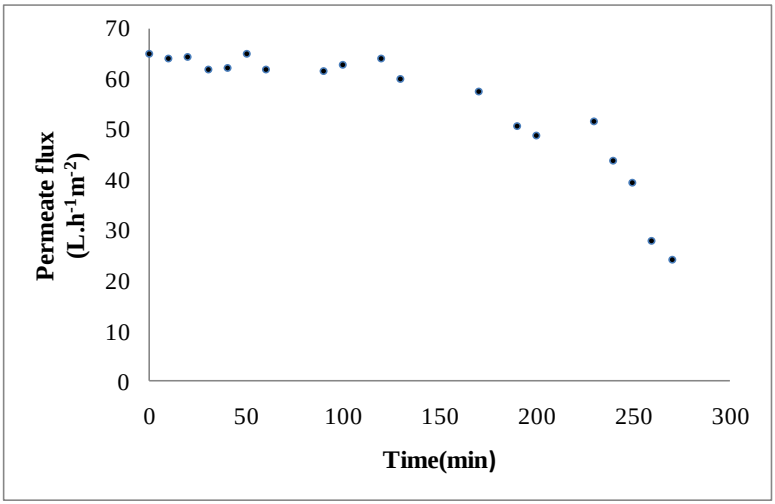
Results shows that the UF process was not influenced by the concentration polarization phenomenon or fouling, since the permeate flux (J) remained stable, i.e., approximately 14.06 L h^{-1} m^{-2} (Fig. 4). However, the concentration of total proteins decreased from 4.40 g L^{-1} to 1.00 g L^{-1}, whose explanation could be the retention of high molar mass compounds, as observed by Matsubara et al. (1996) during the UF of TW.

Figure 4 - Behavior of permeate flux (J) in the ultrafiltration of tofu whey.



The UF permeate from TW was submitted to the NF process. Permeate flux (J) decreased during this process (Fig.5). The same behavior was observed by Matsubara et al. (1996) and Kim et al. (2005) in the NF of TW. Atra et al. (2005) and Wang and Tang (2011) reported that this is due to the formation of a polarization layer and the fouling phenomenon, which are normally associated with the NF process. According to Chan and Chen (2004) the denatured protein in soybean wastewater could influence the J value, since high temperatures induce its aggregation and deposition on the membrane surface.

Figure 5 - Behavior of permeate flux (J) during nanofiltration process of the tofu whey UF permeate.



The average J value was 54 L h^{-1} m^{-2}, which is in agreement with results reported by Kim et al. (2005), Walha et al. (2011), and Cissé et al. (2011) for the NF of TW (40 L h^{-1} m^{-2}), salted tuna cooking juice (40 L h^{-1} m^{-2}) and roselle extract (50 L h^{-1} m^{-2}), respectively. Mulder (1996) and Baker (2004) observed that the operating conditions directly influenced the NF performance. These authors used similar operation parameters during the NF process, i.e., temperature around 30 °C and pressure around 200 kPa. According to Al-Malack and Anderson (1997), modifications in the pressure, temperature, processing time,

membrane type and pore size, raw material, and volume reduction factor (VRF) affect the behavior of the membrane concentration process.

Total isoflavone content of the concentrate gradually increase up to twice (VRF of 2.5), when compared with TW (Table 1). However, long operation times can change the chemical structure of the isoflavones, by de-esterification reaction, as observed by Barbosa et al. (2006). Low VRF values were hence employed in the study. An increase (p<0.05) in the protein content was observed during NF process. According to Wang and Murphy (1996) this behavior can be attributed to the association of isoflavones with the soybean proteins. Benedetti et al. (2013) also observed an increase in the concentration of the isoflavones content during the NF of an aqueous soybean extract. Similar observations were reported by Kim et al. (2005) for the UF process of TW followed by NF, and by Mello et al. (2010) and Murakami et al. (2011) who applied NF to the concentration of bioactive compounds.

As for the isoflavone profile, malonyl and acetyl glucosides were not detected in the TW. Shao et al. (2009) and Belén et al. (2013) observed different profiles for the isoflavones content of TW. These authors reported that factors such as ionic strength, pH and the endogenous β-glucosidase activity are responsible for differences in the TW isoflavone profile. It is also important to note the high content of β-glucosides and aglycones obtained. According to Grün et al. (2001), the TW isoflavone profile is due to the thermal treatment applied during the tofu processing. These authors also noted that the application of moist heat leads to an increase in the content of the β-glucoside conjugates. However, Jackson et al. (2002) affirmed that certain processing methods, such as boiling, milling, and protein coagulation, applied during tofu production do not change the aglycone profile significantly. Concerning the β-glucoside content during the NF process, only an increase (p<0.05) in the daidzin content was observed, while an increase (p<0.05) in all isomers of aglycone occurred during the concentration process. Nurmi et al. (2002) found that the molar mass of the membrane can influence the separation of the compounds.

Table 1 - Isoflavones content (mg isoflavones kg^{-1} TW) and protein content (mg 100 mL^{-1}) in the tofu whey (TW). Concentrates (CNF) and permeates (P) obtained in the three stages of nanofiltration (VRF 1.5; 2.0; 2.5).

Samples	Aglycones[1]			B-glucosides[1]			Total isoflavones[1,2]	Total proteins
	Daidzein	Genistein	Glycitein	Daidzin	Genistin	Glicitin		
TW	1.05(0.07)^{d}	ND	ND	17.85(0.21)^{d}	14.00(0.28)^{d}	6.10(0.00)^{d}	39.00(0.14)^{d}	1.00(0.05)
CNF 1.5	1.50(0.14)^{c}	ND	ND	21.75(1.06)^{c}	17.70(0.28)^{c}	7.60(0.14)^{c}	48.55(1.34)^{c}	1.06(0.01)
CNF 2.0	2.25(0.07)^{b}	ND	ND	30.85(0.21)^{b}	24.00(0.14)^{b}	10.95(0.35)^{b}	68.55(0.64)^{b}	1.46(0.01)
CNF 2.5	2.55(0.07)^{a}	0.6(0.00)^{a}	ND	36.90(0.71)^{a}	28.35(0.35)^{a}	12.70(0.00)^{a}	81.10(0.99)^{a}	1.76(0.09)
P 1.5	ND	ND	ND	ND	ND	ND	ND	<1.00
P 2.0	ND	ND	ND	ND	ND	ND	ND	<1.00
P 2.5	ND	ND	ND	ND	ND	ND	ND	<1.00

ND: não detectado

[a-d] Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$)

[1] Results expressed as means ± SD

[2] Total of isoflavones expressed as the sum of the aglycones and β-glucosides.

3.2 Freeze concentration (FC)

With regard to the isoflavone content of the TW samples, the values for the concentrate fluids (CF1, CF2, CF3) and ice fractions (I1, I2, I3) for the three stages of FC are shown in Table 2. During the FC cycle the isoflavones content of all concentrates increased gradually (p<0.05), and in CF3 the concentration doubled the initial value, notably the aglycone forms. This behavior was also observed in other studies, in which satisfactory results were obtained for the FC of foods and food compounds, such as fruit juices (Sánchez et al., 2010; Auleda et al., 2011), whey (Sánchez et al., 2011), must (Hernández et al., 2010) and sugars (Raventós et al., 2007). During the FC process a gradually decrease (p<0.05) os isoflavones in the ice fractions was also noted.

Burdo et al. (2008) affirm that the chemical structure of certain substances may interfere with the separation of the concentrate from the ice. Only a small part of the isoflavones, principally aglycones, was retained in the ice. This can be explained probably due to the retention of solids during the FC steps. Sánchez et al. (2010) suggested that this behavior may be due to an increase in the solution viscosity. This concentration increase results in the solutes accumulating at the interface, hindering their movement in the solution, and being retained in the ice fraction. Additionally, phenolic compounds are able to bind to a great number of water molecules through hydrogen bonds. By increasing the concentration of isoflavones in the solution, the interstitial water becomes less available for freezing. As a result the isoflavone content remains in the ice and concentrated fluid fractions. Sánchez et al. (2010) suggested that the size of the solutes could also affect their ability to integrate with the ice fraction. According to Gao and Shao (2009), solutes with a small molecular size can be more easily incorporated into the ice structure than those with large molecules in the freeze concentration, as happened to aglycones. Nurmi et al. (2002) stated that the isoflavones groups have different chemical structures and different molar masses, ranging from 400 to 600 g mol^{-1} for β-glucosides and from 200 to 300 g mol^{-1} to aglycones.

Table 2- Isoflavones content (mg isoflavones kg^{-1} TW) in the tofu whey (TW). Concentrates fluids (CF) and ices (I) of each one three stages of freeze concentration (CF1; CF2; CF3).

Samples	Aglycones[1]			B-glucosides[1]			Total isoflavones[2]
	Daidzein	Genistein	Glycitein	Daidzin	Genistin	Glicitin	
TW	1.05(0.07)^{d}	17.85(0.21)^{c}	14.00(0.28)^{b}	6.10(0.00)^{a}	ND	ND	39.00(0.14)^{c}
CF 1	12.90(0.14)^{c}	8.90(0.14)^{d}	6.15(0.07)^{c}	0.50(0.00)^{d}	0.50(0.00)^{c}	0.50(0.50)^{c}	29.45(0.35)^{d}
CF 2	27.00(1.13)^{b}	21.40(0.85)^{b}	13.10(0.57)^{b}	0.85(0.07)^{c}	1.15(0.07)^{b}	1.10(0.14)^{b}	64.60(2.69)^{b}
CF 3	35.25(0.35)^{a}	25.35(0.07)^{a}	17.65(0.21)^{a}	1.20(0.28)^{b}	1.90(0.28)^{a}	1.60(0.14)^{a}	82.95(0.64)^{a}
I 1	1.90(0.00)^{c}	1.70(0.00)^{c}	0.95(0.07)^{c}	ND	ND	ND	6.05(0.07)^{c}
I 2	5.95(0.21)^{b}	3.45(0.35)^{b}	2.90(0.28)^{b}	ND	ND	ND	13.80(0.85)^{b}
I 3	13.15(0.21)^{a}	7.85(0.21)^{a}	6.00(0.14)^{a}	ND	ND	ND	28.50(0.57)^{a}

ND: não detectado

[a-d] Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$)

[1] Results expressed as means ± SD

[2] Total of isoflavones expressed as the sum of the aglycones and β-glucosides.

4 Conclusions

The results of this study verify that the nanofiltration and the freeze concentration processes are very promising techniques for the concentration of tofu whey, aiming to recover bioactive compounds, such as isoflavones. The UF membrane was efficient for the removal of suspended solids and macromolecules of tofu whey which could cause fouling of the NF membrane. The NF membrane allowed the concentration of isoflavones. Similarly, on applying freeze concentration the isoflavone content gradually increased in the concentrates, particularly the aglycone forms.

References

Aider, M., Ounis, W.B., 2012. Skim milk freeze concentration as affected by the thawing mode: gravitational vs. microwave-assisted. Int. J. Food Sci. Tech. 47, 195-202.

Al-Malack, M. H., Anderson, G. K., 1997. Crossflow microfiltration with dynamic membranes. Water Res. 31, 1969-1979.

Alves, R.C., Almeida, I.M.C., Casal, S., Oliveria, M.B.P.P., 2010. Method development and validation for isoflavones quantification in coffee. Food Chem. 122, 914-919.

AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis of the Association Analytical Chemists, 18th ed. Maryland, USA.

Atra, R., Vatai,G., Bekassy-Molnar, E., Balint, A., 2005. Investigation of ultra- and nanofiltration for utilization of whey protein and lactose. J. Food Eng. 67, 325-332.

Auleda, J.M., Raventós, M., Hernández, E., 2011. Calculation method for designing a multi-plate freeze-concentrator for concentration of fruit juices. J. Food Eng. 107, 27-35.

Baker, R. W., 2004. Membrane Technology and Applications, New York.

Barbosa, A. C. L., Lajolo, F. M., Genovese, M. I., 2006. Influence of temperature, pH and ionic strength on the production of isoflavone-rich soy protein isolates. Food Chem. 98, 757-766.

Benedetti, S., Prudêncio, E.S., Mandarino, J.M.G., Rezzadori, K., Petrus, J.C.C., 2013. Concentration of soybean isoflavones by nanofiltration and the effects of thermal treatments on the concentrate. Food Res. Int. 50, 625-632.

Belén, F., Sánchez, J., Hernández, E., Auleda, J.M., Raventós, M., 2012. One option for the management of wastewater from tofu production: Freeze concentration in a falling-film system. J. Food Eng. 110, 364-373.

Belén, F., Benedetti, S., Sánchez, J., Hernández, E., Auleda, J.M., Prudêncio, E.S., Petrus, J.C.C., Raventós, M., 2013. Behavior of functional compounds during freeze concentration of tofu whey. J.Food Eng.116, 681-688.

Burdo, O.G., Kovalenko, E.A., Kharenko, D.A., 2008. Intensification of the processes of low-temperature separation of food solutions. Appl. Therm. Eng. 28, 311- 316.
Bessarabov, D., Twardowski, Z., 2002. Industrial application of nanofiltration- New perspectives. Membrane Tech. 9, 6-9.

Butylina, S., Luque, S., Nystroma, M., 2006. Fractionation of whey-derived peptides using a combination of ultrafiltration and nanofiltration. J. Membrane Sci. 280, 418-426.

Campos, M.G., Matos, M.P., Câmara, M.T., Cunha, M.M., 2007. The variability of isoflavones in soy seeds and the possibility of obtaining extracts for over the counter tablet preparations that can be standardized. Ind. Crop. Prod. 26, 85-92.

Chan, R., Chen. V., 2004. Characterization of protein fouling on membranes: opportunities and challenges. J. Membrane Sci. 242, 169-188.

Chang, S. K. C., 2002. Isoflavones from soybeans and soy foods., in: J. Shi, G. Mazza., M. Lemaguer (Eds.), Functional Foods: Biochemical and Processing Aspects., Boca Raton, pp. 39-69.

Cissé, M., Vaillant, F., Pallet, D., Dornier, M., 2011. Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (Hibiscus sabdariffa L.). Food Res. Int. 44, 2607-2614.

Gao, W., Shao, Y., 2009. Freeze concentration for removal of pharmaceutically active compounds in water. Desalination. 249, 398-402.

Grün, U. I., Li, K. A., Lin, C. B., Zhang, J., Lakdas, F. N., 2001. Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. J. Agr. Food Chem. 49, 2839-2843.

Hernández, E., Raventós, M., Auleda, J.M., Ibarz, A., 2009. Concentration of apple and pear juices in a multi-plate freeze concentrator. Innov. Food Sci. Emerg.Techol. 10, 348-355.

Jackson, C.J.C., Dini, J.P., Labandier, C., Ruspasinghe, H.P.V., Faulkner, H., Poysa, V., Buzzell, D., DeGrandis, S., 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. Process Biochem. 37, 1117-1123.

Kim, W.J., Kim, H.H., Yoo, S.H., 2005. Ultra and nano-filtration process optimation of isoflavones and oligosaccharides from sunmul. Food Sci. Biotechnol. 14, 380-386.

Konar, N., Poyrazoglu, E.S., Demir, K., Artik, N., 2012. Determination of conjugated and free isoflavones in some legumes by LC–MS/MS. J. Food Compos. Anal. 25, 173-178.

Matsubara, Y., Iwasaki, K., Nakajima, M., Nabetani, H., Nakao, S.,1996. Recovery of oligosaccharides from steamed soybean waste water in tofu processing by reverse osmosis and nanofiltration membranes. Biosci. Biotech.Bioch. 60, 421-428.

Mello, B.C.B.S., Petrus, J.C.C., Hubinger, M.D., 2010. Concentration of flavonoids and phenolic compounds in aqueous and ethanolic propolis extracts through nanofiltration. J. Food Eng. 96, 533-539.

Miyawaki, O., 2003. Freeze concentration., in: D.R. Heldman, (Eds.), Encyclopedia of
Agricultural, Food and Biological Engineering., New York, pp. 385-387.

Mulder, M., 2000. Basic Principles of Membrane Technology, Center for Membrane Science and Technology, Enschede.

Murakami, A.N.N., Amboni, R.D.M.C., Prudêncio, E.S., Amante, E.R., Zanotta, L.M., Maraschin, M., Petrus, J.C.C., Teófilo, R.F., 2011. Concentration of phenolic compounds in aqueous mate (Ilex paraguariensis A. St. Hil) extract through nanofiltration. LWT-Food Sci. Technol. 44, 2211-2216.

Nurmi, T., Mazur, W., Heinomen, S., Hokkonen, J., Adlercreutz, H., 2002. Isoflavone content of the soy based supplements. J. Pharmaceut. Biomed. 28, 1-11.

Prabhakaran, M. P., Perera, C.O., 2006. Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. Food Chem. 99, 231-237.

Raventós, M., Hernández, E., Auleda, J. M., Ibarz, A., 2007. Concentration of aqueous sugar solutions in a multi-plate cryoconcentrator. J. Food Eng. 79, 577- 585.

Sánchez, J., Ruiz, Y., Raventós, M., Auleda, J.M., Hernández, E., 2010. Progressive freeze concentration of orange juice in a pilot plant falling film. . Innov. Food Sci. Emerg.Techol. 10, 11, 644-651.

Sánchez, J., Hernández, E., Auleda, J.M., Raventós, M., 2011. Freeze concentration of whey in a falling-film based pilot plant: Process and characterization. J. Food Eng. 103, 147-155.

Shao, S., Duncan, A.M., Yang, R., Marcone, M.F., Rajcan, I., Tsao, R., 2009. Tracking isoflavones: From soybean to soy flour, soy protein isolates to functional soy bread. J. Funct. Foods I. 119-127.

Sudyani Y., Alawiyaf S., Anita, Y., Adilina I.B., 2007. Characterization of waste water from whey tofu. An. Int. Conf. Chem. Sci. ANL 47-6.

Uzzan, M., Labuza, T.P., 2004. Critical Issues in R&D of Soy Isoflavone- enriched Foods and Dietary Supplements. J. Food. Sci. 69, 77-86.

Vacek, J., Klejdus, B., Lojková, L., Kubán, V., 2008. Current trends in isolation, separation, determination and identification of isoflavones: A review. J. Separ. Sci. 31, 2054 -2067.

Van Der Bruggen, B., Mänttäri, M., Nyström, M., 2008. Drawbacks of applying nanofiltration and how to avoid them: A review. Sep. Purif. Technol. 63, 251–263.

Walha, K., Ben Amar, R., Massé, A., Bourseau, P., Cardinal, M., Cornet, J., Prost, C., Jaouen, P., 2011. Aromas potentiality of tuna cooking juice concentrated by nanofiltration. LWT-Food Sci. Technol. 44, 153-157.

Wang, H.J., Murphy, P.A., 1996. Mass balance study of isoflavone during soybean processing. J. Agr.Food Chem. 44, 2377-2383.

Wang, Y.N., Tang, C.Y., 2011. Protein fouling of nanofiltration, reverse osmosis, and ultrafiltration membranes- The role of hydrodynamic conditions,solution chemistry, and membrane properties. J. Membrane Sci. 376, 275-282.

CAPÍTULO 3
Propriedades antioxidantes do soro de tofu concentrado através dos processos de crioconcentração e nanofiltração

Propriedades antioxidantes do soro de tofu concentrado através dos processos de crioconcentração e nanofiltração

Resumo

Um estudo foi realizado visando agregar valor ao soro de tofu, aumentando o seu potencial como fonte de isoflavonas que são associadas com a atividade antioxidante da soja. Os ensaios de concentração foram realizadas usando os processos de crioconcentração em blocos e nanofiltração . Os concentrados obtidos foram avaliados quanto ao seu teor de isoflavonas e atividade antioxidante. O fluido concentrado mostrou um aumento no teor de isoflavonas em as etapas da crioconcentração. Embora as etapas subsequentes tenham alcançado menor eficácia que a primeira, devido à retenção de compostos fenólicos no gelo, a recuperação manteve-se maior que 80 % em todas as fases. Empregando o processo de nanofiltração, os β-glicosídeos e malonil glicosídeos presentes no soro de tofu foram concentrados ($p < 0,05$), o que não ocorreu com as agliconas. Os valores para a atividade antioxidante dos fluidos concentrados determinados pelos métodos FRAP e ABTS, para cada etapa da crioconcentração e da nanofiltração, foram significativamente mais elevados do que no soro de tofu inicial. Além disso, os valores obtidos aplicando os dois métodos antioxidantes foram significativamente correlacionados com o teor de isoflavonas das amostras de soro de tofu.

Palavras-chave: isoflavona, soro de tofu, crioconcentração, nanofiltração, atividade antioxidante.

Antioxidant properties of tofu whey concentrate by freeze concentration and nanofiltration processes

Abstract

A research study was conducted aimed at add value to tofu whey by enhancemen its potential as a source of isoflavones which are associated with antioxidant activity. The concentration assays were carried out using block freeze concentration and nanofiltration processes. The concentrates obtained were evaluated to determine their isoflavone content and antioxidant activity. The concentrated fluid showed an increase in the isoflavone content for all freeze concentration stages. Although the process efficiency reduced significantly compared with the first stage, due to the retention of phenolic compounds in the ice, it remained at > 80% in all stages. The β-glucosides and malonyl glucosides present in the tofu whey were concentrated by nanofiltration process ($p < 0.05$), but not the aglycones. Antioxidant activity of the concentrated fluid obtained from each freeze concentration and nanofiltration stage, measured by FRAP and ABTS assays, were significantly higher than that of the tofu whey. Moreover, the values obtained by both antioxidant assays were significantly correlated with the isoflavone content of the tofu whey samples.

***Keywords**:* isoflavone, tofu whey, freeze concentration, nanofiltration, antioxidant activity.

1 Introduction

Soybean (*Glycine max* L. Merr.) is one of the most important legumes consumed and has long been used as a protein source in Asian countries (Anderson and Wolf, 1995; Yang et al., 2000). It contains several nutrients and bioactive compounds including phytic acids, saponins, oligosaccharides and isoflavones (Anderson and Wolf, 1995; Kwak et al., 2007). In addition, it has been an excellent source of vegetable oil in Latin America countries (Hirakuri and Lazzarotto, 2011). Soybeans can be commercialized as grains or as processed foods such as tofu (Easaki et al., 1999; Kwak et al., 2007; Kim et al., 2008).

Tofu is produced through the coagulation of soymilk and during the processing a liquid by-product called tofu whey (TW) or *sunmul* is generated. The inappropriate discharge of TW in effluents can result in serious environmental problems due to its high organic matter content. Moreover, this residue contains notable concentrations of low molar mass substances, such as isoflavones (Kim et al., 2005; Chung et al., 2006).

It is well known that the main source of the biological activity of soybean food products is isoflavones (Pratt and Birac, 1979), since they exhibit estrogenic, antioxidant, antiosteoporotic and anticarcinogenic activity (Cornwell et al., 2004). Many studies have demonstrated that soybean food products, including non-fermented products, present antioxidant activity (Easaki et al., 1998; Kwak et al., 2007; Kim et al., 2008). Yang et al. (2000), Shon et al. (2007) and Devi et al. (2011) demonstrated that soybean has antioxidant capacity and this fact stimulated the development of health products with functional properties. However, few studies have been carried out to evaluate the antioxidant activity of tofu whey.

It is important to consider that to attain advances in the bio-food industry, effective, low-cost and environmentally-friendly technologies that preserve the functional properties need to be found (Aider, de Halleux and Melnikova, 2009). Reports in the literature have shown that it is possible and advantageous to concentrate the phenolic compounds in vegetal extracts through freeze concentration (Boaventura et al.,2013; Belén et al., 2013) and nanofiltration (Conidi, Cassano, and Drioli, 2011 and Cassano et al., 2013), both processes being able to preserve the biological activity.

The freeze concentration process promotes the concentration of liquid food products by means of freezing with part of the frozen water being further separated from the liquid product (Belén et al., 2013). This

technology has been considered promising for the concentration of functional compounds (Aider and Halleux, 2009). Similarly, the use of nanofiltration to concentrate functional compounds from aqueous streams is also an area of growing interest (Cassano et al., 2013). As described by Conidi, Cassano and Drioli (2011), membrane processes, such as nanofiltration, can operate under mild conditions of temperature, pressure and shear stress and therefore the biological activity and the properties of the original product can be preserved. In addition, solvent extraction is not required.

Considering the positive aspects of the freeze concentration and nanofiltration technologies, the aim of this study was to evaluate the feasibility of these processes to enhance the isoflavones content and antioxidant activity of tofu whey concentrates.

2 Material and methods

2.1 Materials

Tofu whey was supplied by Tofutura Indústria de Alimentos Ltda (Campo Largo, Paraná, Brazil). All reagents used were of analytical grade.

2.2 Preparation of tofu whey

Firstly, the tofu whey (TW) was microfiltered in a pilot filtration unit, using tangential flow and an organic polyimide membrane (PAM Membranas Seletivas, Rio de Janeiro, RJ, Brazil) in a hollow fiber configuration, with an average cross-section diameter of 0.4 μm and a filtration area of 0.7 m^2. The following operating conditions were used in the microfiltration (MF): temperature 22 ±1 °C; transmembrane pressure 1 bar; and tangential velocity 1.0 m/s. MF was used to remove compounds of large molar mass, as recommended by Matsubara et al. (1996). The permeate obtained was used as input in the nanofiltration and freeze concentration processes.

2.3 Protocol for the freeze concentration procedure

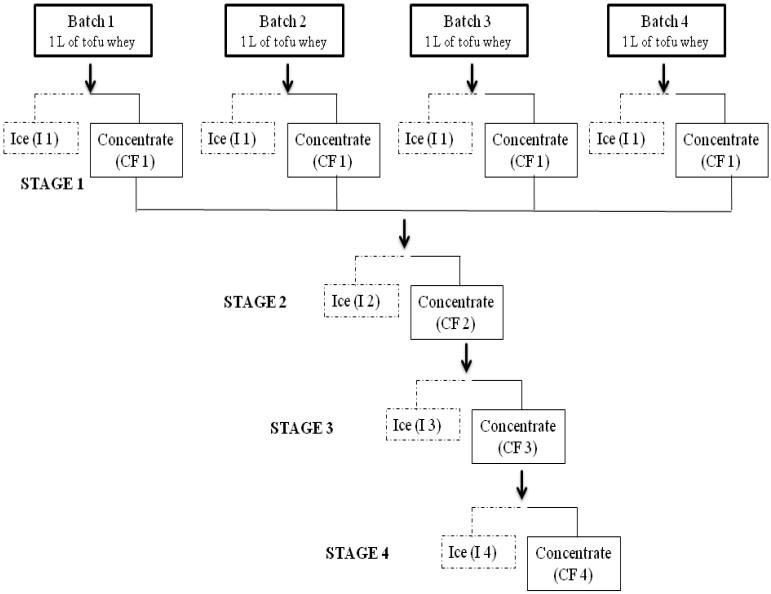
The freeze concentration technique applied was the progressive process, also referred to as block freeze concentration. The experiment was performed according to the methodology proposed by Aider and Ounis (2012), with some modifications. The principle of this method is

based on the total freezing of a food solution followed by a partial defrosting procedure under simple gravitational separation. This method makes it possible to obtain two fractions: the first one is the concentrated fluid (C) and the second one is the ice (I) (Aider, Halleux and Akbache, 2007). A diagram of the freeze concentration procedure applied, is shown in Fig. 1. An initial volume of 4 L of microfiltered tofu whey was divided into four batches of 1 L and then frozen at –24 ± 2 °C. The freezing process was carried out in a freezer unit (Bosch Space REBS37, São Paulo, SP, Brazil) by indirect cooling. Since the feed solution was frozen, 50 % of the initial volume was defrosted at room temperature (17 ± 2 °C). The defrosted liquid consisted in the concentrated fluid of the first freeze concentration stage. This concentrated fluid, obtained in the first stage, was frozen at -24 ± 2 °C and was used as the feed solution in the second stage. At the end of the second freeze concentration stage, 50 % of the frozen solution was defrosted, collected and frozen again. This procedure was repeated in the third and fourth freeze concentration stages. Each concentrate of a given stage was frozen at -24 ± 2 °C and used as the feed solution for the following stage. Samples of ice remaining from each freeze concentration stage and amples of each concentrated fluid was stored at -24 ± 2 °C for further chemical analysis.

2.3.1 Total dry matter

The total dry matter (DM) content of all samples was determined by measuring the weight loss after drying in an oven at 80 °C to constant weight and expressed as dry matter content⁄ total weight (g/100 g) (AOAC, 2005). Analysis of the ice and concentrated fluids was performed in triplicate.

Figure 1- Flowchart of the four concentration stages of TW in the block freeze concentration process.



2.3.2 Freeze concentration performance

The concentration factor (CF) at each freeze concentration stage was calculated in accordance with the methodology proposed by Aider and Ounis (2012), as ratio between the concentration of the solution and the total dry matter content in the original tofu whey. The concentration factor (CF) (%) was obtained as follows:

$$CF = \frac{DM_n}{DM_O} \times 100 \quad (1)$$

where DM_n is the total dry matter content (g) of the concentrated fluid in each freeze concentration stage and DM_0 is the total dry matter content (g) of the original tofu whey.

As described by Belén et al. (2012), the efficiency of the freeze concentration process was determined by the increase in the isoflavone content (IC) of the concentrated fluid with respect to the IC remaining in the ice, as calculated through the following equation:

$$PE = \frac{PCC_n - PCI_n}{PCC_n} \times 100 \quad (2)$$

where PCC_n is the IC of the concentrated fluid (mg) in a given freeze concentration stage and PCI_n is the IC of the ice (mg) in a given freeze concentration stage.

2.4 Nanofiltration process (NF)

The microfiltered tofu whey was concentrated by nanofiltration (NF) using a tangential flow filtration pilot plant equipped with a poly-viny-lidene difluoride (PVDF) filter in the spiral configuration with a molecular weight cut-off (MWCO) ranging between 150-300 g/mol and effective filter area of 0.9 m^2 (GE Osmonics®, Philadelphia, USA). The experiments were carried out in duplicate at 17 ± 2 ºC and with a transmembrane pressure of 6 bar until reaching a volume reduction factor (VRF) of 4. The VRF was calculated as the ratio between the initial volume (L) of the tofu whey used in the feed and the final volume (L) of the concentrate after NF. The permeate flux (J) (L/h m^2) during NF can be calculated as follows:

$$J = \frac{V_p}{t \times A_p} \quad (3)$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and A_p (m^2) is the permeation surface area of the membrane. The efficiency of the filtration process was measured by the isoflavone content in the concentrate. After each experiment, the equipment was cleaned with alkaline solution (0.1%) according to the manufacturer's instructions.

2.5 Isoflavone extraction and quantification

The extraction of isoflavones from the tofu whey samples and the quantification of their components were carried out according to the methodology proposed by Carrão-Panizzi, Favoni, and Kikuchi (2002) with modifications. For the extraction, a 1.5 mL aliquot of the tofu whey

sample was added to 2.5 mL of the extracting solution (70% ethanol and 0.1% acetic acid) in a test tubes. These tubes were stirred in a vortex (Model MA162, Marconi®, Piracicaba, SP, Brazil) and submitted to extraction for 1 h at room temperature (25 °C) with stirring every 15 min. The test tubes were then placed in an ultrasound bath (Model USC5000, Unique®, Indaiatuba, SP, Brazil) for 30 min. A 1.5 mL aliquot of this extract was transferred to a refrigerated microcentrifuge (dimensions of 31 x 60 x 25 cm, 35 kg, Model 5417R 230 V/50 Hz, Eppendorf®, São Paulo, Brazil) and centrifuged at 20,800 *g* for 15 min at 5 °C. The supernatant was filtered through 0.45 µm filters (Millipore®, Billerica, MA, USA) and 20 µL was used to analyse the isoflavones by high performance chromatography.

The separation and quantification of the isoflavones were performed using HPLC, as proposed by Berhow (2002), with a photodiode array detector (Model 996) and an automatic sample injector (Model 717 Plus), both manufactured by WATERS® (Milford, USA). In this stage, a reverse phase column (YMC-Pack ODS-AM, C18, S-5 µm, diameter of 250 x 4.6 mm) was used. For the separation of isoflavones, the binary linear gradient system was used and the mobile phases were: (a) methanol containing 0.025% trifluoroacetic acid (TFA) (Phase A) and (b) ultrapure water (Millipore®, Billerica, MA, USA) containing 0.025% TFA (Phase B). The initial condition of the gradient was 20% of Phase A, reaching 90% in 35 min, followed by cleaning of the column with 100% of Phase A for 5 min and subsequently returning to 20% and then mantaining these conditions for to 60 min. The mobile phase flow rate was 1 mL min^{-1} and the temperature during the analysis was 25 ºC. For the isoflavone detection the wavelength of the detector was set at 254 nm. The software used to control the equipment and for the data acquisition was Millennium 32 (version 3.05.01) (GCLC® Toronto, Pickering, ON, Canada). For the identification and quantification of the peaks corresponding to each one of the isoflavones, calibration curves obtained by linear regression based on the peak areas were used. These calibration curves were constructed using external standards of daidzin, daidzein, genistin, genistein, glycitin, glycitein, malonyl daidzin, malonyl genistin, malonyl glicitin, acetyl daidzin, acetyl genistin and acetyl glycitin. All standards were solubilized in methanol (chromatographic grade) to give the following concentrations: 0.00625 mg/mL, 0.0125 mg/mL, 0.0250 mg/mL, 0.0500 mg/mL and 0.1000 mg/mL. The results for the isoflavones were expressed as mg of isoflavones per liter of tofu whey (mg/L). All analysis were performed in triplicate.

2.6 Determination of antioxidant activity

2.6.1 Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996). The FRAP reagent was freshly prepared from 300 mM acetate buffer, pH 3.6, with 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) made up in a solution comprised of 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$. All three solutions were mixed together in a ratio of 10:1:1 (v/v/v). A 40-µL aliquot of each sample (with appropriate dilution) was added to 1.2 mL of the FRAP reagent. The absorption of the reaction mixture was measured at 593 nm after 2 min incubation at 37 °C. Measurements were performed in triplicate. Fresh working solutions of known Fe (II) concentrations ($FeSO_4 \cdot 7H_2O$) of 0–2 mM were used for the calibration. The antioxidant capacity based on the ability to reduce the ferric ions in the samples was calculated from the linear calibration curve and expressed as Trolox equivalent in µmol per mL of tofu whey.

2.6.2 ABTS•+ radical cation decoloration assay

The ABTS free radical-scavenging activity of each sample was determined according to the method described by Re et al. (1999). The $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate; the mixture must stand in the dark at room temperature for 16 h before use. A working solution was diluted with ethanol to an absorbance of 0.70 (±0.02) nm (constant initial absorbance value used for standard and samples) at 734 nm and 30°C. An aliquot (30 µL) of each sample or Trolox standard was mixed with the working solution (3 mL) of $ABTS^{\bullet+}$, and the decrease in the absorbance was measured after 6 min at 734 nm using a Shimadzu LC-20AT system (Shimadzu, Kyoto, Japan) equipped with a UV-visible detector with DAD (Shimadzu, SPD-M20A, Kyoto, Japan). Measurements were performed in triplicate. The ABTS•+scavenging rate was calculated to express the antioxidant ability of the sample and the results were expressed in terms of Trolox equivalent antioxidant capacity (µmol per mL of tofu whey).

2. 7 Statistical analysis

The results were reported as the mean (± standard deviation) of at least three experiments. The significance of the difference between the results obtained for the various treatments was determined by analysis of variance (ANOVA), followed by the Tukey test. A p value of < 0.05 was considered to be statistically significant. Pearson's correlation test was used to determine the correlation between the antioxidant activity determined applying the two independents tests (ABTS and FRAP) and the isoflavone contents. The data analysis was carried out using STATISTICA 7.0 software (2004) (StatSoft Inc., Tulsa, Ok, USA).

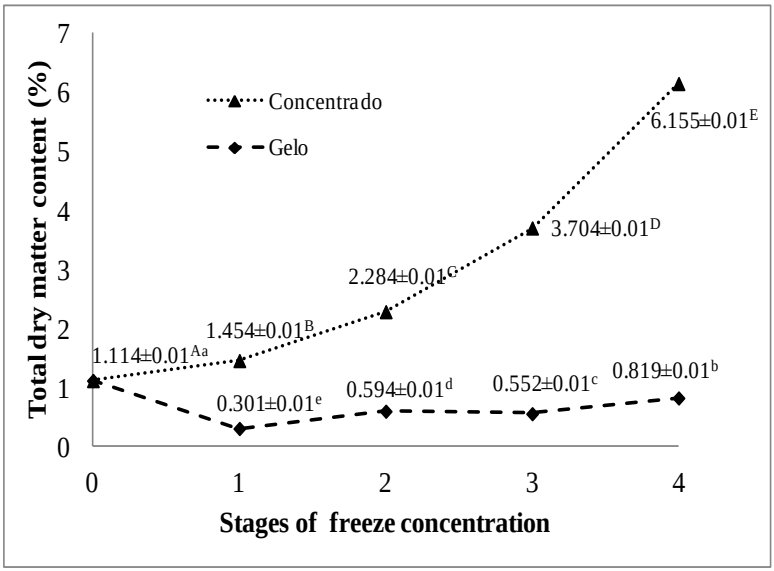
3 Results and discussion

3.1 Freeze concentration (FC) process

As described by Belén et al. (2013), for the concentration of the tofu whey (TW) isoflavones the freeze concentration (FC) process was conducted in four stages. Shimoni (2009) and Alothman, Bhat and Karim (2009) reported that shorter processing times provide ideal conditions for preserving the phenolic compounds, which showed functional properties. Belén et al. (2013) and Moreno et al. (2014) noted that the FC process is able to maintain the functional properties because of the use of low temperatures.

During the FC, the total dry matter (DM) content of the concentrated fluids increased ($p < 0.05$) (Fig. 2), resulting in an increase in their concentration factor (CF) ($p < 0.05$). The CF values were 130.52(±1.30)%, 205.02(±1.10)%, 332.49(±2.10)% and 552.51(±3.34)% for the first, second, third and fourth stages, respectively. The same behavior was observed by Aider, Halleux and Akbache (2007) for whey proteins and by Boaventura et al. (2013) for aqueous extracts of mate, both submitted to FC. These authors obtained CF values of up to 500 %. On the other hand, a decrease in the DM values was observed for the remaining ice in the first three stages (I1, I2 and I3). However, the DM increased in the fourth stage (I4). The increase in the DM content of the ice can be attributed to the high content of total solids entrapped in the ice fractions, as detected by Aider and Ounis (2012) and Boaventura et al. (2013), in the final stages of FC.

Figure 2- Total dry matter content evolution of the ice and concentrated fluids as a function of the freeze concentration stages.



Data are expressed as mean±SD (n=3) of the total dry matter content in the concentrated fluid and ice of each freeze concentration stage. Different superscript uppercase letters indicate significant difference ($p < 0.05$) between the feed extract and the concentrated fluid of each freeze concentration stage. Different superscript lowercase letters indicate significant difference ($p < 0.05$) between the feed extract and the ice of each freeze concentration stage.

As verified for the DM values observed during the FC process, the isoflavone content (IC) of the concentrated fluids (C1, C2, C3 and C4) and ice fractions (I1, I2, I3 and I4) also increased ($p < 0.05$) (Table 1). Boaventura et al. (2013) noted that the phenolic compounds of the aqueous extract of mate also increased during the FC process. These authors associated this finding with the hydrogen bonds, since the phenolic compounds are able to bind a great number of water molecules. Thus, during the concentration of isoflavones in a solution, the interstitial water becomes less available for freezing. For this reason, the process efficiency was reduced, as also observed in the fourth stage (Table 1).

Table 1- Isoflavone content (IC) (mg isoflavone/ L tofu whey) of the feed tofu whey, concentrated fluid and ice at each freeze concentration stage and the process efficiency in relation to IC.

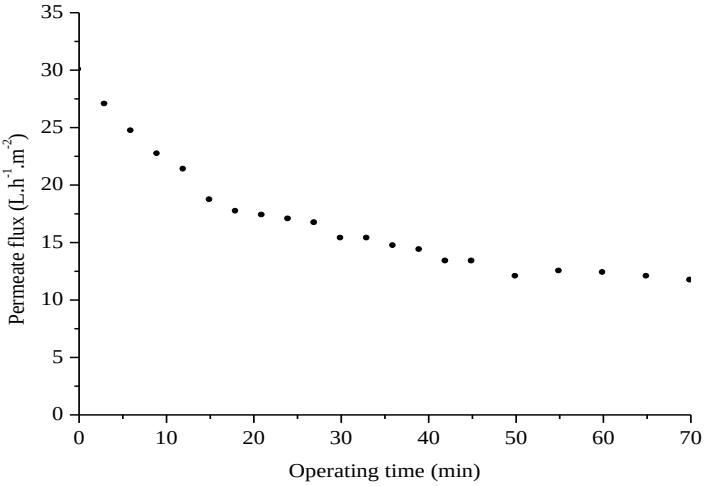
Samples	β-glucosides			Malonil glucosides			Aglycones			Total	Efficiency process (%)
	G-dai	G-gli	G-gen	M-dai	M-gli	Mgen	Dai	Gli	Gen		
Initial TW	2.02^{e} ±0.04	4.07^{e} ±0.12	1.13^{e} ±0.02	7.70^{e} ±0.20	9.73^{c} ±0.10	12.78^{d} ±0.10	4.01^{c} ±0.06	12.43^{c} ±0.24	1.69^{b} ±0.02	55.74^{e} ±0.78	-
C1	6.72^{d} ±0.05	5.84^{d} ±0.05	5.32^{d} ±0.08	10.64^{d} ±0.12	9.41^{c} ±0.17	11.80^{d} ±0.23	1.61^{d} ±0.06	8.43^{d} ±0.21	0.92^{c} ±0.01	60.67^{d} ±0.98	81,44 ±0.87
C2	4.99^{c} ±0.09	8.60^{c} ±0.05	3.61^{c} ±0.07	21.95^{c} ±0.47	19.33^{c} ±0.27	25.73^{c} ±0.13	5.94^{b} ±0.09	15.24^{b} ±0.00	1.60^{b} ±0.06	107.00^{c} ±1.23	83,59 ±1.12
C3	7.92^{b} ±0.14	11.76^{b} ±0.05	4.73^{b} ±0.18	27.78^{b} ±0.85	24.83^{b} ±0.08	32.84^{b} ±0.50	6.27^{b} ±0.08	16.22^{b} ±0.72	1.51^{b} ±0.03	133.85^{b} ±2.62	87.41 ±0.75
C4	11.62^{a} 0.08	18.04^{a} ±0.46	7.91^{a} ±0.01	44.12^{a} ±0.36	38.94^{a} ±0.26	52.63^{a} ±1.76	9.56^{a} ±0.15	34.32^{a} ±0.54	2.19^{a} ±0.09	219.33^{a} ±3.18	83.62 ±1,23
I1	1.70^{C} ±0.03	1.51^{D} ±0.04	1.43^{C} ±0.01	1.12^{C} ±0.04	1.71^{D} ±0.03	1.37^{D} ±0.04	0.49^{C} ±0.01	1.55^{C} ±0.01	0.39^{C} ±0.01	11.26^{C} ±0.05	-
I2	2.26^{B} ±0.00	2.70^{B} ±0.02	1.73^{B} ±0.06	1.45^{BC} ±0.01	2.04^{C} ±0.02	1.89^{C} ±0.03	1.31^{A} ±0.03	3.24^{B} ±0.12	0.92^{A} ±0.03	17.56^{B} ±0.14	-
I3	1.03^{D} ±0.01	1.61^{C} ±0.00	0.55^{D} ±0.05	1.96^{B} ±0.05	3.00^{B} ±0.01	3.16^{B} ±0.01	1.19^{B} ±0.01	3.39^{B} ±0.11	0.69^{B} ±0.03	16.59^{B} ±0.12	-
I4	4.88^{A} ±0.02	4.16^{A} ±0.05	3.91^{A} ±0.04	5.60^{A} ±0.16	5.37^{A} ±0.09	5.70^{A} ±0.19	1.09^{B} ±0.02	4.49^{A} ±0.01	0.72^{B} ±0.00	35.92^{A} ±0.32	-

Data are expressed as mean±SD (n=3). ^{a,b} Different superscript lowercase letters indicate significant difference ($p < 0.05$) between the feed tofu whey and the concentrated fluid of each freeze concentration stage. ^{A,B}Different superscript uppercase letters indicate significant difference ($p < 0.05$) between the feed tofu whey and the ice of each freeze concentration stage; CF, concentrated fluid; I, ice.

3.2 Nanofiltration (NF) process

As expected, a decrease in the permeate flux (J) was observed over time (Fig. 3). This behavior is similar to that observed by Wang and Tang (2011) and Ranamukhaarachchi, Meissner, and Moresoli (2013) during the membrane filtration of soy protein and protein solutions, respectively. These authors attributed this fact mainly to concentration polarization, a phenomenon which typically occurs during the membrane filtration process. Noordman et al. (2003) reported that liquid protein foods, such as TW, tend to exhibit this phenomenon during the membrane filtration process. According to Kelly, Opong, and Zydney (1993), the deposition of large protein aggregates on the membrane surface commonly occurs during this process. The decrease in the J value is greater in the case of proteins that contain a free thiol group, which, according to Kelly and Zydney (1997), is responsible for the formation of the intermolecular thiol-disulfide bonds among the protein molecules, and the resulting protein aggregates can not pass through the membrane.

Figure 3- Permeate flux (J) during nanofiltration of tofu whey.



The average J value obtained in this study was around 17.3 L/h m^2. It is noteworthy that this value was higher than that obtained by

Murakami et al. (2011) for an aqueous mate extract (4.53 L/h m^2), where similar operating conditions, such as pressure and type of membrane, were applied. However, similar J values (between 10 and 20 L/h m^2) were observed by Mello et al. (2010) in the concentration of phenolic compounds in an aqueous solution of propolis.

Isoflavone content increased in the NF concentrates ($p < 0.05$) with an increase in the volume reduction factor (VRF), and were higher than the corresponding values obtained for the TW (Table 2).

Table 2- Isoflavone content (IC) (mg isoflavone/L tofu whey) of the feed tofu whey and concentrated at each VRF of nanofiltration.

Samples	β-glucosides			Malonil glucosides			Aglycones			Total
	G-dai	G-gli	G-gen	M-dai	M-gli	Mgen	Dai	Gli	Gen	
Initial TW	2.02^{d} ±0.04	4.07^{d} ±0.12	1.13^{d} ±0.02	7.70^{d} ±0.20	9.73^{d} ±0.10	12.78^{d} ±0.10	4.01^{a} ±0.06	12.43^{a} ±0.24	1.69^{a} ±0.02	55.74^{d} ±0.78
FRV 2	13.97^{c} ±0.25	10.21^{c} ±0.21	10.81^{c} ±0.07	22.30^{c} ±0.53	20.65^{c} ±0.07	33.94^{c} ±0.14	1.11^{b} ±0.04	4.43^{d} ±0.01	0.26^{b} ±0.04	117.68^{c} ±0.94
FRV 3	18.47^{b} ±0.21	13.06^{b} ±0.03	13.51^{b} ±0.13	25.82^{b} ±2.05	25.94^{b} ±0.43	40.89^{b} ±0.96	1.07^{b} ±0.08	6.18^{c} ±0.00	0.32^{b} ±0.02	145.27^{b} ±3.71
FRV 4	28.10^{a} ±0.45	19.32^{a} ±0.25	19.16^{a} ±0.37	40.53^{a} ±1.24	39.46^{a} ±0.44	61.27^{a} ±1.82	1.17^{b} ±0.14	7.24^{b} ±0.00	0.38^{b} ±0.00	216.63^{a} ±3.93

Data are expressed as mean±SD (n=3).

[a,b]Different superscript lowercase letters indicate significant difference ($p < 0.05$) between the feed tofu whey and the concentrated fluid of each VFR of nanofiltration.

Based on the results obtained it was verified that a VRF of 4 showed the best concentration performance for TW applying the NF process, presenting the highest IC value ($p < 0.05$). In relation to the isoflavone forms, the greatest increase was observed for the conjugates ($p < 0.05$), while the aglycones decreased ($p < 0.05$). However, isoflavones were not detected in the permeate. According to Schausberger et al. (2009), this is due to two mechanisms, i.e., deposition or adsorption on the membrane surface resulting from the interactions between the proteins and isoflavones. According to Siebert (1999) and Speroni et al. (2010), during the processing of tofu the isoflavones may be involved in different types of interactions with proteins because of their diverse polarity and hydrophobicity characteristics, as well as their ability to form hydrogen bonds. On the other hand, Garem et al. (1998) noted that the presence of high molar mass compounds, such as proteins and peptides, in the concentration polarization layer could influence the selectivity of the NF membrane as well as the accumulation and/or adsorption of these proteins at the membrane surface. This phenomenon would amplify the membrane charge density and, in turn, affect the transmission of smaller positive or negative species. Finally, the deposition or adsorption of isoflavones on the membrane may occur through their binding with soy proteins.

3.3 Evaluation of antioxidant activity (FRAP and ABTS assays)

The antioxidant activity of soybean products has been previously investigated through numerous studies (Yang et al., 2000; Shon et al., 2007; Moktan, Saha, and Sarkar, 2008; Kim et al., 2008; Devi et al., 2009; Chaiyasut et al., 2010). The results for the antioxidant activity of the TW and the concentrated fluids of each FC and NF stage, determined via ABTS and FRAP assays, are shown in Table 3. An improvement ($p < 0.05$) in the antioxidant potential was observed for all stages of the FC and NF processes. The maximum values obtained for the final concentrates obtained from the FC and NF, respectively, were 0.25 and 0.30 μmol/mL for FRAP, and 1.99 and 1.68 μmol/mL for ABTS. Conidi, Cassano and Drioli (2011) and Cassano et al. (2013) also verified an increase in the antioxidant activity for vegetal extracts concentrated applying the NF process. The same behavior was noted for the concentrates obtained for all FC stages, similarly to the results obtained by Boaventura et al. (2013) for the FC of aqueous extract of mate.

Chaiyasut et al. (2010) stated that the antioxidant activity of soybean is related mainly to the presence of isoflavones. Therefore, the correlation between the total isoflavone contents and the FRAP and ABTS values was investigated. A correlation ($p < 0.05$) was found between the IC and the antioxidant activity for the concentrates obtained from both the FC (r^2=0.872 and r^2=0.939) and NF processes (r^2=0.986 and r^2=0.968). With regard to the isoflavone isomers, it was verified that during the FC an increase ($p < 0.05$) in the aglycones content occurred, and correlations with the FRAP and ABTS values (r^2=0.71 and r^2=0.81, respectively) were observed. A similar finding was reported by Izumi et al. (2000) and Rao and Muralikrishna (2002), who found that aglycones have a higher antioxidant activity than their conjugated form. Other studies have shown that the aglycone form can present higher antioxidant activity against ABTS, higher ABTS radical scavenging activity, and a more prolonged lag time for low-density lipoprotein oxidation than its conjugates, such as malonyl, acetyl and β-glucosides (Ruiz-Larrea et al., 1997; Takahashi et al., 2005). It is noteworthy that for both processes an increase ($p < 0.05$) was also observed in the malonyl glucosides concentration (Tables 1 and 2). In addition, the malonyl glucosides were also correlated ($p < 0.05$) with the antioxidant activity observed for the FC and NF concentrates, determined by FRAP (r^2=0.89 and r^2=0.98, respectively) and ABTS (r^2=0.94 and r2=0.97, respectively). As noted by Fleury et al. (1992) and Kwak, Lee and Park (2007), malonyl glucosides can exhibit strong antioxidant activity.

Table 3 - Antioxidant activities of tofu whey and concentrates obtained by freeze concentration (FC) and nanofiltration (NF) processes determined by ABTS cation radical scavenging and FRAP method.

Samples		FRAP (µmol/ mL)	ABTS (µmol /mL)
FC concentrated	Initial TW	0.03^{c}±0.01	0.75^{e}±0.04
	CF1	0.11^{b}±0.01	1.17^{d}±0.05
	CF2	0.13^{b}±0.01	1.28^{c}±0.05
	CF3	0.24^{a}±0.01	1.64^{b}±0.03
	CF4	0.25^{a}±0.02	1.99^{a}±0.03
NF concentrated	Initial TW	0.03^{c}±0.01	0.66^{d}±0.02
	NF2	0.12^{b}±0.01	1.26^{c}±0.01
	NF3	0.14^{b}±0.01	1.39^{b}±0.04
	NF4	0.30^{a}±0.03	1.68^{a}±0.05

Data are expressed as mean±SD (n=3).
^{a,b}Different superscript lowercase letters indicate significant difference ($p < 0.05$) between the feed tofu whey and the concentrated fluid of freeze concentration stage and each VFR of nanofiltration.

Finally, the results showed that, in the case of tofu whey, FC and NF can be considered as good alternatives for isoflavone concentration and for improving the antioxidant activity. This provides a means to reuse and add value to a liquid by-product from the soybean industry, since the isoflavones present in the soybean are bioactive compounds of major importance.

4 Conclusions

The freeze concentration process promoted a high concentration factor for the total dry matter content of tofu whey. The concentrated fluid showed an increase in the isoflavone content, for all freeze concentration stages, mainly the fourth stage. Although the process efficiency decreased in the fourth stage, due to the high retention of isoflavones in the ice, it remained at > 80% in all four stages. Similarly, the concentrate obtained through nanofiltration presented high isoflavone content, mainly in the volume reduction factor equal to 4. The antioxidant activity determined applying the FRAP and ABTS

methods increased after all freeze concentration and nanofiltration stages, and was mainly correlated to the aglycone and malonyl gucosides present in the tofu whey.

References

Aider, M., de Halleux, D., Akbache, A., 2007. Whey cryoconcentration and impact on its composition. J Food Eng. 82, 92-102.

Aider, M., de Halleux, D., 2009. Cryoconcentration technology in the bio-food industry: Principles and applications. LWT- Food Sci Technol. 42, 679-685.

Aider, M., de Halleux, D., Melnikova, I., 2009. Skim milk whey cryoconcentration and impact on the composition of the concentrated and ice fractions. Food Bioprocess Technol. 2, 80-88.

Aider, M., Ounis, W. B., 2012. Skim milk cryoconcentration as affected by the thawing mode: Gravitational vs. microwave-assisted. Int J Food Sci Technol. 47, 195-202.

Alothman, M., Bhat, R., Karim, A.A., 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food SciTech.20, 201-212.

Anderson, R. L. and Wolf, W. J., 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J Nutr. 125, 581-588.

Belén, F., Sánchez, J., Hernández, E., Auleda, J. M., & Raventós, M., 2012. One option for the management of wastewater from tofu production: Freeze concentration in a falling-film system. J Food Eng. 110, 364-373.

Belén, F., Benedetti, S., Sánchez, J., Hernández, E., Auleda, J.M., Prudêncio, E.S., Petrus, J.C.C., Raventós, M., 2013. Behavior of functional compounds during freeze concentration of tofu whey. J Food Eng. 116, 681–688.

Benzie, I. F., Strain, J. J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem. 239, 70-76.
Berhow, M.A., 2002. Modern analytical techniques for flavonoid determination. In B.S. Buslig, and J.A. Manthey (Eds.). Flavonoids in the living cell. Advances in experimental medicine and biology, New York: Klusher academic, 61-76.

Boaventura, B.C.B., Murakami, A.N.N., Prudêncio, E.S., Maraschin, M., Murakami, F.S., Amante, E.R., Amboni, R.D.M.C, 2013. Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (*Ilex paraguariensis* A. St. Hil.) through freeze concentration technology. Food Res Int. 686–692.

Carrão-Panizzi, M.C., Favoni, S.P.G., Kikuchi, A., 2002. Extraction time for soybean isoflavone determination. Braz Arch Biol Technol. 45, 515-518.

Cassano, A., Conidi, C., Giorno, L., Drioli, E., 2013. Fractionation of olive mill wastewaters by membrane separation techniques. J Hazard Mater. 248-249, 185- 193.

Chaiyasut, C., Kumar, T., Tipduangta, P., Rungseevijitprapa, W., 2010. Isoflavone content and antioxidant activity of Thai fermented soybean and its capsule formulation. Afr J Biotechnol. 9, 4120-4126.

Chung, H.J., Eom, K.Y., Kim, W.J., 2006. Evaluation of physicochemical properties of muffins made with ultrafiltered sunmul powder. J Food Sci Nutr. 11, 333-338.

Conidi, C., Cassano, A., Drioli, E., 2011. A membrane-based study for the recovery of polyphenols from bergamot juice. J Membrane Sci. 375, 182-190.

Cornwell, T., Cohick, W., Raskin, I., 2004. Dietary phytoestrogens and health. Phytochemistry, 65, 995-1016.

Devi, M.K.A., Gondi, M., Sakthivelu, G., Giridhar, P., Rajasekaran,T., Ravishankar, G.A., 2009. Functional attributes of soybean seeds and products, with reference to isoflavone content and antioxidant activity. Food Chem. 114, 771–776.

Easaki, H., Watanabe, R., Onozaki, H., Kawakishi, S., Osawa, T., 1999. Formation mechanism for potent antioxidative o-Dihydroxyisoflavones in soybeans fermented with *Aspergillus saitoi*. Biosci Biotech Bioch. 63, 851-858.

Fleury, Y., Welti, D.H., Philippossian, G., Magnolato, D., 1992. Soybean (malonyl)isoflavones: characterization and antioxidant properties.ACS Symp Ser. 507, 98-113.

Garem, A., Daufin, G., Maubois, J.L., Chaufer, B., Leonil, J., 1998. Ionic interactions innanofiltration of β-casein peptides, Biotech. Bioeng. 57,109.

Hirakuri, M.H., Lazzarotto, J.J., 2011. Evolução e Perspectivas de Desempenho Econômico Associadas com a Produção de Soja nos Contextos Mundial e Brasileiro. Embrapa Soja-Documentos, 319, 23-25.

Izumi, T., M.K. Piskula, S. Osawa, A. Obata and K. Tobe et al., 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. J Nutr. 130, 1695-1699.

Kelly, S.T., Opong, W.S., Zydney, A.L., 1993. The influence of protein aggregates on the fouling of microfiltration membranes during stirred cell filtration. J. Membr.Sci. 80, 175-187.

Kelly, S.T., Zydney, A.L., 1997. Protein fouling during microfiltration: comparative behavior of different model proteins. Biotech. Bioeng. 55, 91-100.

Kim, W.J., Kim, H.H., Yoo, S.H., 2005. Ultra and nano-filtration process optimation of isoflavones and oligosaccharides from sunmul. Food Sci Biotechnol. 14, 380-386.

Kim, N.Y., Song, E.J., Kwon, D. Y., Kim, H.P., Heo, M.Y., 2008. Antioxidant and antigenotoxic activities of Korean fermented soybean. Food Chem Toxicol. 46, 1184-1189.

Kwak, C.S., Lee, M.S., Park, S.C., 2007. Higher antioxidant properties of Chungkookjang, a fermented soybean paste, may be due to increased

aglycone and malonylglycoside isoflavone during fermentation. Nutr Res. 27, 719-727.

Moktan, B., Saha, J., Sarkar, P.K., 2008. Antioxidant activities of soybean as affected by Bacillus-fermentation to kinema. Food Res Int. 41, 586–593.

Mello, B.C.B.S., Petrus, J.C.C. & Hubinger, M.D. (2010). Concentration of flavonoids and phenolic compounds in aqueous and ethanolic propolis extracts through nanofiltration. J. Food Eng. 96, 533-539.

Moreno, F.L., Raventós, M., Hernández, E., Ruiz, Y., 2014. Block freeze-concentration of coffee extract: Effect of freezing and thawing stages on solute recovery and bioactive compounds. J Food Eng. 120, 158-166.

Murakami, A.N.N., Amboni, R.D.M.C., Prudêncio, E.S., Amante, E.R., Zanotta, L.M., Maraschin, M., Petrus, J.C.C. & Teófilo, R.F. (2011). Concentration of phenolic compounds in aqueous mate (*Ilex paraguariensis* A. St. Hil) extract through nanofiltration. LWT- Food Sci Technol. 44, 2211-2216.

Noordman, T.R., Kooiker, K., Bel. W., Dekker, M., Wesselingh, J.A., 2003. Concentration of aqueous extracts of deffated soy flour by ultrafiltration: Effect of suspended particles on the filtration flux. J Food Eng. 58, 135-141.

Nurmi, T., Mazur, W., Heinomen, S., Hokkonen, J., Adlercreutz, H. (2002). Isoflavone content of the soy based supplements. J Pharmaceut Biomed. 28, 1-11.

Pratt, D. E.; Birac, P. M., 1979. Source of antioxidant activity of soybeans and soy products. J Food Sci. 44, 1720-1722.

Ranamukhaarachchi, S., Meissner, L., Moresoli. C., 2013. Production of antioxidant soy protein hydrolysates by sequential ultrafiltration and nanofiltration. J Membrane Sci. 429, 81-87.

Rao, M., Muralikrishna, G, 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted

finger millet (Ragi, Eleusine coracana Indaf-15). J Agr Food Chem. 50, 889-892.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorizationassay. Free Radical Biol. Med. 26, 1231-1237.

Ruiz-Larrea, M. B., Mohan, A., Miller, N. J., Bolwell, G. P., Rice-Evans, C. A., 1997. Antioxidant activity of phytoestrogenic isoflavones. Free Radic. Res. 26, 63-70.

Schausberger, P., Norazman, N., Li, H., Chen, V., Friedl, A., 2013. Simulation of protein ultrafiltration using CFD: Comparison of concentration polarisation and fouling effects with filtration and protein adsorption experiments. J Membrane Sci. 337, 1-8.

Shimoni, E., 2009. Stability and shelf life of bioactive compounds during food processing and storage: soy isoflavones. J Food Sci. 69, 160-166.

Shon, M-Y., Lee, J., Choi, J-H., Choi, S-Y., Nam, S-H., Seo, K.I., Lee, S-W., Sung, N.J., Park, S-K., 2007. Antioxidant and free radical scavenging activity of methanol extract of chungkukjang. J Food Compos Anal. 20, 113-118.

Siebert, K.J., 1999. Effects of protein–polyphenol interactions on beverage haze, stabilization, and analysis. J Agr Food Chem. 47, 353–362.

Speroni, F., Milesi, V., Añón, M.C., 2010. Interactions between isoflavones and soybean proteins: applications in soybean–protein isolate production. LWT- Food Sci Technol. 43, 1265–1270.

Takahashi, R. Ohmori, R., Kiyose, C., Momiyama, Y., Ohsuku, F., Kondo, K., 2005. Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. J Agric Food Chem. 53, 4578-82.

Wang, Y-W., Tang, C.Y., 2011. Protein fouling of nanofiltration, reverse osmosis, and ultrafiltration membranes- The role of

hydrodynamic conditions, solution chemistry, and membrane properties. J Membrane Sci. 376, 275-282.

Yang, J-H., Maub, J-L., Kob, P-T., Huang, L-C., 2000. Antioxidant properties of fermented soybean broth. Food Chem. 71, 249-254.

CAPÍTULO 4
Utilização do soro de tofu concentrado na obtenção de bebida láctea fermentada

Utilização do soro de tofu concentrado na obtenção de bebida láctea fermentada

Resumo

O soro de tofu foi concentrado através do processo de nanofiltração até atingir fator de redução volumétrica igual a 4,5. O concentrado foi usado na produção da bebida láctea fermentada 1 (10 % de soro de tofu concentrado + 90 % de leite) e bebida láctea fermentada 2 (20 % de soro de tofu concentrado + 80 % de leite). Ambas as bebidas (1 e 2) foram abaliadas quanto à contagem de bactérias ácido-láticas e suas propriedades físico-químicas, cor, índice de sinerese, reológicas e funcionais durante o período de estocagem (30 dias). Observou-se que a contagem total de bactérias ácido-láticas foi maior do que 8 log UFC mL^{-1}. O uso do soro de tofu concentrado contribuiu para o processo de pós-acidificação, para a redução do teor de sólidos totais e proteínas e pelo aumento do índice de sinerese. Todas as amostras apresentaram tendência a coloração amarelo-esverdeado. A viscosidade aparente de todas as amostras diminuiu com o aumento da taxa de cisalhamento, apresentando comportamento shear thinning e propriedades tixotrópicas. O modelo Lei da Potência foi apropriado para descrever o comportamento reológico. O conteúdo de isoflavonas totais foi maior para a bebida 2, cujo conteúdo conteúdo permaneceu inalterado, enquanto o conteúdo de oligossacarídeos diminuiu durante o período de estocagem.

***Palavras-chave**:* nanofiltração, soro de tofu, bebida láctea fermentada, isoflavonas, oligossacarídeos.

Utilization of tofu whey concentrate by nanofiltration in fermented lactic beverage formulation

Abstract

Tofu whey was concentrated by nanofiltration process up to a volume reduction factor of 4.5. The concentrate was used to manufacture the fermented lactic beverage 1 (10 % of concentrate tofu whey + 90 % of milk) and fermented lactic beverages (20 % of concentrate tofu whey + 80 % of milk). Both beverages (1 and 2) were evaluated for total lactic acid bacteria count and their physicochemical, color, syneresis index, rheological and functional properties during the storage time (30 days). It was observed that the total lactic acid bacteria count was greater than 8 log CFU mL^{-1}. The use of concentrate tofu whey contributed for the post-acidification process, for the decrease of total solids and protein contents and for the increase of the syneresis index. All samples showed a tendency to the greenish yellow color. The apparent viscosity of all samples decreased with an increase in the shear rate, showing shear rate-thinning and thixotropic properties. The Power Law model was appropriate to describe the rheological behavior. The total isoflavones content was greater for the beverage 2, and their content remained unchanged, while the oligosaccharides content decrease, during the storage time.

***Keywords**:* tofu whey, nanofiltration, fermented lactic beverage, isoflavones, oligossacharides.

1 Introduction

During the last decade, fundamental studies opened a new field of research dealing with bioactive compounds used in functional foods processing. The bioactive compounds, as isoflavones present mainly in soy products, deserve a large interest since they are mainly associated with the prevention of some diseases. In this sense, the consume of soy- products has been associated with a reduced prevalence of various chronic diseases, such as breast and prostate cancers, cardiovascular diseases and osteoporosis (Adlercreutz, Hamalainen, Gorbach, & Goldin, 1992; Ganry, 2002; Reynolds et al., 2006). Besides, other important components of soybean are oligosaccharides, that have prebiotic effects. Studies have shown that their consumption is related to several health benefits, such as lowering blood cholesterol, reducing blood pressure and preventing some types of cancer (Roberfroid, 2007).

The isoflavones and oligossacharides have been found in soy-products as tofu and its liquid waste, called of tofu whey, which also contains these components that remains soluble after coagulation (Sobral & Wagner, 2007). Currently, the tofu whey is a residual liquid that represents an important problem due to its negative environmental impact. A strategy to minimize this impact and allow its utilization in formulated foods should include a concentration stage (Sobral & Wagner, 2007). Nanofiltration is a membrane process based on the principle of selective permeation of the solute molecules through semi-permeable membranes, with the advantage of operating at lower temperatures and thus preserves the chemical structure and biological properties of the bioactive compounds of interest. This process has been successfully employed for concentrating phenolic compounds extracted from natural products (Murakami, et al., 2011; Prudêncio et al., 2012). However, the utilization of the tofu whay concentrate for special products through addition of exogenous bioactive compounds is still scarce. Thus, dairy products with bioative compounds from natural sources appears to be a convenient food format to satisfy the consumer interested in health benefits.

The objective of the present study was to investigate the use of concentrate tofu whey by nanofiltration to forumulate a functional fermented lactic beverage and evaluate its microbiological, physical, chemical, rheological and functional properties during the storage time.

2 Material and Methods

2.1 Materials

Tofu whey (TW) supplied by Tofutura Indústria de Alimentos LTDA (Campo Largo, Paraná, Brazil) with 0.35 g 100 g^{-1} of protein, 1.00 g 100 g^{-1} of lipids and 0.85 g 100 g^{-1} of sugars; homogenized pasteurized cow's milk with 3.5 g 100 g^{-1} of protein, 3 g 100 g^{-1} of lipids and 10 g 100 g^{-1} of sugars; milk thermophilic culture (YC-X11 Yo Flex®, Chr. Hansen, Hønsholm, Denmark) composed of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* were used in the production of fermented lactic beverages. All the reagents were of analytical grade.

2.2 Membrane processes

Aiming to remove the compounds with large molar mass for clarify and avoid fouling in the nanofiltration process (Matsubara et al., 1996), TW was first submitted to the microfiltration process in a pilot filtration unit, using tangential flow and an organic polyimide membrane (PAM Membranas Seletivas, Rio de Janeiro, RJ, Brazil) in a hollow fiber configuration, with an average cross-section diameter of 0.4 µm and a filtration area of 0.7 m^2. The following operating conditions were used in the microfiltration: temperature 22±1 °C; transmembrane pressure equal to 100 kPa; and tangential velocity of 1.0 m s^{-1}. The permeate resulting from the microfiltration process was used as the nanofiltration (NF) feed material. Yet, the NF was performed using a polyvinylidene (PVDF) membrane in the spiral configuration with molecular weight cut-off (MWCO) ranging between 150 and 300 g mol^{-1} and effective area of 1.2 m^2 (GE Osmonics®, Philadelphia, USA). MF and NF experiments were carried out in triplicate at 22 ± 1 °C. However, for NF process the transmembrane pressure was 600 kPa. As long operation times can change the molecular conformation of the isoflavones, as observed by Barbosa, Lajolo and Genovese (2006), low VRF values, as used in the present study are desirable. NF process was performed to achieve a volume reduction factor (VRF) equal to 4.5. This processing result in a concentrated tofu whey, with 6.12 g 100 g^{-1} of total solids and 1.20 g 100 g^{-1} of proteins. The VRF was calculated as the ratio between the initial volume (L) of tofu whey used in the feed and the final volume (L) of the concentrate after NF. During NF process

the permeate flux (J) (L h^{-1} m^{-2}) was calculated at each 3 min, as follow in the Eq. (1):

$$J = \frac{V_p}{tA_p} \quad (1)$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and A_p (m^2) is the permeation surface area of the membrane. After each processing, the pilot unit and the membranes were cleaned and hygienized, according to the manufacturer's instructions.

Thus, this concentrate was added to to the formulation of a fermented lactic beverages.

2.3 Production of fermented lactic beverages

Two fermented lactic beverages with the following formulations: 10 % concentrate tofu whey and 90 % milk (beverage 1); and 20 % concentrate tofu whey and 80 % milk (beverage 2), were manufactured according to the procedures described by Najgebauer-Lejko, Grega, and Walczycka (2011), with modifications. Milk and concentrated tofu whey were heated at 42 ±1 °C and then mixed in the proportions mentioned above. The lactic culture (5 g 100 g^{-1} of inoculums) was added before incubation (42 ± 1 °C) until pH of 4.6 ± 0.2 was reached. After fermentation, the lactic beverages were cooled to 10 ± 1 °C, gently stirred, and then conditioned into polypropylene cups sealed and stored at 5 ± 1 °C, until for them analysis. Following the same procedure, a control sample was run containing only milk (100 %).

2.4 Microbiological analysis

In order to assess the influence of the concentrate tofu whey over lactic acid bacteria (LAB) counting was carried out in beverage 1, beverage 2 and in the control sample (only milk). Theses counts were made in triplicate according to the methodology described in APHA (2001). The results were expressed as logarithm of colony counts per mL of beverage (log CFU mL^{-1}).

2.5 Physicochemical analysis

Beverage 1, beverage 2 and control were analyzed for total solids content (g 100 g^{-1}), by drying samples until constant weight. The

samples were also analyzed to the protein content (g 100 g^{-1}) by Kjeldahl method (N x 6. 38) (AOAC, 2005). The acidity (g 100 g^{-1} of lactic acid) of the three samples were determined according to the methodology described by Analytical Norms of the Adolfo Lutz Institute (IAL, 2008). The measurements of pH values were carried out with a pH meter (MP220, Metler-Toledo, Greinfensee, Switzerland).

2.6 Syneresis index

The syneresis index for beverages 1 and 2 and the control sample, was determined in accordance with the method proposed by Riener et al. (2010). Thirty grams of each sample were spread evenly on to Whatman No. 1 filter paper (Whatman Ltd., Maidstone, England) inside a funnel placed on the top of a 50 mL graduated cylinder to collect the liquid after 3 h of draining at 5 (± 1) °C. The syneresis index was calculated, using the following Eq. (2):

$$\text{Syneresis index (\%)} = \frac{\text{Supernatant (mL)}}{\text{Fermented lactic beverage (mL)}} \times 100 \qquad (2)$$

2.7 Color measurements

Measurement of the samples color (beverages 1 and 2, and control) were carried out with a Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Osaka, Japan). This apparatus was previously calibrated and adjusted to operate with D65 illuminant and observation angle of 10°.The CIElab color scale was used to measure the L*, a* and b* parameters. The L* parameters indicate luminosity hile, the a* axis shows the variation from red (+a*) to green (-a*) and the b* axis is the variation from yellow (+b*) to blue (-b*). The measurements were performed four times for each sample.

2.8 Rheological analysis

The rheological measurements (beverages 1 and 2 and control) were carried out using a Thermo Haake DC 10 rotational viscosimeter (model VT 550, Thermo Haake, Karlsruhe, Germany), with concentric cylinders (NV ST 807-0713 CE and NV 807-0702). Data were collected using the software program Pro Rheowin® (version 2.93, Haake). The flow curves were generated by shear rate increased linear from 0.02 s^{-1} to 200 s^{-1} first 5 min (upward curve) and returned to 0.02 s^{-1} in the

following 20 min (downward curve). Constant temperature (5.0 ± 0.1 °C) was maintained using a circulating water bath (Phoenix P1, Thermo Haake, Karlsruhe, German) coupled to the viscosimeter. The rotational speed was increased from 2 rpm to 41 rpm, at a rate of 2 rpm per minute.

The flow behavior was described through the Power Law model according to Eq. (3):

$$\sigma = K(\gamma)^n \quad (3)$$

where σ is shear stress (Pa), γ is shear rate (s^{-1}), K is consistency index (Pa s^{-1}), *n* is flow behavior index). Viscosity values on the downward (viscosity/shear rate) curves at a rate of 50 s^{-1} were considered as the apparent viscosity (g) of the beverages 1 and 2 and control, which according to Bourne (2002), represent the approximate viscosity perceived on the palate. The thixotropic behavior of the beverages and control were evaluated by calculating the hysteresis loop area between the upward and downward flow curves.

2.9 Extraction and determination of isoflavones

Extraction of isoflavones and the determination of their components were carried out with lyophilized samples (beverage 1 and beverage 2), in accordance with methodology proposed by Carrão-Panizzi, Favoni, and Kikuchi (2002), with modifications. One hundred grams of each sample was transferred to a 10 mL test tube, into which 4 mL of an extracting solution (70 % ethanol and 0.1 % acetic acid) were added. The test tubes with the samples and the extracting solution were stirred in a Vortex (Model MA162, MARCONI®, Piracicaba, SP, Brazil). The extraction was realized during 1 h at 25 °C, with stirring at each 15 min. The test tubes were then placed into an ultrasound bath (Model USC5000, UNIQUE®, Indaiatuba, SP, Brazil) and left for 30 min. A 1.5 mL aliquot of this extract was transferred to a refrigerated microcentrifuge (dimensions of 31 x 60 x 25 cm, 35 kg, Model 5417R 230 V/ 50 Hz, EPENDORFF®, São Paulo, Brazil) and centrifuged at 20,800 x g for 15 min at 5 ± 1°C. The supernatant was filtered through 0.45 µm filters (MILLIPORE®, Billerica, MA, USA) and 20 µL was used to separate and quantify the isoflavones through chromatography. The separation and the quantification of the isoflavones were performed using HPLC, as proposed by Berhow (2002), with a photodiode array

detector (Model 996) and an automatic sample injector (Model 717 Plus), both manufactured by WATERS® (Milford, USA). In this stage, a reverse phase column (YMC-Pack ODS-AM, C18, S-5 lm, diameter of 250 x 4.6 mm) was used. For the isoflavones separation, the binary linear gradient system was used and the mobile phases were: (a) methanol containing 0.025 % trifluoroacetic acid (TFA) (Phase A) and (b) ultrapure water (MILLIPORE®, Billerica, MA, USA) containing 0.025 % TFA (Phase B). The initial condition of the gradient was 20 % in Phase A, reaching 90 % in 35 min, followed by cleaning of the column with 100 % of Phase A for 5 min and subsequently return to 20 %, retaining these conditions for up to 60 min. The mobile phase flow was of 1 mL min^{-1} and the temperature during the analysis was 25 °C. For the isoflavone detection, the wavelength of the detector was adjusted to 254 nm. The software used to control the equipment and the data acquisition was Millennium 32 (version 3.05.01) (GCLC®, Toronto, Pickering, ON, Canada). For the identification and quantification of the peaks corresponding to each one of the isoflavones, calibration curves with linear regression based on the peak areas were used. These calibration curves were constructed with external standards of daidzin, daidzein, genistin, genistein, glycitin, glycitein, malonyl daidzin, malonyl genistin, malonyl glicitin, all of which were solubilized in methanol (chromatographic grade) to the following concentrations: 0.00625 mg mL^{1}; 0.0125 mg mL^{-1}; 0.0250 mg mL^{-1}; 0.0500 mg mL^{-1} and 0.1000 mg mL^{-1} (for each external standard). The results of isoflavones were expressed as mg of isoflavones 100 g^{-1} of solid sample. This assay was repeated 4 times for each sample.

2.10 Determination of oligosaccharides

Oligosaccharides were determined only in beverage 2. This determination was carried out in triplicate with lyophilized samples as methodology proposed by Kennedy, Mwandemelet, and McWhirtert (1985), Burgner and Feinberg (1992) and Cicek (2001), with modifications. Two grams of each sample were transferred to a 100 mL wide mouth glass flask, into which 20 mL of an extracting solution (50 % ethanol and 50 % distilled water) were added. The glass flasks with the samples and the extracting solution were stirred during 2 h. Then 2.0 mL of potassium ferricyanide 0.25 M and 2.0 mL of zinc acetate were added, and the glass flasks were stirred again. The supernatant was filtered through paper filter Whatman No. 1 filter paper (Whatman Ltd., Maidstone, England) and PVDF membrane of 0.22 µm and 13 mm of

diameter. The clarified filtrate was analyzed by HPLC (Model YL 9100, Young Lin Instrument Co., Anyang, Henan, Korea), equipped with injection valve Reodyne with loop of 20 µL, quaternary pump (Model YL 9110, Young Lin Instrument Co., Anyang, Henan, Korea) and refractive index detector (Model YL 9170, Young Lin Instrument Co., Anyang, Henan, Korea), using column NH_2 Luna (Phenomenex, 250 x 4.6 mm, 5 µm). The mobile phase flow was of 1.5 mL min^{-1} and the temperature during the analysis was 40 °C. For the oligosaccharides separation, a binary system was used and the mobile phases were: (a) 75 % acetonitrile and (b) 25 % ultrapure water (MILLIPORE®, Billerica, MA, USA).

For the identification and quantification of the each oligosaccharide peak, calibration curves with linear regression based on the peak areas were used. These calibration curves were constructed with external standards of raffinose and stachyose, which were solubilized in methanol (chromatographic grade) at concentrations ranging between 0.02 and 1.0 g 100 mL^{-1}. The results were expressed as g of oligosaccharides 100 g^{-1} of sample.

2.11 Statistical analysis

Data were expressed as means and standard deviation. One-way ANOVA and Tukey's test (5 % significance) were carried out to evaluate the results, using the software STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA).

3 Results and discussion

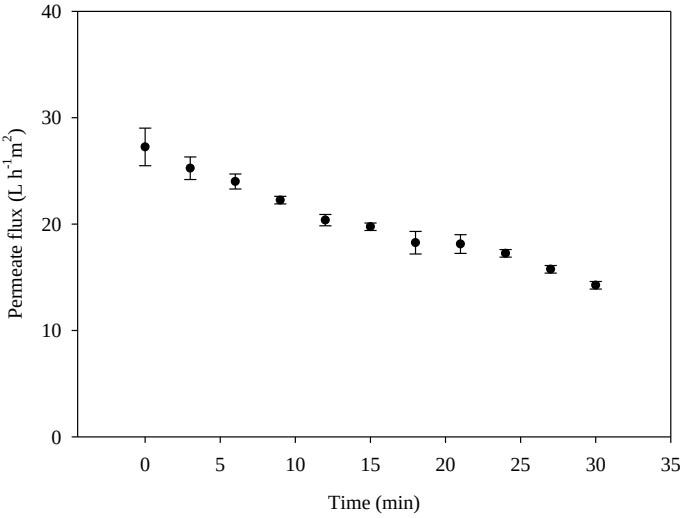
3.1 Nanofiltration process

In Fig. 1 it is possible to see that the permeate flux (J) decreased during the nanofiltration (NF) process, with an average J value equal to 20.25 L $h^{-1}m^{-2}$. The same behavior was also observed by Kim, Kim and Yoo (2005) during the NF process of tofu whey, with J values ranging from 15 to 35 L $h^{-1}m^{-2}$. Several factors can influence the NF performance (Baker, 2004), such as the increase of the osmotic pressure of the feed solution (Suárez et al., 2009), concentration polarization phenomena (Rinaldoni, Tarazaga, Campderrós, & Pérez Padilla, 2009; Pan, Yan, Zhu, & Li, 2013), adsorption, fouling and gel-layer formation due to presence of protein (Rinaldoni et al., 2009). Furthermore, Al-Malack and Anderson (1997) stated that modifications in the pressure,

temperature, duration of the process, type and pore size of the membrane, raw material, and the VRF used could affect the membrane process.

Regarding to the NF process, Wang and Tang (2011) affirm that apart of the type of raw material, normally are detected the formation of a polarization layer and the fouling phenomenon. Yet, Suárez et al. (2009) verified that the proteins are able to form a gel layer on the membrane surface. On the other hand, Chan and Chen (2004) associated the high temperatures used during the tofu manufacture with the proteins aggregation and therefore, with the deposition of these aggregates on the membrane surface, resulting in lower J values.

Figure 1- Permeate flux (J) (mean ± standard deviation) of tofu whey submitted to nanofiltration (NF), up to volume reduction factor equal to 4.5.



3.2 Lactic Acid Bacteria (LAB) Count

In the present study, the inclusion of tofu whey concentrate in the elaboration of fermented lactic beverages (1 and 2) didnot affect the survival of lactic acid bacteria (LAB) (Table 1) count greater than 8 log CFU mL^{-1}, including during all storage time. These results are in

accordance with Codex Alimentarius (2011), which establishes that this count must be greater than 7 log CFU mL^{-1}.

During the storage time, it was possible to observe that in the beverage 2 there was a greater reduction ($p < 0.$ in the LAB count. According to Farnworth et al. (2007) this fact can be associated with the further reduction in pH (Table 2), by the increase of organic acids concentration. These authors consider this is one of the more important factors that can dramatically affect bacterial. These results are also in agreement with those obtained by Lee, Jenner, Low, and Lee (2006) and Jaziri et al. (2009) by tea phenolic compounds.

Table 1- Lactic Acid Bacteria (LAB) count of control sample and fermented lactic beverages (1 and 2) during storage time, expressed as logarithms of colony counts per mL of beverage (log CFU mL^{-1}).

Sample	Day 1	Day 15	Day 30
Control	8.90 ± 0.04^{Ba}	9.10 ± 0.04^{Aa}	9.08 ± 0.04^{Aa}
Beverage 1	9.00 ± 0.06^{Aa}	9.01 ± 0.06^{Aa}	8.90 ± 0.01^{Ab}
Beverage 2	8.69 ± 0.01^{Bb}	8.99 ± 0.01^{Aa}	8.40 ± 0.06^{Cc}

Control sample (only milk); Fermented lactic beverage 1 (10 % of concentrate tofu whey and 90 % of milk); Fermented lactic beverage 2 (20 % of concentrate tofu whey and 80 % of milk).
Results expressed as mean ± standard deviation (n=3).
A,B,C Within a line, different superscript uppercase letters denote significant differences ($p < 0.05$) among the different storage day, for each sample.
A,B,C Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) among the different samples (control, beverage 1, beverage 2) for the same storage time.

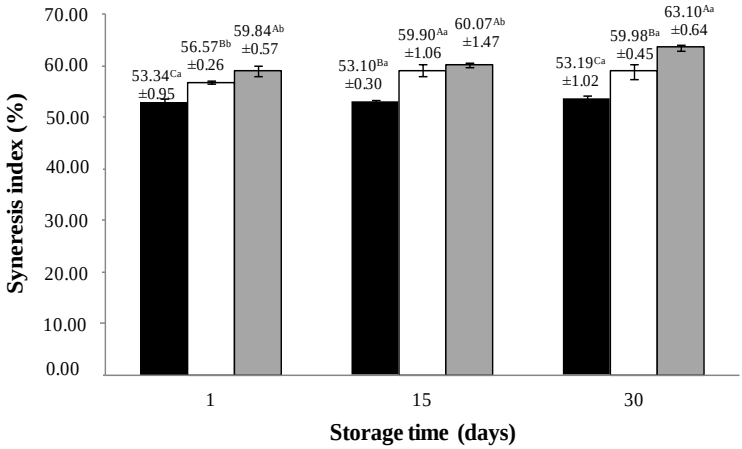
3.3 Physical and chemical properties

The use of concentrate tofu whey decreased the total solids content, reflecting also in a decrease of the protein contents ($p < 0.05$) (Table 2), but this behavior was more evident in beverage 2 (with 20 % of concentrate tofu whey). However, at the end of storage time (30 days) it was possible to verify that total solids and protein contents remained unchanged ($p < 0.05$). The same behavior was observed by Cunha et al. (2008) for lactic beverages produced with different contents of whey and Debon et al. (2012) for prebiotic fermented milk.

On day 1 of storage, acidity and pH values for beverage 2 were lower ($p < 0.05$) than the beverage 1 and the control sample. However, acidity increased and pH values decreased ($p < 0.05$) (Table 2), throughout the storage time (up to 30 day) for all samples, i. e., it was observed a post-acidification process. Kailasapathy and Sultana (2003) stated that the post-acidification of fermented dairy products occurred during refrigeration storage because of the residual metabolic activity of lactic acid bacteria (LAB). The activity of β-galactosidase released by the LAB to cleave lactose is still active even at refrigerated storage temperature (0 - 5 ºC) (Kailasapathy & Sultana, 2003). This contributes to the accumulation of lactic acid, acetic acid, citric acid, butyric acid, acetaldehyde and formic acid produced by starter culture as metabolic by-products (Kailasapathy, 2006). Acidity and pH values obtained were in agreement with those recommended by Costa et al. (2013) and Tamime and Robinson (2007), which should be in the range acidity of 0.6 and 1.5 % lactic acid and pH equal to 4.6, respectively. Furthermore, Tamime and Robinson (2007) related that fermented milks obtained at this pH range would exhibit in a better rearrangement and aggregation of casein particles.

The syneresis index data for these beverages (1 and 2) and control (Fig. 2) show that higher the amount of concentrate tofu whey used, within the levels studied, the greater the syneresis. This influence was also observed by Castro et al. (2009) for lactic beverages. At the end of storage time (30 days), for the same sample, it was possible to verify that the syneresis index showed differences ($p < 0.05$). The instability of syneresis index during the storage time could be related with the pH values observed in all samples evaluated. As observed by Castro et al. (2009), in the present study the increase of concentrate tofu whey content in fermented lactic beverage contributes to the formation of acid gels with open structure, due to reduction of intermolecular interactions and, therefore, more susceptible to the syneresis effect.

Figure 2- Results of the average ± (standard deviation) (⊥) syneresis index of controle sample and fermented lactic beverages (beverage 1 and beverage 2), during storage time at 5±1 °C.



Control sample (■); Beverage 1 (□); Beverage 2 (▒). ^{A,B}Different superscript uppercase letters denote significant differences ($p < 0.05$) among the different storage time, for each sample. ^{a,b}Different superscript lowercase letters denote significant differences ($p < 0.05$) among the different samples (control, beverage 1 and beverage 2) for the same storage time.

Table 3 shows the parameters L*, a*, and b* for both beverages (1 and 2) and for the control sample, during the storage time. For all samples, at the the same day of storage time, it was possible to note that the parameter a* increased, while the parameter b* decreased ($p < 0.05$). For these parameters the same behavior was observed during the storage time. Despite these variations, it was possible to note that all samples showed a tendency to the greenish yellow color. The same behavior was observed by Poloseli-Scopel, Hernández-Herrero, Guamis, and Ferragut (2013) in soymilk. Parameter L* decreased ($p < 0.05$) during the storage time for beverages 1 and 2. This decrease could be due to oxidation of th concentrated tofu whey, which is undesirable and may influence the consumer acceptability. According Kong, Chan, Liu, and Wilson (2008), this change in the color of soybean products could be attributed to nonenzymatic reactions.

Table 2 - Results of physicochemical composition of control sample and fermented lactic beverages (beverage 1 and beverage 2) during storage time at 5 ± 1ºC.

Samples	Day	Total solids (g 100 g^{-1})	Proteins (g 100 g^{-1})	Acidity (g 100 g^{-1} of lactic acid)	pH
Control	1	11.62 ± 0.03^{Aa}	3.25 ± 0.12^{Aa}	0.67 ± 0.01^{Ba}	4.50 ± 0.02^{Ab}
	15	11.36 ± 0.02^{Ab}	3.26 ± 0.04^{Aa}	0.69 ± 0.01^{Bb}	4.38 ± 0.01^{Ba}
	30	11.64 ± 0.02^{Aa}	3.31 ± 0.07^{Aa}	0.72 ± 0.00^{Aa}	4.40 ± 0.03^{Ba}
Beverage 1	1	10.93 ± 0.06^{Bb}	3.14 ± 0.03^{Aa}	0.66 ± 0.01^{Ba}	4.53 ± 0.01^{Aa}
	15	10.79 ± 0.03^{Bb}	3.05 ± 0.03^{Bb}	0.71 ± 0.01^{Aa}	4.33 ± 0.01^{Bb}
	30	11.06 ± 0.01^{Ba}	3.13 ± 0.04^{Ba}	0.72 ± 0.00^{Aa}	4.38 ± 0.03^{Ca}
Beverage 2	1	10.36 ± 0.04^{Ca}	2.92 ± 0.03^{Ba}	0.61 ± 0.00^{Cb}	4.48 ± 0.00^{Ac}
	15	10.02 ± 0.03^{Cb}	2.69 ± 0.02^{Cb}	0.67 ± 0.01^{Bb}	4.30 ± 0.01^{Cc}
	30	10.36 ± 0.05^{Ca}	2.91 ± 0.03^{Ca}	0.71 ± 0.01^{Aa}	4.35 ± 0.01^{Ba}

Control sample (only milk); Fermented lactic beverage 1 (10 % of concentrate tofu whey and 90 % of milk); Fermented lactic beverage 2 (20 % of concentrate tofu whey and 80 % of milk).

Results expressed as mean ± standard deviation, among three batches realized in triplicate for each type of beverage, with three repetitions for physicochemical analyzes.

^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) among the different samples (control, beverage 1, beverage 2)
for the same storage time.

^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) among the different storage time, for each sample.

Table 3- Color parameters (L*, a* and b*) for the control samples and fermented lactic beverage 1 and beverage 2 during storage time at 5 ±1 °C.

Sample	Day	Parameters		
		L*	a*	b*
Control	1	82.50 ± 0.57^{Aa}	-3.35 ± 0.04^{Aa}	9.31 ± 0.13^{Ba}
	15	82.97 ± 0.91^{Aa}	$-3.11\pm0.03_{Ab}$	8.99 ± 0.08^{Ab}
	30	81.70 ± 0.99^{Aa}	-2.79 ± 0.07^{Ac}	8.02 ± 0.29^{Ac}
Beverage 1	1	82.55 ± 0.23^{Aa}	-3.28 ± 0.35^{Aa}	9.49 ± 0.06^{ABa}
	15	82.82 ± 0.86^{Aa}	-2.97 ± 0.02^{Bb}	9.01 ± 0.09^{Ab}
	30	78.75 ± 1.08^{ABb}	-2.45 ± 0.02^{Bc}	7.60 ± 0.10^{Ac}
Beverage 2	1	81.02 ± 0.98^{Aa}	-3.13 ± 0.02^{Ba}	9.56 ± 0.07^{Aa}
	15	82.00 ± 0.52^{Aa}	-2.85 ± 0.03^{Cb}	9.06 ± 0.08^{Ab}
	30	75.84 ± 2.91^{Bb}	-2.21 ± 0.11^{Cc}	7.38 ± 0.33^{Ac}

Control sample (only milk); Fermented lactic beverage 1 (10 % of concentrate tofu whey and 90 % of milk); Fermented lactic beverage 2 (20 % of concentrate tofu whey and 80 % of milk).

Results expressed as mean ± standard deviation (n=3).

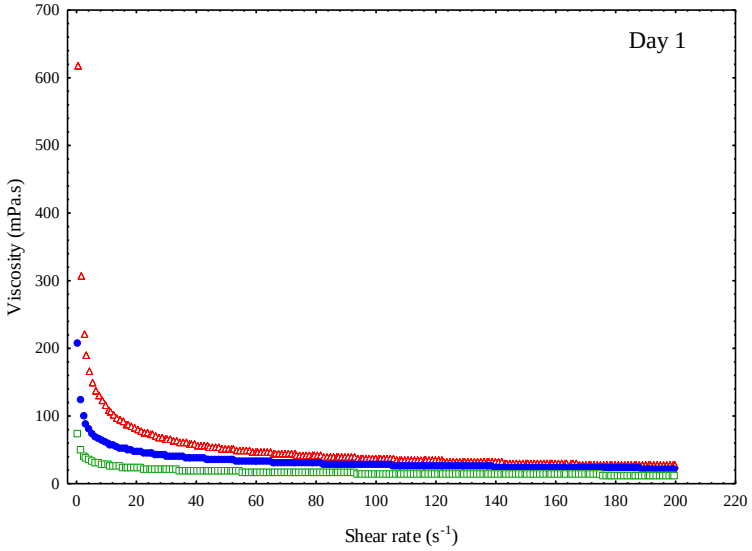
^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) among the different samples (control, beverage 1, beverage 2) for the same storage time.

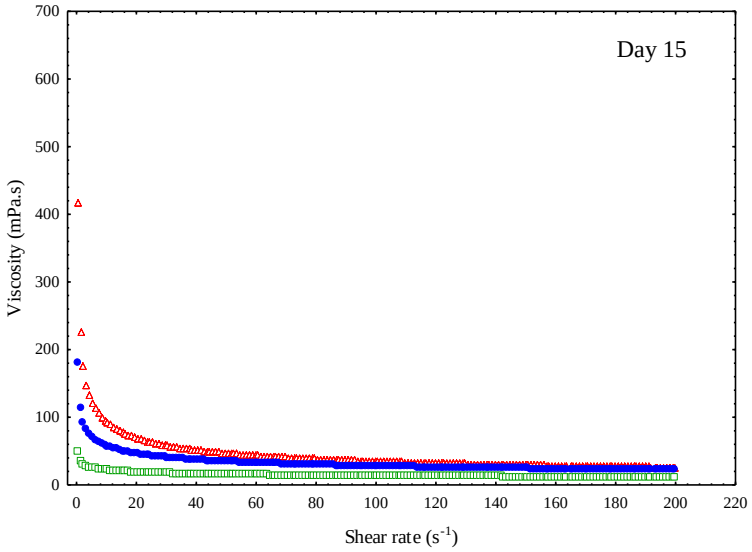
^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) among the different storage time, for each sample.

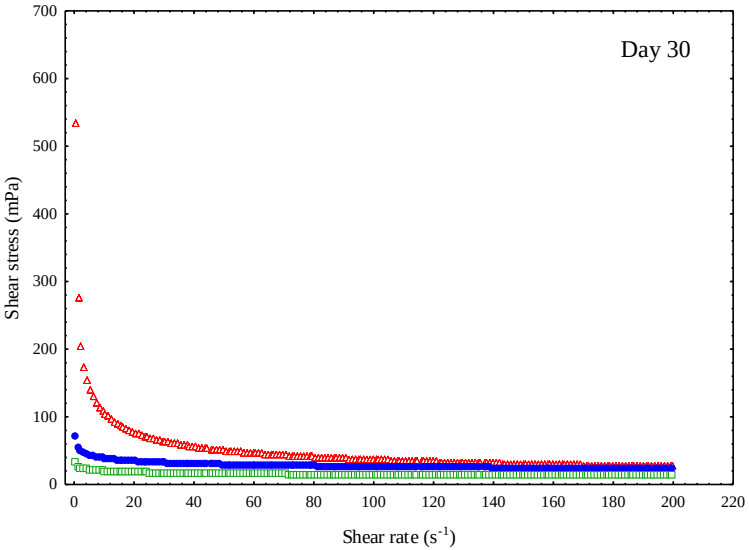
3.4 Rheology properties

The apparent viscosity of beverage 1 and 2 and control decreased with an increase in the shear rate, indicating a non-Newtonian fluid behavior (Fig. 3a-c), with shear thinning characteristics. This is in agreement with results obtained by Rinaldoni, Campderrós, and Pérez Padilha (2012) for fermented milk from ultrafiltreted soy milk. Karazhiyan et al. (2009) explained that the increase in shear rate and decrease in viscosity occur by a breaking of the macromolecular structures in solution, due to the shear force. Because the hydrodynamic forces are more intense at the beginning of shearing, greater rupture occurs generating system stretching allowing, over time, the alignment with the flow and, consequently, a reduction in the viscosity values, as related by Castro (2003).

Figure 3- Apparent viscosity versus shear rate on (a) 1 (b) 15 and (c) 30 storage days for control sample (△) and fermented lactic beverages (beverage 1(●) and beverage 2 (□). Results expressed as mean (n=3).







During the storage time, it can be verified that the control sample showed a higher initial viscosity, with a steep decrease more pronounced for both beverages (1 and 2). However, the viscosity was lower for beverage 2 (Table 4). According to Ramaswamy and Basak (1992), this behavior can be credited to the addition of aqueous extracts, which generally decreased the viscosity of the products due to reduced water-binding capacity of proteins. Jung, Chung, and Kim (2005) also observed a reduction in the viscosity values for fermented milk mixed with concentrate tofu whey.

The rheological parameters evaluated for beverages 1 and 2 and for the control, during 30 days of storage, are given in Table 4. For the Power Law model, correlation coefficients (R) values were greater than 0.9. Thus, this model was considered appropriate to describe the rheological behavior of these beverages, during the storage time (30 days). The flow behavior index (n) for these samples obtained through the model (Table 3), showed characteristics of a shear thinning fluid ($n < 1$), thus confirming the non-Newtonian behavior. The storage time contributed to the decrease of the beverages apparent viscosity (Table 4). This fact can also be credited to the addition of the aqueous extract. Since Ross-Murphy (1990) states that this behavior is common in acid gels during storage.

The decrease in K values (Table 4), combined to the partial structure recovery between the two cycles of shear rate (Fig. 4a-c), indicate that all samples show shear rate-thinning and thixotropic properties, as stated by McCann et al. (2011), Rawson and Marshall (1997) and Staffolo et al. (2004), which it is typical for fluid fermented dairy products.

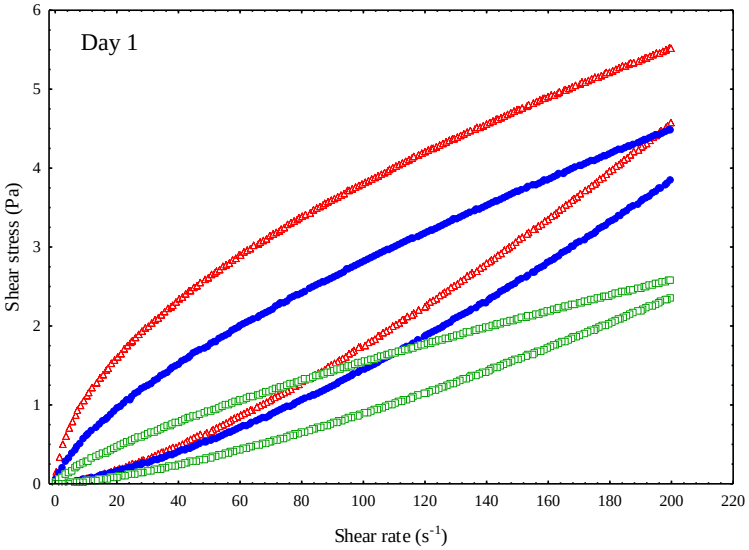
Table 4 - Rheological parameters obtained using Power Law model ($\eta = K(\gamma)^{n-1}$) and apparent viscosity for the control samples and fermented lactic beverage 1 and beverage 2 during storage time at 5 ±1 °C.

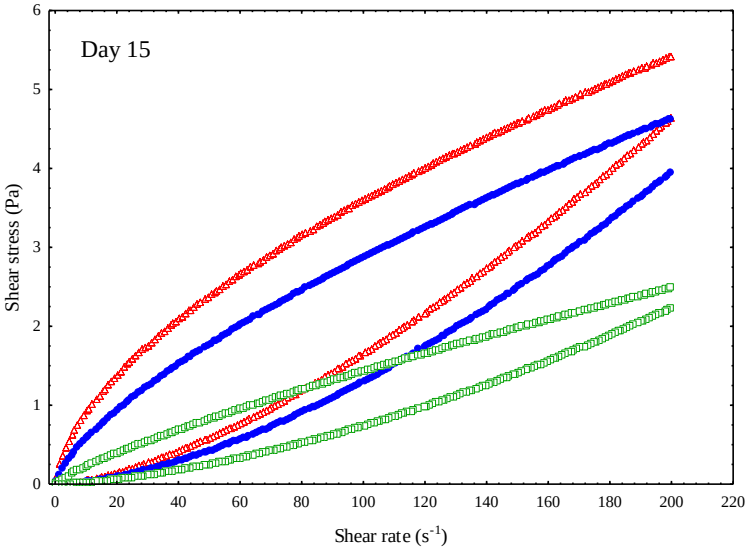
Sample	Day	Power Law model			
		K (Pa s^n)	n	η (mPa.s)	R
Control	1	0.328	0.533	52.75	0.989
	15	0.293	0.557	51.45	0.984
	30	0.239	0.589	47.94	0.993
Beverage 1	1	0.131	0.675	35.28	0.989
	15	0.119	0.692	35.56	0.990
	30	0.057	0.835	29.95	0.987
Beverage 2	1	0.051	0.740	18.51	0.990
	15	0.037	0.794	16.53	0.991
	30	0.028	0.867	16.51	0.991

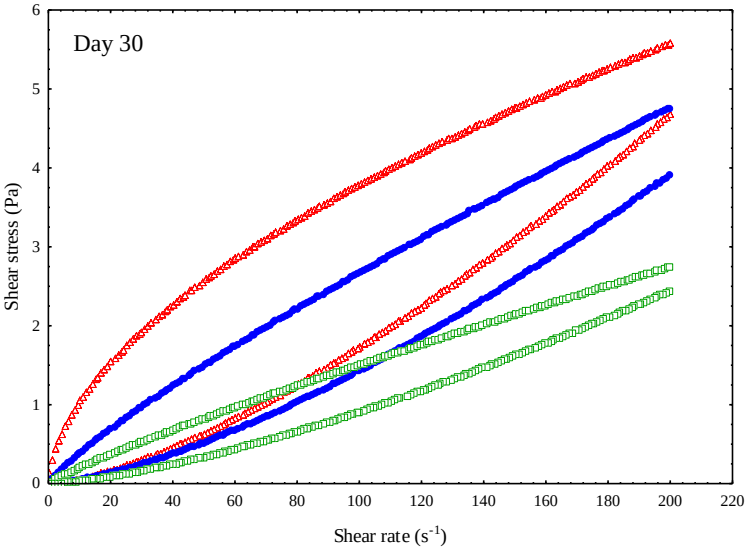
Control sample (only milk); Fermented lactic beverage 1 (10 % of concentrate tofu whey and 90 % of milk); Fermented lactic beverage 2 (20 % of concentrate tofu whey and 80 % of milk).
K, consistency index; n, flow behavior index; η, apparent viscosity; R, correlation coefficient.

Toneli, Mürr, and Park et al. (2005) reported that the formation of an hysteresis curve reflects changes in the rheological behavior of the product. This behavior is of a product with shear thinning characteristics, which exhibits thixotropic behavior. Therefore, the rheograms (Fig. 4a-c) indicate that all samples evaluated showed hysteresis, and thus, they are dependent of the storage time. It is noteworthy, that this behavior occurs when products with fragile agglomerated particles are submitted to a shear force. In this case, Oliveira et al. (2002) stated that the tridimensional structures of fluid fermented dairy products are lost, but they are practically regained after a period of rest.

Figure 4- Flow curves, shear stress versus shear rate, for control samples △ and fermented lactic beverages (beverage 1 ● and beverage 2 □) on (a) 0, (b) 15 and (c) 30 storage days.

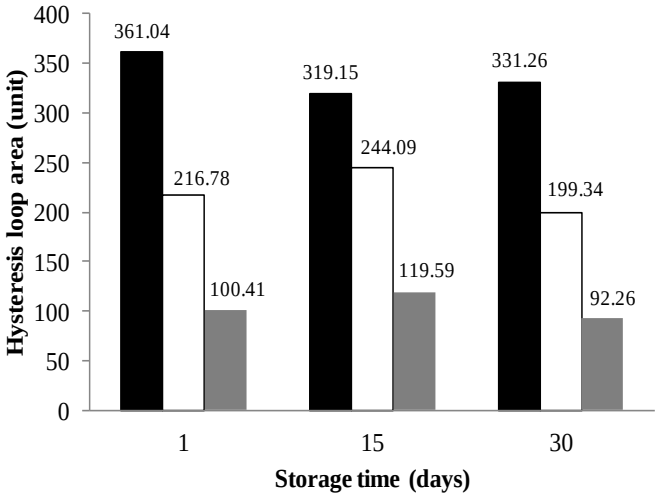






Thus, in the Fig. 5 it is possible to verify that the control sample showed the greatest hysteresis values, followed by the beverage 1 and beverage 2. In accordance with Mohameed et al. (2004) changes in the rheological properties of fluid fermented dairy products can be associated to their solids concentration. Therefore, the greatest hysteresis observed for the control sample may be related to its total solids content, as shown in Table 1. This behavior can be also associated to the viscosity of sample, i. e., as stated by Hernández (1996) a thixotropic fluid of higher viscosity must have a greater hysteresis area than a fluid with lower viscosity, even when an increase occurs in the breaking of the structure of the less viscous product.

Figure 5 - Hysteresis area for control sample (■), beverage 1 (□) and beverage 2 (▨) during storage time at 5 ± 1ºC.



3.5 Functional properties assessment of the fermented lactic beverages

As expected, beverage 2 presented the greatest isoflavone content ($p < 0.05$), that remained unchanged ($p > 0.05$), during the storage time. However, storage time affected beverage 1, so it was possible to note an increase in its total isoflavone content ($p < 0.05$), due to increase in the M-daidzin, M-glicitin and M-genistin contents ($p < 0.05$). Already, the total isoflavone content remained unchanged ($p > 0.05$), during the storage time of beverage 2. In beverage 2, the increase of β-glucosides (daidzin, glicitin and genistin) and of aglycones (daidzein and genistein), and the decrease of malonil glucosides (M-daidzin and M-genistin) were responsible for the unchanged total isoflavones content.

It was also possible to verify that after storage time (30 days) of the beverage 2 (with 20 % of concentrate tofu whey), the daidzein and genistein contents increased ($p < 0.05$). According to Li-Jun et al. (2004) this fact could be due to enzymatic hydrolysis produced by the starter microorganism. However, it was not observed the conversion from β-glucosides into the corresponding aglycones, because both values increased (Table 5). Therefore, there was not enough enzyme

amount or activity, under hydrolysis by β-glucosidase. For the other hand, Fletcher (2003) stated that the diversity of processing techniques has a major effect on the level of isoflavone. The effects of soybean processing techniques on the distribution of isoflavones were also investigated by Chien, Huang and Chou (2006), Li-Jun et al. (2004) and Wang and Murphy (1996), for soybean products. As affirmed by Yin et al. (2005), in the present work the conversion of isoflavones structures was perhaps due to storage conditions than by glucosidase hydrolysis. Rossi et al. (2004) neither observed the isoflavone hydrolysis in soybean yogurt. However, Cassidy et al. (2006) emphasize that the bioavailability of isoflavones are influenced mainly by the type of food matrix or form in which they are ingested. These authors also stated that a liquid matrix, such as soy yogurt, yields a faster absorption rate than a solid matrix, whereas aglycones in a fermented food are absorbed more rapidly than glucosides conjugates. Therefore, the technological and cost advantages of the use of concentrate tofu whey make possible a wider range of foods, such as the fermented lactic beverage, offering potential health benefits.

As observed by Kano et al. (2006) fermentation products (lactic acid) can influence the absorption or the metabolism of isoflavones. It is noteworthy, that it reinforces the need to accurately determine the isoflavone content of foods used in dietary intervention studies while exposing the limitations for estimating daily isoflavone intakes.

As well as the isoflavones, Gomes and Malcata (1999) stated that products with oligosaccharides may positively affect human health. Thus, the oligosaccharides contents were determined in beverage 2. During its storage time, it was observed that the oligosaccharides contents decreased ($p < 0.05$) (Table 4). De Vuyst (2000) verified that ever technological conditions of food processing must affect the oligosaccharides content. However, this author also reported that post-acidification is responsible by hydrolysis of the oligosaccharides present in the food matrix, and that this behavior results in the loss of the nutritional properties of the food. Furthermore, De Vuyst (2000) also affirms that the stability of these compounds is strongly dependent on the nature and type of the oligosaccharides; in ring structure, their anomeric configuration, and the type of bonds. Finally, it has to be underlined that oligosaccharides are substrates for microorganisms, so that they may be fermented and consumed during storage time (Buono, Erickson, & Fung, 1990).

Table 5- Isoflavone content (mg isoflavone 100 g^{-1} dry sample) for the fermented lactic beverage 1 and beverage 2 during storage time and oligosaccharides content (g oligosaccharide 100 g^{-1} dried sample) for the beverage 2 on days 1 and 30 of storage.

Sample	Day	Isoflavones								Total	Oligosaccharides		
		β-glucosides			Malonil glucosides			Aglycones					
		Daidzin	Glicitin	Genistin	M-daidzin	M-glicitin	M-genistin	Daidzein	Genistein		Raffinose	Stachyose	Total
Beverage 1	1	11.56 ±0.24^{Ab}	2.13 ±0.13^{Ab}	6.93 ±0.17^{Ab}	16.22 ±0.50^{Cb}	4.42 ±0.23^{Bb}	24.50 ±0.52^{Cb}	0.47 ±0.05^{Ab}	0.34 ±0.03^{Ab}	66.59 ±1.53^{Cb}	-	-	-
	15	11.19 ±0.32^{Ab}	2.18 ±0.09^{Ab}	6.77 ±0.18^{Ab}	20.02 ±0.14^{Bb}	5.02 ±0.17^{Ab}	27.76 ±0.60^{Bb}	0.41 ±0.05^{Ab}	0.33 ±0.05^{Ab}	73.68 ±1.18^{Bb}	-	-	-
	30	11.63 ±0.47^{Ab}	2.34 ±0.23^{Ab}	6.95 ±0.40^{Ab}	22.29 ±1.27^{Ab}	5.33 ±0.35^{Ab}	30.16 ±1.53^{Ab}	0.44 ±0.04^{Ab}	0.34 ±0.01^{Ab}	79.49 ±3.99^{Ab}	-	-	-
Beverage 2	1	23.62 ±0.26^{Ca}	4.74 ±0.35^{Ba}	13.92 ±0.27^{Ca}	38.55 ±0.93^{Aa}	10.33 ±0.38^{Aa}	58.47 ±1.37^{Aa}	1.00 ±0.09^{Ba}	0.68 ±0.07^{Ba}	151.31 ±3.25^{Aa}	0.22 ±0.01^{A}	1.79 ±0.03^{A}	2.02 ±0.04^{A}
	15	24.79 ±0.36^{Ba}	4.98 ±0.08$^{A}_{Ba}$	14.88 ±0.07^{Ba}	37.61 ±0.31^{Aa}	10.31 ±0.47^{Aa}	58.54 ±0.26^{Aa}	1.09 ±0.02^{Ba}	0.71 ±0.06^{Ba}	152.92 ±0.43^{Aa}	-	-	-
	30	27.71 ±0.80^{Aa}	5.17 ±0.10^{Aa}	16.68 ±0.39^{Aa}	35.20 ±0.53^{Ba}	10.35 ±0.24^{Aa}	55.55 ±1.18^{Ba}	1.31 ±0.14^{Aa}	0.92 ±0.03^{Aa}	152.89 ±2.82^{Aa}	0.12 ±0.01^{B}	0.98 ±0.01^{B}	1.10 ±0.01^{B}

Beverage 1 (10 % of concentrated tofu whey and 90 % of milk); Beverage 2 (20 % of concentrated tofu whey and 80 % of milk). ^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) among the different storage day, for each sample. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) among different samples (beverage 1 and beverage 2) for the same storage time.

4 Conclusions

The inclusion of concentrate tofu whey in the manufacture of fermented lactic beverages did not affect the survival of lactic acid bacteria during all storage time. It was observed a decrease in the total solids content, proteins content and in the apparent viscosity and highest syneresis index in the fermented lactic beverage 2, i. e., with 20 % of concentrate tofu whey. Besides, it was possible to note that all samples showed a tendency to the greenish yellow color. The power-law model was applied successfully to describe the flow properties of fermented lactic beverages. Both types of beverages showed shear rate-thinning and thixotropic properties, confirming a non-Newtonian behaviour. The total isoflavones remained in the fermented lactic beverages, while the oligosaccharides (evaluated only in the beverage 2) decreases over storage time. Finally, the use NF process to concentrate the tofu whey and the use the concentrate in the manufacture of l fermented lactic beverage offer an opportunity for an entirely new approach regarding the utilization of tofu whey.

References

Adlercreutz, H., Hamalainen, E., Gorbach, S., & Goldin, B. (1992). Dietary phytoestrogens and the menopause in Japan. *Lancet*, 339, 1233.

Al-Malack, M. H., & Anderson, G. K. (1997). Crossflow microfiltration with dynamic membranes. *Water Research*, 31, 1969-1979.

APHA, 2001. *Compendium of Methods of the Microbiological Examination of Foods*, fourth ed. American Public Health Association, Washington, DC.

Association of Official Analytical Chemists (AOAC). (2005). *Official methods of analysis of the association analytical chemists* (18th ed.). Maryland, DC: AOAC.

Baker, R. W. (2004). *Membrane Technology and Applications*. (2nd ed). New York: John Wiley and Sons.

Berhow, M.A., 2002. Modern analytical techniques for flavonoid determination. In B.S. Buslig, and J.A. Manthey (Eds.). F*lavonoids in*

the living cell. Advances in experimental medicine and biology, New York: Klusher academic.

Bourne, M.C. (2002). *Food Texture and Viscosity: Concept and Measurement*. New York: Academic Press.

Buono, M.A., Erickson, L.E., & Fung, D.Y.C. (1990). Carbohydrate utilization and growth kinetics in the production of yogurt from soymilk. Part II: experimental and parameter estimation results. *Journal of Food Processing and Preservation*, 14, 179-204.

Burgner, E., & Feinberg, M. (1992). Determination of mono - and disaccharides in foods by interlaboratory study: Quantitation of Bias components for liquid chromatography. *Journal of AOAC International*, 75, 443-464.

Carrão-Panizzi, M.C., Favoni, S.P.G., & Kikuchi, A. (2002). Extraction time for soybean isoflavone determination. *Brazilian Archives of Biology and Technology*, 45, 515-518.

Cassidy, A.,. Brown, J.E., Hawdon, A., Faughnan,M.S., King, L.J., Millward,J., Zimmer-Nechemias, L., Wolfe, B., & Setchell, K.D.R. (2006). Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *The Journal of Nutrition*, 136, 45-51.

Castro, A.G., 2003. *A química e a reologia no processamento de alimentos.* Ciência e Técnica. Instituto Piaget, Lisboa. pp. 37–61.

Castro, F.P., Cunha, T.M., Ogliari, P. J., Teófilo, R.F., Ferreira, M.M.C., & Prudêncio. E.S. (2009). Influence of different content of cheese whey and oligofructose on the properties of fermented lactic beverages: Study using response surface methodology. *LWT - Food Science and Technology*, 42, 993-997.

Chien, H-L., Huang, H-Y., & Chou, C-C. (2006). Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and bifidobacteria. *Food Microbiology*, 23, 772-778.

Cicek, M. *Genetic marker analysis of three major carbohydrates in soybean seeds.* Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University for the degree of Doctor of Philosophy in Crop and Soil Environmental Sciences. Blacksburg, Virginia, 2001. Cap III Determination of carbohydrate content in soybean seeds using high pressure liquid chromatography, 33-52.

Costa, A.V.S., Nicolau, E.S., Torres, M.C.L., Fernandes, P.R., Rosa, S.I.R., & Nascimento, R.C. (2013). Desenvolvimento e caracterização físico-química, microbiológica e sensorial de bebida láctea fermentada elaborada com diferentes estabilizantes/espessantes. *Semina: Ciências Agrárias*, 34, 209-226.

Cunha, T.M., Castro, F.P., Barreto, P.L.M., Benedet, H.D., & Prudêncio, E.S. (2008). Avaliação físico-química, microbiológica e reológica de bebida láctea e leite fermentado adicionados de probióticos. *Semina: Ciências Agrárias*, 29, 103-116..

Debon, J., Prudêncio, E.S., Petrus, J.C.C., Fritzen-Freire, C.B., Müller, C.M.O., Amboni, R.M.C., &Vieira, C.R.W. (2012). Storage stability of prebiotic fermented milk obtained from permeate resulting of the microfiltration process. *LWT - Food Science and Technology*, 47, 96-102.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology*, 38, 105-112.

Farnworth, E.R., Mainville., Desjardins, M.-P., Gardner., & Fliss, C.I. (2007) Growth of probiotic bacteria and bifidobac-teria in a soy yogurt formulation. *International Journal of FoodMicrobiology*, 116, 174-181.

Fletcher, R.J. (2003). Food sources of phyto-oestrogens and their precursors in Europe. *British Journal of Nutrition*, 89, 39-43.

Ganry, O. (2002). Phytoestrogen and breast cancer prevention. *European Journal of Cancer Prevention*, 11, 519-22.

Gomes, A.M.P., & MALCATA, F.X. (1999). Bifidobacterium spp. and Lactobacillus acidophilus: biochemical, technological and therapeutical

properties relevant for use as probiotics. *Trends in Food Science and Technology*, 10, 139-157.

Hernández, M.J. (1996). *Caracterización reológica de hidrogeles de MCCNaCMC + almidón. Tixotropía y sinergismo*. Ph.D. Thesis. Universitat de València, Valência, Spain.

Howard, A. N., Nigdikar, S. V., Rajput-Williams, J., & Williams, N. R. (2000). *Food supplements*. United States Patent, 6086910.

Instituto Adolfo Lutz (IAL). (2005). *Normas Analíticas do Instituto Adolfo Lutz* (4thed.). São Paulo, DC: IAL.

Jaziri, I., Ben Slama, M., Mhadhbi, H., Urdaci, M. C., & Hamdi, M. (2009). Effect of green and black teas (Camellia sinensis L.) on the characteristic microflora ofyogurt during fermentation and refrigerated storage. *Food Chemistry*, 112, 614e620.

Jung, J-Y., Chung, H-J., & Kim, W-J. (2005). Influence of Addition of Membrane-Filtered Powder of Sunmul on the Quality Characteristics of Yogurt. *Journal of the Korean Society of Food Science and Nutrition*, 34, 1579-1585.

Kailasapathy, K., & Sultana, K. (2003). Survival and β-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream. *Australian Journal of Dairy Technology*, 58, 223-227.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT - Food Science and Technology*, 39, 1221-1227.

Kano, M., Takayanagi, T., Harada, K., Sawada, S., & Ishikawa, F. (2006). Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *The Journal of Nutrition*, 136, 2291-2296.

Karazhiyan, H., Razavi, S. M. A., Phillips, G. O., Fang, Y., Al-Assaf, S., Nishinari, K., & Farhoosh, R. (2009). Rheological properties of *Lepidium sativum* seed extract as a function of concentration, temperature and time. *Food Hydrocolloids*, 23, 2062-2068.

Kennedy, I.R., Mwandemele, O.D., & McWhriter, K.S. (1985). Estimation of sucrose, raffinose and stachyose in soybean seeds. *Food Chemistry,* 17, 85-93.

Kim, W.J., Kim, H.H., & Yoo, S.H. (2005). Ultra and nano-filtration process optimation of isoflavones and oligosaccharides from sunmul. *Food Science and Biotechnology,* 14, 380-386.

Kong, F., Chang, S.K.C., Liu, Z., & Wilson, L.A. (2008). Changes of soybean quality during storage as related to soymilk and tofu making. *Journal of Food Science*, 73, 134-144.

Lee, H. C., Jenner, A. M., Low, C. S., & Lee, Y. K. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in Microbiology*, 157, 876-884.

Li-Jun, Y., Li-Te, L., Zai-Gui, L., Tatsumi, E., & Saito, M. (2004). Changes in isoflavone contents and composition of sufu (fermented tofu) during manufacturing. *Food Chemistry*, 87, 587-592.

McCann, T. H., Fabre, F., & Day, L. (2011). Microstructure, rheology and storage stability of low-fat yogurt structured by carrot cell wall particles. *Food Research International*, 44, 884–892.

Matsubara, Y., Iwasaki, K., Nakajima, M., Nabetani, H., & Nakao, S. (1996). Recovery of oligosaccharides from steamed soybean waste water in tofu processing by reverse osmosis and nanofiltration membranes. *Bioscience, Biotechnology and Biochemistry*, 60, 421-428.

Mohameed, H.A., Abu-Jdayil, B., & Al-Shawabkeh, A. (2004). Effect of solidsconcentration on the rheology of labneh (concentrated yogurt) produced from sheep milk. *Journal of Food Engineering*, 61, 347–352.

Murakami, A.N.N., Amboni, R.D.M.C., Prudêncio, E.S., Amante, E.R., Zanotta, L.M., Maraschin, M., Petrus, J.C.C. & Teófilo, R.F., 2011. Concentration of phenolic compounds in aqueous mate (Ilex paraguariensis A. St. Hil) extract through nanofiltration. *LWT-Food Science and Technology*, 44, 2211-2216.

Najgebauer-Lejko, D., Sady, M., Grega, T., & Walczycka.M. (2011). The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt. *International Dairy Journal*, 21, 568-574.

Pan, B., Yan, P., Zhu, L., & Li, X. (2013). Concentration of coffee extract using nanofiltration membranes. *Desalination*, 317, 127-131.

Prudêncio, A.P.P., Prudêncio, E.S., Amboni, R.D.M.C., Murakami, A.N.N., Maraschin, M., Petrus, J.C.C., Ogliari, P.J. & Leite, R.S. (2012). Phenolic composition and antioxidant activity of the aqueous extract of bark from residues from mate tree (Ilex paraguariensisSt. Hil.) bark harvesting concentrated by nanofiltration. *Food and Bioproducts Processing*, 90, 399-405.

Ramaswamy, H.S., & Basak, S. (1992). Pectin and Raspberry Concentrate Effects on the Rheology of Stirred Commercial Yogurt. *Journal of Food Science*, 57, 357-360.

Rawson, H. L., & Marshall, V. M. (1997). Effect of 'ropy' strains of *Lactobacillus delbrueckii* ssp.*bulgaricus* and *Streptococcus thermophilus* on rheology of stirred yogurt. *International Journal of Food Science and Technology*, 32, 213-220.

Reynolds, K., Chin, A., Lees, K.A., Nguyen, A., Buinowski, D., & He, J. (2006). A meta-analysis of the effect of soy protein supplementation on serum lipids*The American Journal of Cardiology*, 98, 633–640.

Riener, J., Noci, F., Cronin, D.A., Desmond, J.M., & Lyng, J.G. (2010). A comparison of selected quality characteristics of yoghurts prepared from thermosonicated and conventionally heated milks. *Food Chemistry*, 119, 1108-1113.

Rinaldoni, A. N., Tarazaga, C. C., Campderrós, A., & Pérez Padilla, A. (2009). Assessing performance of skim milk ultrafiltration by using technical parameters. *Journal of Food Engineering*, 92, 226-232.

Rinaldoni, A.N., Campderrós, M.E., & Pérez Padilha, A. (2012). Physico-chemical and sensory properties of yogurt from ultrafiltreted soy Milk concentrate added with inulin. *LWT - Food Science and Technology*, 45, 142-147.

Roberfroid, M. (2007). Prebiotics: The concept revisited. *The Journal of Nutrition*, 137, 830S–837S.

Robinson, R. K. (2002). Yoghurt types and manufacture. In H. Roginsky, J.W. Fuquay, & P. F. Fox (Eds.), *Encyclopedia of dairy sciences*. London: Academic Press.

Ross-Murphy, S.B., 1990. In: Dickinson, E. (Ed.), *Food Polymer*, Gels and Colloids. University of Leeds, United Kingdom.

Rossi, E.A., Rosier, I., Dâmaso, A.R., Carlos, I.Z., Vendramini, R.C., Abdalla, D.S.P., Talarico, V.H., & Minto, D.F. (2004). Determinação de isoflavonas nas diversas etapas do processamento do "iogurte" de soja. *Alimentos e Nutrição*, 15, 93-99.

Servilli, M., Rizallo, C.G., Taticchi, A., Esposto, S., Urbani, S., Mazzacan, F., Di Maio, I., & Selvaggini, R. (2011). Functional milk beverage fortified with phenolic compounds extracted from olive vegetation water, and fermented with functional lactic acid bacteria. *International Journal of Food Microbiology*, 147, 45-52.

Sobral, P.A., & J.R. Wagner. (2007). Thermal properties of soybean whey and its protein. In *Functional Properties of Food Components*. Kerala: Lupano. pp. 57-76.

Staffolo, M. D., Bertola, N., Martino, M., & Bevilacqua, A. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal*, 14, 263–268.

Suárez, E., Lobo, A., Alvarez, S., Riera, F.A., & Alvarez, R. (2009). Demineralization of whey and milk ultrafiltration permeate by means of nanofiltration. *Desalination*, 241, 272-280.

Toneli, J.T.C.L., Mürr, F.E.X., & Park, K.L., 2005. Revisão: Estudo da reologia de polissacarídeos utilizados na indústria de alimentos. *Revista Brasileira de Produtos Agroindustriais*, 7181,–204.

Varga , L. (2006) Effect of acacia (*Robinia pseudo-acacia* L.) honey on the characteristic microflora of yoghurt during refrigerated storage. *International Journal of Food Microbiology*, 108, 272 -275.

Wang, H-J., & Murphy, P.A. (1996). . Mass Balance Study of Isoflavones during Soybean Processing. *Journal of Agricultural and Food Chemistry*, 44, 2377-2383.

Wang, Y.N., & Tang, C.Y. (2011). Protein fouling of nanofiltration, reverse osmosis, and ultrafiltration membranes- The role of hydrodynamic conditions,solution chemistry, and membrane properties. *Journal of Membrane Science*, 376, 275-282.

Yin L.J., Li, L.T., Liu, H., Saito, M., & Tatsumi E (2005) Effects of fermentation temperature on the content and composition of isoflavones and β-glucosidase activity in sufu. Bioscience, Biotechnology and Biochemistry, 69, 267-272.

CONSIDERAÇÕES FINAIS

A crioconcentração e a nanofiltração mostraram-se técnicas promissoras para a concentração de soro de tofu, visando a recuperação dos compostos bioativos. Em ambos os processos, observou-se um aumento gradual no conteúdo de isoflavonas ao longo das etapas de concentração.

Os processos de crioconcentração e nanofiltração também possibilitaram o aumento do potencial antioxidante do soro de tofu através da sua concentração, estando positivamente correlacionado com o aumento do conteúdo de isoflavonas. A crioconcentração promoveu o aumento do conteúdo de matéria seca total e apresentou eficiência de concentração superior a 80 % em todas as etapas. Na nanofiltração, o maior potencial antioxidante foi observado no concentrado obtido no fator de redução volumétrica igual a 4. A atividade antioxidante em ambos os concentrados foi correlacionada principalmente com as agliconas e malonil glicosídeos presentes no soro de tofu.

A utilização do soro de tofu concentrado na elaboração de bebidas lácteas fermentadas com propriedades biológicas não afetou a sobrevivência das bactérias ácido-láticas, embora tenha contribuído para o processo de pós-acidificação das bebidas ao longo do período de armazenamento.

A adição de soro de tofu concentrado na bebida láctea fermentada 2 (20 % de soro de tofu concentrado + 80 % de leite) contribuiu para a redução do teor de sólidos totais, proteínas e aumento do índice de sinerese. Além disso, foi possível detectar tendência à coloração amarelo-esverdeada em todas as amostras. O modelo Lei da Potência foi adequado para descrever as propriedades de fluxo das bebidas lácteas fermentadas. Ambas apresentaram comportamento *shear thinning* e propriedades tixotrópicas, típico de fluido não-newtoniano.

As isoflavonas totais permaneceram inalteradas, enquanto o conteúdo de oligossacarídeos diminui ao longo do período de armazenamento na bebida láctea fermentadas 2. Finalmente, o uso da nanofiltração para concentrar o soro de tofu e o uso do seu concentrado na elaboração de uma bebida láctea fermentada especial com atividade biológica oferece uma oportunidade ainda não explorada de utilização do soro de tofu, agregando valor a esse resíduo agroindustrial de processamento de soja.

SUGESTÕES PARA TRABALHOS FUTUROS

a) Otimizar as condições operacionais da nanofiltração e crioconcentração para os ensaios de concentração do soro de tofu;
b) Desenvolver novas formulações de bebidas lácteas fermentadas saborizadas adicionadas de soro de tofu concentrado;
c) Realizar a análise sensorial das bebidas lácteas produzidas;
d) Propor a aplicação do soro de tofu concentrado em outros tipos de alimentos.

ANEXOS

ANEXO A

Journal of Food Engineering 116 (2013) 681–688

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Behavior of functional compounds during freeze concentration of tofu whey

F. Belén [a], S. Benedetti [b], J. Sánchez [a], E. Hernández [a], J.M. Auleda [a], E.S. Prudêncio [c], J.C.C. Petrus [b], M. Raventós [a,*]

[a] Agri-Food Engineering and Biotechnology Department, Technical University of Catalonia (UPC), Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
[b] Department of Chemistry and Food Engineering, Technology Center, Federal University of Santa Catarina, Trindade, 88040-900 Florianópolis, SC, Brazil
[c] Department of Food Science and Technology, Agricultural Sciences Center, University of Santa Catarina, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001 Florianópolis, SC, Brazil

ARTICLE INFO

Article history:
Received 15 March 2012
Received in revised form 12 December 2012
Accepted 18 January 2013
Available online 1 February 2013

Keywords:
Isoflavones
Freeze concentration
Wastewater tofu

ABSTRACT

The liquid waste generated from tofu production, denominated as Tofu Whey (TW), contains important amounts of isoflavones that can be evaluated by various separation techniques. In this work TW was concentrated by falling-film freeze concentration from 1.9 to 15.5 °Brix, up to levels of 208 mg of isoflavones per 100 g solids. The levels of isoflavones, proteins, sugars, calcium and magnesium were determined both in the ice and in the concentrate obtained. It was found by rheological characterization that TW shows a Newtonian behavior at concentrations between 1.9 and 15.5 °Brix with freezing points of −0.6 to −2.7 °C.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Tofu is the main processed soybean product in the world and the TW, the liquid that oozes out of soybean curd during processing, is therefore an important soybean processing by-product. This represents an environmental problem for direct disposal as the TW deteriorates very quickly because of its high water content and its high content of nutritious substances for bacteria. Although TW contains high quantities of beneficial nutrients, most tofu by-products are used as animal feed, fertilizer or simply disposed of as liquid waste (Matemu et al., 2009). Jackson et al. (2002) reported that this effluent contains soluble salts and carbohydrates (oligosaccharides) and also significant levels of isoflavones resulting from the dissolution of these in the water during processing. According to Wang and Murphy (1996), the loss of isoflavones in the liquid waste during the tofu processing reaches 44%.

Isoflavones are a group of naturally occurring hetero cyclic phenols, called phytoestrogens, found mainly in soybean. Isoflavones have been claimed to perform several health-promoting functions. Kim et al. (2005) reported that these health claims place soybean products into a select category of functional foods that possess good overall nutritional values apart from the specific health benefits. The benefits provided by the isoflavones and oligosaccharides from soybeans include positive effects on patients with the following diseases: cancer of breast, prostate, and colon (Espinosa-Martos et al., 2006; Ounis et al., 2008; Kennedy, 1993; Nagata et al., 2007), cardiovascular diseases (Sacks et al., 2006), osteoporosis (Taku et al., 2011) and menopausal symptoms (Taku et al., 2010). Oldoni et al. (2011) reported that a dietary consumption of foods and food additives containing isoflavone phytoestrogens have been associated with several beneficial properties to human health, such as prevention of coronary heart disease and osteoporosis; and reduction of menopausal symptoms.

Soybean contains 12 different phytoestrogens, divided into the following four chemical forms: aglycones (genistein, daidzein and glycitein), β-glucosides (genistin, daidzin and glycitin), malonyl glucosides (6′-O-malonilgenistin, 6′-O-malonildaidzin and 6′-O-malonilglycitin) and acetyl glucosides (6′-O-acetilgenistin, 6′-O-acetildaidzin and 6′-O-acetil glycitin). However, Shao et al. (2009) reported that it has been established that the bioavailability of isoflavones can be influenced by their chemical form. Already, Jackson et al. (2002) shown that some isoflavones could be lost during the processing steps, as obtention of soy products. Since some studies have shown that the chemical form and the content of isoflavones may depend of process used, Rostagno et al. (2005) highlights the importance of studies about the influence of processing and behavior of isoflavones aimed at industrial use.

The composition and properties of TW make it a reusable effluent with potential applications (Espinosa-Martos et al., 2006). The management of this waste with high water content represents an economic problem because of the high transportation costs for its disposal, treatment and/or use. The concentration of TW thus becomes a necessary first step for its waste management and evaluation. In principle one could use evaporation as concentration process, but unfortunately this can cause damage to heat sensitive components or loss of volatile compounds (Bakshi and Johnson,

* Corresponding author.
E-mail address: mercedes.raventos@upc.edu (M. Raventós).

1983), so that alternative concentration processes are needed. One possible option is the use of freeze concentration, a process operated at low temperatures, which favors a good retention of thermosensible components.

The literature usually indicates that the concentration of isoflavones in soy foods and soy ingredients may vary with the genetics of soybean cultivars and climatic conditions during the cultivation. In addition, processing techniques also may affect the concentration of isoflavones. Reports in the literature usually indicate that isoflavones are rather stable compounds affected by heat only with regard to their specific conjugated form and there is little evidence for thermal degradation of these compounds. The change of the isoflavone aglycones in soymilk during heat processing is of particular interest, because there are indications that the aglycones, especially genistein, show the greatest biological activity (Huang et al., 2006). Thus, the use of low temperature is important to maintain the functional properties of the isoflavones during the concentration process (Góes-Favoni et al., 2004). Freeze concentration, as solution for separations of most the substances from the solution, has been studied from year ago. This would be a good alternative for treatment of wastewater the food and chemical industrially. This method require small amount of power compared with the high temperature technology such as evaporations.

By falling-film freeze concentration technology tofu industry can recover soluble solids in the effluents, the TW volume can be reduced and in this way its environmental management can be improved (Beléin et al., 2012). The aim of this study was to identify the effect of falling-film freeze concentration of TW on the content of isoflavones, proteins, sugars, calcium and magnesium in both the ice and concentrate obtained. Furthermore, the freezing point and rheological behavior of the TW during the steps of freeze concentration was determined.

2. Materials and methods

2.1. Samples of Tofu Whey (TW)

Fresh TW was supplied by NATURSOY SL, Alimentos Naturales and Biológicos (Castelltercol, Barcelona-Spain). 840 kg of fresh TW, at an initial concentration of 1.9 °Brix, were freeze concentrated. The TW freeze-concentration process was carried out in three stages maintaining the average flow rate at $1 \pm 0.2\ L\,s^{-1}$ to ensure good contact between the evaporator plates and the fluid being concentrated. The equipment used in this study was described in detail by Sánchez et al. (2010) and is shown in Fig. 1. Freeze concentrated samples of TW (6.0–15.5 °Brix) and ice were obtained from the test developed by Beléin et al. (2012), according to Fig. 2. Samples of concentrate and ice were collected after each step of freeze concentration.

For each freeze concentration test, the following samples were analyzed: fresh TW in stage 0, the final concentrates (CF1, CF2, and CF3) and ice fractions (I1, I2, and I3) for stages 1, 2 and 3 respectively. Analyses included the determination of concentration of proteins, sugars, calcium, magnesium and isoflavones. Moreover, the freezing point depression and the rheological behavior were determined for fresh TW, CF1, CF2 and CF3. The soluble solid concentration in each concentrates was measured with an Atago refractometer (model DBX-55; Barcelona, Spain), and in accordance with Beléin et al. (2012), they were equal to 1.9, 6.0 and 11.0 and 15.5 °Brix to TW, CF1, CF2 and CF3, respectively.

2.2. Physicochemical analysis

The protein content ($g\,100\,g^{-1}$) in the various samples was determined by Kjeldahl analysis ($N \times 5.70$) in an automatic Kjeldahl distillation equipment (Pro-Nitro II-measuring range 1–140 mg of nitrogen), according to AOAC (2005). Following Sánchez et al. (2010), with modifications, the total sugar content ($g\,L^{-1}$) were determined through High Performance Liquid Chromatography (HPLC, Beckman, San Ramon, USA) equipped with two Beckman 110B pumps; injector Hewlett Packard Series 1100; refractive index detector Beckman 156; Hewlett Packard ChemStation software; and Phenomenex Luna NH2 100 Å (250 × 4.6 mm) column, 5 μm particles. The mobile phase used was acetonitrile: water (75:22); while the flow rate was 1.2 mL min^{-1} and the injection volume was equal to 20 μL. All these analyses were carried out in triplicate.

The concentration of calcium and magnesium in the various samples was measure by flame atomic adsorption, according to AOAC (2005). The equipment used was supplied by Varian, model spectra AA110. The references used for calcium and magnesium were solutions of 1–10–25–100 mg L^{-1} and 1–5–10–20 mg L^{-1} respectably. 10 mL of liquid was used both for the samples and for the references; and 100 μL of strontium chloride ($SrCl_2$) was added at 200 g L^{-1}. The wavelengths used were 422.7 nm and 285.2 nm for calcium and magnesium, respectively.

2.3. Isoflavone extraction and determination

Twenty milliliter of each sample were lyophilized firstly (freeze-dryer; CRYODOS-45, Telstar, Barcelona, Spain), in triplicate. The extraction of isoflavones and the determination of their components were carried out with samples dried in accordance with methodology proposed by Carrão-Panizzi et al. (2002), with modifications. One hundred of lyophilized samples were transferred to a 10 mL test tube, into which 4 mL of an extracting solution (70% ethanol and 0.1% acetic acid) were added. The test tubes with the samples and the extracting solution were stirred in a Vortex (Model MA162, MARCONI®, Piracicaba, SP, Brazil). The extraction was realized during 1 h at 25 °C, with stirring at each 15 min. The test tubes were then placed into an ultrasound bath (Model USC5000, UNIQUE®, Indaiatuba, SP, Brazil) and left for 30 min. A 1.5 mL aliquot of this extract was transferred to a refrigerated microcentrifuge (dimensions of 31 × 60 × 25 cm, 35 kg, Model 5417R 230 V/50 Hz, EPENDORF®, São Paulo, Brazil) and centrifuged at 20,800 g for 15 min at 5 °C. The supernatant was filtered through 0.45 μm filters (MILLIPORE®, Billerica, MA, USA) and 20 μL was used to separate and quantify the isoflavones through chromatography. The separation and the quantification of the isoflavones were performed using HPLC, as proposed by Berhow (2002), with a photodiode array detector (Model 996) and an automatic sample injector (Model 717 Plus), both manufactured by WATERS® (Milford, USA). In this stage, a reverse phase column (YMC-Pack ODS-AM, C18, 5–5 μm, diameter of 250 × 4.6 mm) was used. For the separation of isoflavones, the binary linear gradient system was used and the mobile phases were: (a) methanol containing 0.025% trifluoroacetic acid (TFA) (Phase A) and (b) ultrapure water (MILLIPORE®, Billerica, MA, USA) containing 0.025% TFA (Phase B). The initial condition of the gradient was 20% in Phase A, reaching 90% in 35 min, followed by cleaning of the column with 100% of Phase A for 5 min and subsequently return to 20%, retaining these conditions for up to 60 min. The mobile phase flow was of 1 mL min^{-1} and the temperature during the analysis was 25 °C. For the isoflavone detection, the wavelength of the detector was adjusted to 254 nm. The software used to control the equipment and the data acquisition was Millennium 32 (version 3.05.01) (GCIC® Toronto, Pickering, ON, Canada). For the identification and quantification of the peaks corresponding to each one of the isoflavones, calibration curves with linear regression based on the peak areas were used. These calibration curves were constructed with external standards of daidzin, daidzein, genistin,

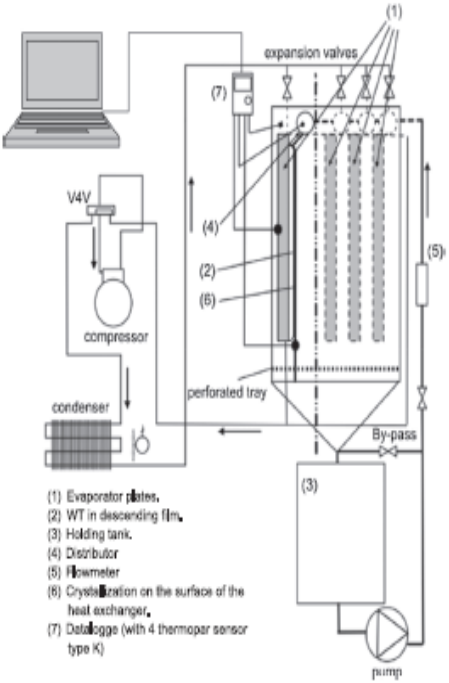


Fig. 1. Schematic experimental equipment for freeze concentration.

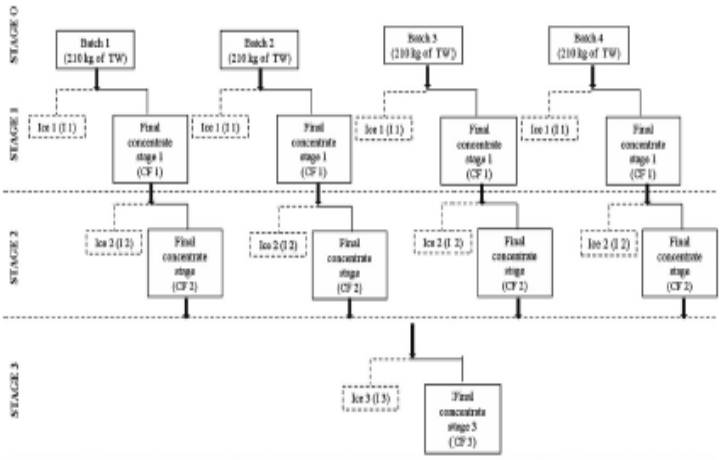


Fig. 2. Flowchart of the three concentration stages of TW.

genistein, glycitin, glycitein, malonyl daidzin, malonyl genistin, malonyl glicitin, acetyl daidzin, acetyl genistin and acetyl glycitin, all of which were solubilized in methanol (chromatographic grade) to the following concentrations: 0.00625 mg mL^{-1}; 0.0125 mg mL^{-1}; 0.0250 mg mL^{-1}; 0.0500 mg mL^{-1} and 0.1000 mg mL^{-1}. The results of isoflavones were expressed as mg of isoflavones per 100 g of solid sample. All these analyses were performed in duplicate.

2.4. Rheological behavior

A rotational viscosimeter with coaxial cylinder (Visco Star plus, Fungilab, S.A., Barcelona, Spain) equipped with an adapter LCP/B for low viscosities, a spindle with a radius of 9.5 mm and overall length of 65 mm. The viscometer contained an adapter LCP/B for low viscosities that works with a sample volume of 18 mL. The temperature of the samples was controlled whit a Data Logger, Testo 1777-T4; Barcelona-Spain, connected by a sensor type K probe (NiCr–NiAl sensor). Data analysis was performed using the data logger "Testo Comfort Software". The measures of viscosity was carried out at constant rotational speed with a value in the range of 50–200 rpm and at temperatures of −2 °C; −1 °C; 0 °C; 1 °C; 2 °C and 4 °C respectably. The cryostat used was Polyscience, model 9505; USA using a mixture of ethylene glycol and water. An Arrhenius-type model, given in Eq. (1), was used to describe the effect of temperature on the viscosity of TW and its concentrates (at 1.9; 6.0; 11.0 and 15.5 °Brix).

$$\eta = k_0 \exp\left(\frac{E_a}{RT}\right) \quad (1)$$

where η represents the dynamic viscosity (mPa s), K_0 is a constant (mPa s), E_a is the activation energy for viscous flow (KJ mol^{-1}), R is the gas constant (KJ mol^{-1}) and T the absolute temperature (K). From the slope of the plot of ln η versus $1/RT$, the E_a was determined for TW and all concentrates. These analyses were repeated four times.

2.5. Determination of freezing point

Cooling curves were obtained from samples of fresh TW at 1.9 °Brix and of freeze-concentrated TW samples at 6.0, 11.0, and 15.5 °Brix. For each concentration the freezing point depression (Δt) was identified, based on the cooling curve (Fig. 3). The experimental set up and the method used have been described by Sánchez et al. (2011). The freezing point measures were repeated four times.

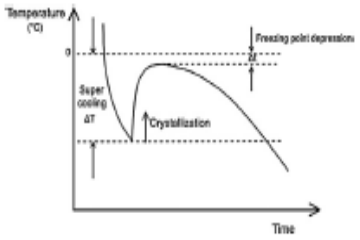


Fig. 3. Schematic diagram of cooling curve.

2.6. Statistical analysis

Statistical data analysis was carried out using the multivariate statistical software package Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA; 2004). Analysis of variance (ANOVA) and Tukey's studentized range test were carried out to detect any significant differences ($p < 0.05$). The correlation coefficient (R^2) was used to determine the best fit for the curves.

3. Results and discussion

3.1. Physicochemical analysis

As expected, the protein content increased in the concentrates 1 and 2 (CF1 and CF2), i.e. the concentrates obtained in stage 1 and 2 of the freeze concentration process. However, in stage 3 the protein content of the concentrate (CF3) decreased (Fig. 4). Noh et al. (2006) reported that the coagulation of soymilk, in which TW is obtained, involves a two-step process: first protein denaturation by heating, followed by hydrophobic coagulation promoted by coagulants. Cruz et al. (2007) and Tang (2007) reported that the details of these two process steps, together with the details of the soybean composition, and the processing methods that are used to obtain the soymilk could influence the stability of the proteins. In addition to these various processing steps, the TW was submitted to three stages of freeze concentration. Thus it seems reasonable to assume that these stages have caused some changes in the protein characteristics.

The increase in protein content for CF1 and CF2 could be due to the fact that the protein becomes partially insoluble in water and thus remaining in higher concentration in the concentrates (CF1 and CF2) than in the ice. According to Noh et al. (2006) upon freezing a protein solution, the soy protein became partially insoluble due to the polymerization of protein molecules through the formation of intermolecular disulfide bonds. Thus, it appears that the two first stages of freeze concentration process increase the hydrophobicity of soy proteins. During these stages the proteins possibly interact with each other and form larger aggregates. According to Chen et al. (2000) smaller molecules in solution are more easily trapped inside the ice structure, whereas larger molecules are less likely to be included in the ice fraction.

The decrease observed in the protein content of CF3 might indicate that the freezing of soy proteins causes a change in conformation of soy proteins, as observed by Vishwanathan et al. (2011) for soybean products. This result suggests that during the freezing of CF2 large aggregates of proteins are destroyed thus causing a decrease in mean particle size, leading to the decrease in protein con-

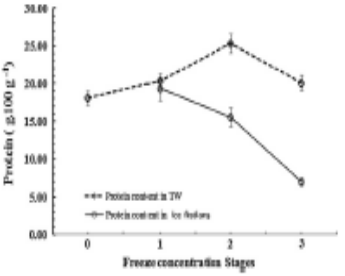


Fig. 4. Mean protein content (±standard deviation) in: fresh TW, freeze concentrated TW (CF1, CF2, CF3) and ice fractions (I1, I2, I3).

tent of CF3. Moreover, Li et al. (2009) reported that larger aggregates preferentially form a separate phase because of depletion interaction.

The results of total sugar content of concentrates and ices fractions are shown in Fig. 5. The concentration of total sugars in the concentrate show a trend of linear growth (R^2 = 0.94) during the entire process. The concentration of total sugars in the ice follows a logarithmic trend (R^2 = 0.94). Poysa and Woodrow (2002) reported that the process of making tofu involves a complex interaction of many factors, including the chemical composition and physical attributes of the soybean, processing techniques and processing conditions. According to Pednekar et al. (2010), the interactions between all these factors could cause changes to the sugar content of the soybean. Karr-Lilienthal et al. (2005) reported that during processing, barriers are removed and the carbohydrates of soybean undergo transformations that include a modification of their crystallinity and a depolymerization. This behavior is possibly due to the arrangement of these sugars and their affinity for other components of TW. Hydrophilic matrices can be formed in the fluid, through covalent bonds between sugars and these molecules (Ricklert et al., 2004). Gu et al. (2009) indicated that a change in the net charge of proteins is observed, caused by the glycation reaction.

The results of magnesium content of the concentrate and ice fractions are shown in Fig. 6. As expected the magnesium level increased in the concentrate during all stages of freeze concentration. Prabhakaran and Perera (2006) reported that the coagulation of soymilk is the most important step in the production process of tofu. There are different types of coagulants used on industrial scale. The TW used in this study had been coagulated with "Nigari", a salt extracted from sea water composed primarily of magnesium chloride. The magnesium present in the TW is probably an excess of coagulant from the coagulation process. According to Chai et al. (1999), the divalent cations added to soy milk as coagulant make that the wastewater from this process requires further treatment before discharging. Ounis et al. (2008) demonstrated the feasibility to recover the salts of the TW and use these as growth medium to microorganisms in fermentation processes.

Fig. 7 shows the content of calcium in the concentrate and ice fractions. In the concentrate an increase in calcium content is observed until the second stage, followed by a reduction in the third stage, as observed for the proteins. The calcium present in the TW results mainly from the soymilk and a minor part could be from the nigari too. According to Liu (1997), during the heating and coagulation processes, the calcium present in soymilk possibly competes with the hydrogen ions for the same binding sites on the soy proteins between pH 3 and 7. The bond between calcium and proteins can explain the similarities observed for their behavior in freeze concentration.

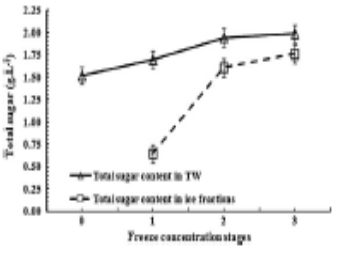


Fig. 5. Mean total sugar content (standard deviation) in: fresh TW, freeze concentrated TW (CF1, CF2, CF3) and ice fractions (I1, I2, I3).

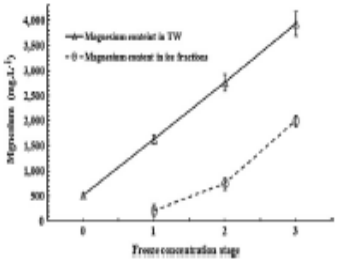


Fig. 6. Mean magnesium content (standard deviation) in: fresh TW, freeze concentrated TW (CF1, CF2, CF3) and ice fractions (I1, I2, I3).

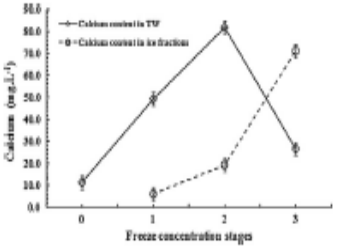


Fig. 7. Mean calcium content (standard deviation) in: fresh TW, freeze concentrated TW (CF1, CF2, CF3) and ice fractions (I1, I2, I3).

3.2. Isoflavone contents

The isoflavone contents in the samples of tofu whey (TW), in the concentrates fluids (CF1, CF2, CF3) and ices fractions (I1, I2, I3) for each of the three stages of freeze concentration are shown in Table 1. In the TW were not detected β-glucosides. Different profile and content of isoflavones were obtained by Shao et al. (2009). These authors reported that factors such as ionic strength, pH and the endogenous β-glucosidase activity will be responsible by these behaviors. The results obtained still shows that TW has high content of isoflavones, highlighting the content of malonyl glucosides and aglycones. These results are also in accordance with that obtained by Sudyani et al. (2007), which reported that TW has a representative content of genistin and daidzin.

Although, the evidence in the literature suggests that the biological effects of soy isoflavones depend upon the aglycone form, Lee and Choung (2011) affirm that the recent trend has been to quantify all type of derivative consumed, as these may have differing biological activities. Kao et al. (2004) reported that differences in the content of isoflavones could be credited to the type of coagulant used in the preparation of tofu, which is responsible by interactions between proteins and isoflavones. Already, Speroni et al. (2010) affirm that the final isoflavone content in a given soybean-derived product depends on the association/release of isoflavones and proteins during each step of production. According to these authors during the process of tofu the various isoflavones may establish different kinds of interactions with proteins because of their diverse polarity and hydrophobicity as well as their ability to form hydrogen bonds. Siebert (1999) also noted that an important role in this molecular linkage would be the degree of stacking between the polyphenol rings and the aliphatic moieties of the proline residues of proteins. In this regard, Siebert (1999) also cited

Table 1
Mean of results (standard deviation) of isoflavones content (mg isoflavones 100 g^{-1} LDM) in the TW, concentrates and ices fractions of each one three stages of freeze concentration process.

Samples	β-glucosides[b]			Malonyl glucosides[c]			Aglycones[d]			Total[e]
	Daidzin	Glycitin	Genistin	Malonyl daidzin	Malonyl glycitin	Malonyl genistin	Daidzein	Glycitein	Genistein	
TW	ND	ND	ND	24.56(0.06)^{a}	10.61(0.02)^{a}	19.13(0.01)^{a}	25.38(0.99)^{a}	27.95(2.63)^{a}	13.80(0.74)^{a}	121.51 (4.29)^{a}
CF1	10.49 (0.05)^{d}	5.56(0.10)^{e}	5.18(0.40)^{d}	14.57(0.01)^{d}	6.54(0.71)^{e}	8.84(0.21)^{d}	25.32 (0.52)^{a}	31.94(0.78)^{a}	13.51(0.37)^{a}	121.84(1.27)^{a}
CF2	17.42(0.08)^{a}	6.87(0.22)^{a}	9.20(0.34)^{a}	10.42(0.46)^{e}	5.24(0.02)^{e}	8.34(0.64)^{d}	20.42 (0.24)^{a}	44.41(0.86)^{a}	11.90(0.11)^{a}	134.23 (2.00)^{a}
CF3	13.97(0.11)^{b}	6.22(0.08)^{d}	8.57(1.93)^{b}	5.53(0.30)^{g}	2.52(0.03)^{f}	4.21(0.12)^{f}	20.79 (0.05)^{a}	31.29(2.27)^{a,d}	11.59(0.00)^{a}	104.68(4.50)^{b}
I1	ND	5.58(0.33)^{e}	2.29(0.01)^{h}	14.86(0.19)^{d}	4.88(0.21)^{g}	17.37(0.19)^{b}	54.34 (0.19)^{b}	80.67(4.64)^{b}	28.06(0.29)^{b}	203.01(5.08)^{a}
I2	12.81 (0.45)^{c}	9.33(0.59)^{b}	6.16(0.01)^{c}	17.65(0.09)^{b}	7.90(0.10)^{b}	14.19(0.01)^{c}	26.86 (0.36)^{a}	29.72(0.01)^{a}	15.74(0.03)^{a}	140.35(0.25)^{b}
I3	13.22 (0.16)^{c}	7.59(0.35)^{b}	7.64(1.23)^{b}	7.66(0.61)^{f}	2.32(0.10)^{g}	4.18(0.01)^{f}	21.93 (0.31)^{a}	32.16(0.73)^{a}	10.87(0.09)^{a}	107.57 (0.70)^{a}

ND = not detected.
[a–g] Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$).
[b] Total of β-glucosides expressed as the sum of the conjugates daidzin, genistin and glycitin.
[c] Total of malonyl glucosides expressed as the sum of the conjugates malonyl daidzin, malonyl glycitin and malonyl genistin.
[d] Total of aglycones expressed as the sum of the daidzein, glycitein and genistein.
[e] Total isoflavones content.

that the phenolic hydroxyl group is an excellent hydrogen donor for the formation of hydrogen bonds with the amide carbonyl of the peptide backbone of proteins.

It was verified a decrease in the isoflavones content ($p < 0.05$), as well as observed with the protein content of CF3. As related by Achouri et al. (2005) and Speroni et al. (2010), this fact also could be due to the interaction between the isoflavones, a polyphenolic compound, with components in the food matrix, such as the proteins. In the concentrates fluids higher isoflavones content ($p < 0.05$) was observed in the second stage of freeze concentration, which was possible to detect the presence of β-glucosides. Benedetti et al. (2011) also observed an increase in the content of these compounds after the concentration of aqueous extract of soybean.

In the present study, the highest content ($p < 0.05$) of isoflavones was observed in the ice from the first stage of freeze concentration (I1). This behavior could be due to the compounds of TW. As observed by Ayel et al. (2006) these compounds could be a negative impact on the freeze concentration resulting in the formation of a porous ice layer on the chilled surface (dendritic ice), with the solute retention in the dendritic ice layer. Besides, the size of the solutes could also affect their ability to integrate into the ice phase. The smaller molecules can be incorporated more easily into the ice structure than larger molecules in the freeze concentration (Gao and Shao, 2009). This may explain the behavior of isoflavones, which are molecules of low molar mass.

The difference ($p > 0.05$) between total isoflavones content of CF3 and I3 was not observed, because according to Burdo et al. (2008) the complete separation between concentrated and the pure water is not possible. According to Raventós et al. (2007), the freezing temperature of a solution is affected above all by the presence of solutes with low molar mass, since, for a given mass, the molar fraction of such compounds is higher than the molar fraction of a solute with a high molar mass. Besides, these authors also reported that although some substances have the same molar mass, the difference of the thermal conductivity can be explained the difference in the formation of ice, since it affects the heat transfer. Furthermore, the efficient separation of the compounds could be also related to the presence of low molar mass co-solute such as sugars. Gu et al. (2009) cited that the sugars can alter the conformation and interactions of proteins by binding to protein surface groups, or they may indirectly influence these characteristics by altering the physico-chemical properties of water. However, Baier and McClements (2005) emphasize that the interactions and their influence on protein functionality depend on the type and concentrations of co-solutes present.

The ice formed during all three stages of freeze concentration also showed a high content of isoflavones. The concentrations of β-glucosides and malonyl glucosides increased ($p < 0.05$) in the ice of the second stage of freeze concentration, agreeing with Sánchez et al. (2010), who claimed that the impurity of the ice increases with increasing concentration of the solution due to the retention of solids in the ice. As with other products with high levels of protein, an explanation for this is that soy proteins are able to bind with water molecules via hydrogen bonds. Yee et al. (2003) suggested that an alternative for recovery of this solids on the ice is the using the technique of fractional thawing.

The acetyl conjugates were not detected in the samples analyzed. According to Jung et al. (2008), the formation of these isomers occur only when the soy or soy-based products are subjected to high temperatures, as in the drying of soybeans, which was not used in the present study.

Table 2
Viscosities of TW samples.

Concentration (°Brix)	1.9	6.0	11.0	15.5
Temperature (°C)	η (mPa s)			
4	1.68	1.98	2.67	3.58
2	1.69	2.09	2.85	3.85
1	1.83	2.16	2.92	3.94
0	1.87	2.24	2.98	4.09
−1	1.91	2.29	3.07	4.29
−2	1.98	2.38	3.09	4.54

Table 3
Parameters of the Arrhenius model for freeze-concentrated TW in the temperature range from 4 °C to −2 °C.

Concentration (°Brix)	K_0 (mPa s)	E_a (kJ mol^{-1})	R^2
1.9	7.46×10^{-4}	17.74	0.93
6.0	3.76×10^{-4}	19.72	0.99
11.0	2.95×10^{-4}	20.03	0.99
15.5	1.68×10^{-4}	22.95	0.99

3.3. Rheological behavior

The increase in fluid viscosity during freeze concentration has been considered as one of the factors that limit the process (Raventós et al., 2007; Zhang and Hartel, 1996). To the knowledge of the present authors there has not been any published study on the rheological behavior of freeze-concentrated TW at temperatures near the freezing point. However, there have been some studies on the effect of freezing on the rheological properties of whey protein concentrate suspensions and freeze-concentrated whey (Meza et al., 2010; Bhargava and Jelen, 1995; Sánchez et al., 2011). Table 2, shows the experimental results for viscosity of freeze-concentrated

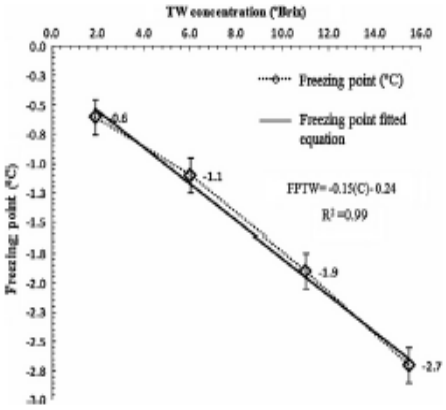


Fig. 8. Freezing point Vs. TW concentration.

TW. It was found that TW behaved as a Newtonian fluid over the entire range of temperatures and concentrations tested. Even at a concentration of solutes of 15.5 °Brix the obtained viscosity values were less than 5 mPa s. To show the temperature dependency of viscosity in terms of the Arrhenius equation a logarithmic plot of viscosity versus reciprocal temperature has been made. The Arrhenius equation fits well for all concentrations as shown by the correlation coefficients obtained. At increasing concentrations a clear increase in the flow activation energy (E_a) was observed (Table 3).

3.4. Determination of freezing point

The freezing point of a liquid depends on the concentration and type of solutes present in the solution (Hernández et al., 2009). It is well known that the presence of low-molecular-weight solutes (salts, sugars, etc.) cause a change in the freezing point of liquid foods. The higher the level of dissolved solids, the lower the freezing point (Gabas et al., 2003). The freezing point depression (Δt) was determined for TW and concentrates at various solid concentrations (Fig. 8). The experimentally determined freezing point depression increased with concentration of solids, as expected. Similar results were obtained by Sánchez et al. (2011) and Auleda et al. (2011a). The values of electrical conductivity of the concentrated phase and of the ice indicate a higher concentration of salts in the concentrated TW than that in the ice (Belén et al., 2012). This can explain the increase of Δt. Combining the graph of freezing point versus concentration with the correlation of Choi and Okos (1986), one can obtain the values of physico-chemical properties such as thermal conductivity, specific heat, thermal diffusivity and density (Ibarz and Barbosa, 2005). Once these properties have been determined, simulations can be made of the process of falling-film freeze concentration, which include the estimation of energy consumption and comparison with other processes, as suggested by Auleda et al. (2011b). Moreover, obtaining the values of these properties is of great importance for process design in the food industry.

4. Conclusions

Tofu whey (TW) contains isoflavones at levels around 120 mg 100 g^{-1} lyophilized dry matter. A relationship is observed between the concentration of isoflavones and proteins both in the freeze-concentrate of TW as in the ice layer obtained in each process stage. The ice of the first stage of freeze concentration retained about 208 mg isoflavones 100 g^{-1} lyophilized dry matter. TW has a Newtonian rheological behavior in the concentration range of 1.9–15.5 °Brix. Freezing points in this concentration range are between –0.6 °C and –2.7 °C.

Acknowledgements

The author Freddy A. Belén is grateful to FUNDAYACUCHO (Foundation Gran Mariscal de Ayacucho) of the Bolivarian Republic of Venezuela. Contact by e-mail: *freddy.belen@gmail.com*. Silvia Benedetti wish to thank Capes (Process number BEX 5166/11-0). José Sánchez is grateful to FONACIT (National Fund for Science and Technology) of the Bolivarian Republic of Venezuela. The company "NATURSOY". Hispano-Brazilian program of universitary cooperation, reference: PHB-20100017PC. The financial support in the Digite um texto ou endereço de um site ou traduza um documento. Cancelar Cooperation Project Capes-DGU 245/11, Brazil-Spain. Embrapa Soja (Empresa Brasileira de Pesquisa Agropecuária) and Silliker Ibérica, S.A. (Barcelona-Spain).

References

Achouri, A., Boye, J.I., Belanger, D., 2005. Soybean isoflavones: efficacy of extraction conditions and effect of food type on extractability. Food Research International 38, 1189–1204.

AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis of the Association Analytical Chemists, 18th ed. Maryland, USA.

Auleda, J.M., Raventós, M., Hernández, E., 2011a. Calculation method for designing a multi-plate freeze-concentrator for concentration of fruit juices. Journal of Food Engineering 107, 27–35.

Auleda, J.M., Raventós, M., Sánchez, J., Hernández, E., 2011b. Estimation of the freezing point of concentrated fruit juices for application in freeze concentration. Journal of Food Engineering 105, 289–294.

Ayel, V., Lottin, O., Faucheux, M., Sallier, D., Peerhossaini, H., 2006. Crystallisation of undercooled aqueous solutions: experimental study of free dendritic growth in cylindrical geometry. International Journal of Heat and Mass Transfer 49, 1876–1884.

Baier, K.S., McClements, D.J., 2005. Influence of cosolvent systems on the gelation mechanism of globular protein: thermodynamic, kinetic, and structural aspects of globular protein gelation. Comprehensive Reviews in Food Science and Food Safety 4, 43–54.

Bakshi, A.S., Johnson, R.M., 1983. Calorimetric studies on whey freeze concentration. Journal of Food Science 48 (4), 1279–1283.

Belén, F., Sánchez, J., Hernández, E., Auleda, J.M., Raventós, M., 2012. One option for the management of wastewater from tofu production: freeze concentration in a falling-film system. Journal of Food Engineering 110, 364–373.

Benedetti, S., Prudêncio, E.S., Mandarino, J.M.G., Rezadori, K., Petrus, J.C.C., 2011. Concentration of soybean isoflavones by nanofiltration and the effects of thermal treatments on the concentrate. Food Research International. http://dx.doi.org/10.1016/j.foodres.2011.04.03.

Berhow, M.A., 2002. Modern analytical techniques for flavonoid determination. In: Rusling, B.S., Manthey, J.A. (Eds.), Flavonoids in the Living Cell. Advances in Experimental Medicine and Biology, vol. 505. Kluwer Academic, New York, pp. 61–76.

Bhargava, A., Jalan, P., 1985. Freezing of whey protein concentrate solutions and its effect on protein functionality indicators. International Dairy Journal 5, 533–541.

Burdo, O.G., Kovalenko, E.A., Khanevko, D.A., 2008. Intensification of the processes of low-temperature separation of food solutions. Applied Thermal Engineering 28, 311–316.

Carrão-Panizzi, M.C., Favoni, S.P.G., Kikuchi, A., 2002. Extraction time for soybean isoflavone determination. Brazilian Archives of Biology and Technology 45 (4), 515–518.

Chai, X., Mi, Y., Yue, P.L., Chen, G., 1998. Beer curd wastewater treatment by membrane separation. Separation and Purification Technology 15, 175–180.

Chen, P., Chen, X.D., Free, K.W., 2000. An experimental study on the spatial uniformity of solute inclusion in ice formed from falling film flows on a subcooled surface. Journal of Food Engineering 39, 101–105.

Choi, Y., Okos, M.R., 1986. Effect of temperature and composition on the thermal properties of foods. In: le Maguer, M., Jelen, P. (Eds.), Food Engineering and Process Applications, Transport Phenomena, vol. 1. Elsevier, London, pp. 93–101.

Cruz, N., Capellas, M., Hernández, M., Trujillo, A.J., Guamis, B., Ferragut, V., 2007. Ultra high pressure homogenization of soymilk: microbiological, physicochemical and microstructural characteristics. Food Research International 40, 725–732.

Espinosa-Martos, I., Prieto-Sierra, G., Rupérez, P., 2006. Composición y propiedades del suero de soja, un subproducto de la elaboración del tofu. Alimentaria 371, 72–73.

Galvão, A.L., Tello-Romero, J., Tello, N.V.R., 2003. Influenced of fluid concentration on freezing point depression and thermal conductivity of frozen orange juice. International Journal of Food Properties 6 (3), 543–556.

Gao, W., Shao, Y., 2008. Reverse concentration for removal of pharmaceutically active compounds in water. Desalination 249, 388–402.

Góes-Favoni, S., Belêia, A.D.P., Carrão-Panizzi, M.C., Mandarino, J.M.G., 2004. Isoflavonas em produtos comerciais de soja. Ciência e Tecnologia de Alimentos 24 (4), 582–586.

Gu, X., Campbell, L.J., Euston, S.R., 2009. Influence of sugars on the characteristics of glucono-d-lactone-induced soy protein isolate gels. Food Hydrocolloids 23, 314–326.

Hernández, E., Raventós, M., Asúnda, J.M., Ibarz, A., 2009. Concentration of apple and pear juices in a multi-plate freeze concentrator. Innovative Food Science and Emerging Technologies 10, 348–355.

Huang, H., Liang, H., Kwok, K.C., 2006. Effect of thermal processing on genistein, daidzein and glycitein content in soymilk. Journal of the Science of Food and Agriculture 86, 1110–1114.

Ibarz, A., Barbosa, G., 2005. Operaciones unitarias en la ingeniería de alimentos. Ediciones Mundi-Prensa, vol. 12, pp. 339–350. (ISBN: 84-8476-163-0).

Jackson, C.J.C., Dini, J.P., Lahandier, C., Rupasinghe, H.P.V., Faulkner, H., Poysa, V., Buzzell, D., DeGrandis, S., 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. Process Biochemistry 37, 1117–1123.

Jung, S., Murphy, P.A., Sala, I., 2008. Isoflavones profile of soymilk as affected by high-pressure treatments of soymilk and soybeans. Food Chemistry 111, 592–598.

Kao, T.H., Ju, Y.F., Hsieh, H.C., Chen, B.H., 2004. Stability of isoflavone glucosides during processing of soymilk and tofu. Food Research International 37 (9), 891–900.

Kam-Lilienthal, L.K., Kadzere, C.T., Grimshop, C.M., Fahey, G.C., 2005. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: a review. Livestock Production Science 97, 1–12.

Kennedy, A.R., 1993. Anticarcinogenic activity of protease inhibitors. In: Troll, W., Kennedy, A.R. (Eds.), Protease Inhibitors as Cancer Chemopreventive Agents. Plenum Press, New York, pp. 9–64.

Kim, J.J., Kim, H.S., Hahn, J.S., Chung, M.J., 2005. Changing soybean isoflavone composition and concentrations under two different storage conditions over three years. Food Research International 38, 435–444.

Lee, J.H., Choung, M.G., 2011. Determination of optimal acid hydrolysis time of soybean isoflavones using drying oven and microwave assisted methods. Food Chemistry 129, 577–582.

Li, X., Cheng, Y., Yi, C., Hua, Y., Yang, C., Cui, S., 2009. Effect of ionic strength on the heat-induced soy protein aggregation and the phase separation of soy protein aggregate/dextran mixtures. Food Hydrocolloids 23, 1015–1023.

Liu, K., 1997. Soybeans: Chemistry, Technology and Utilization. Chapman & Hall, New York, p. 532.

Matsumi, A., Kayahara, H., Murasawa, H., Nakamura, S., 2009. Importance of size and charge of carbohydrate chains in the preparation of functional glycoproteins with excellent emulsifying properties from tofu whey. Food Chemistry 114, 1328–1334.

Meza, B.E., Verdini, R.A., Rubiolo, A.C., 2010. Effect of freezing on the viscoelastic behaviour of whey protein concentrate suspensions. Food Hydrocolloids 24, 414–423.

Nagata, Y., Sinoda, T., Mori, M., Miyanaga, N., Okumura, K., Goto, K., Naito, S., Fujimoto, K., Hirao, Y., Takahashi, A., Tsukamoto, T., 2007. Dietary isoflavones may protect against prostate cancer in Japanese men. The Journal of Nutrition Nutritional Epidemiology 137, 1974–1979.

Noh, B.J., Kang, C., Hong, S.T., Yun, S.E., 2006. Freezing of soybeans influences the hydrophilicity of soy protein. Food Chemistry 97, 212–216.

Oldoni, T., Cabral, L., Regitano, M., Rosales, P., Bonafé, M., Nascimento, A., Alencar, S., 2011. Isolation and analysis of bioactive isoflavonoid and chalcone from a new type of Brazilian propolis. Separation and Purification Technology 77, 208–213.

Ozuna, W.B., Champagne, C.P., Makhlouf, J., Barrette, L., 2001. Utilization of tofu whey pre-treated by electromembrane process as a growth medium for *Lactobacillus plantarum* UI 17. Desalination 228, 192–203.

Pednekar, M., Das, A.K., Rajalakshmi, V., Sharma, A., 2010. Radiation processing and functional properties of soybean (*Glycine max*). Radiation Physics and Chemistry 79, 490–494.

Poysa, V., Woodrow, L., 2002. Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. Food Research International 35, 337–345.

Prabhakaran, M.P., Perera, C.O., 2006. Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. Food Chemistry 98, 231–237.

Raventós, M., Hernández, E., Asúnda, J.M., Ibarz, A., 2007. Concentration of aqueous sugar solutions in a multi-plate cryoconcentrator. Journal of Food Engineering 79, 577–585.

Rickert, D.A., Meyer, M.A., Murphy, P.A., 2004. Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production. Food Chemistry and Toxicology 69 (8), 623–631.

Rostagno, M., Palma, M., Barroso, C., 2005. Short-term stability of soy isoflavones extractic sample conservation aspect. Food Chemistry 93, 557–564.

Sacks, F.M., Lichtenstein, A., Horn, L.V., Harris, W., Kris-Etherton, P., Winston, M., 2006. Soy protein, isoflavones, and cardiovascular health: a summary of a statement for professionals from the American heart association nutrition committee. Journal of the American Heart Association 113, 1034–1044.

Sánchez, J., Ruiz, Y., Raventós, M., Asúnda, J.M., Hernández, E., 2010. Progressive freeze concentration of orange juice in a pilot plant falling film. Innovative Food Science and Emerging Technologies 11, 644–651.

Sánchez, J., Hernández, E., Asúnda, J.M., Raventós, M., 2011. Freeze concentration of whey in a falling-film based pilot plant: process and characterization. Journal of Food Engineering 103, 147–155.

Shao, S., Duncan, A.M., Yang, R., Marcone, M.F., Rajcan, I., Tsao, R., 2009. Trading isoflavones: from soybean to soy flour, soy protein isolates to functional soy bread. Journal of Functional Foods 1, 119–127.

Siebert, K.J., 1999. Effects of protein–polyphenol interactions on beverage haze, stabilization, and analysis. Journal of Agricultural and Food Chemistry 47, 353–362.

Speroni, F., Milesi, V., Añón, M.C., 2010. Interactions between isoflavones and soybean proteins: applications in soybean-protein isolate production. LWT – Food Science and Technology 43, 1265–1270.

Sudyani, Y., Alawiyah, S., Anita, Y., Adilina, I.B., 2007. Characterization of waste water from whey tofu. Annals of International Conference on Chemical Sciences, ANS. 47–6.

Taku, K., Melby, M., Kurzer, S.M., Mizuno, S., Watanabe, S., Ishimi, Y., 2010. Effects of soy isoflavone supplements on bone turnover marker in menopausal women: systematic review and meta-analysis of randomizado controlled trials. Bone 47, 413–423.

Taku, K., Melby, M., Nishi, N., Omori, T., Kurzer, M., 2011. Review: soy isoflavones for osteoporosis: an evidence-based approach. Maturitas 70, 333–338.

Tang, C.H., 2007. Effect of thermal pretreatment of raw soymilk on the gel strength and microstructure of tofu induced by microbial transglutaminase. LWT – Food Science and Technology 40, 1403–1409.

Vishwanathan, K.H., Singh, V., Subramanian, R., 2011. Influence of particle size on protein extractability from soybean and okara. Journal of Food Engineering 102, 240–246.

Wang, H.J., Murphy, P.A., 1996. Mass balance study of isoflavone during soybean processing. Journal of Agricultural and Food Chemistry 44, 2377–2383.

Yee, P.L., Wakisaka, M., Shirai, Y., Hassan, M.A., 2003. Effects of single food components on freeze concentration by freezing and thawing technique. Japan Journal of Food Engineering 4 (3), 77–83.

Zhang, Z., Hartel, R.W., 1996. A multilayer freezer for freeze concentration of liquid milk. Journal of Food Engineering 29, 23–28.

ANEXO B

Trabalho oral apresentado no VII Congreso Español de Ingenieria de Alimentos- VII CESIA, Ciudad Real, Espanha, 2012**:**

RECUPERACIÓN DE ISOFLAVONAS A PARTIR DE LA CRIOCONCENTRACIÓN DE RESÍDUO LÍQUIDO DE LA PRODUCCIÓN DE TOFU

Benedetti, S.; Belén, F.; Prudêncio, E.S.; Petrus, J.C.C.; Hernández, E.; Auleda, J.M., Raventós, M.*

*Departamento de Ingeniería Agroalimentaria y Biotecnología, Universidad Politécnica de Cataluña (UPC), c/Esteve Terradas 8, 08860 Castelldefels, Barcelona, España
e-mail: merce.raventos@upc.edu.

Palabras clave: efluente de soja, concentración por congelación, propiedades funcionales

Resumen

El tofu es un alimento obtenido a través de la coagulación de la leche soja y su producción genera importantes volúmenes de suero. Este residuo contiene isoflavonas, compuestos funcionales de gran interés para la salud. El objetivo de este trabajo fue analizar la composición del suero de tofu con relación a la concentración de isoflavonas para su recuperación a través del proceso de crioconcentración. Los ensayos fueran realizados en tres etapas de crioconcentración en placa y las isoflavonas fueron analizadas de acuerdo con la metodología de la AOAC 2001.10 (2005). Los resultados muestran que el suero de tofu contiene cerca de 10,7 mg isoflavona kg^{-1} suero. Al final de la tercera etapa de la crioconcentración, se obtuvo un concentrado con 82,95 mg isoflavona kg^{-1} suero, demostrando que la mayoría de las isoflavonas permanece en el concentrado mientras que el hielo presenta bajos valores de retención. Las agliconas, las isoflavonas con las propiedades funcionales más importantes, aumentan de 6,9 hasta 61,5 mg isoflavona kg^{-1} de suero. En conclusión, se observa que el suero de tofu presenta un gran potencial para el aprovechamiento y la recuperación de isoflavonas y que el proceso de crioconcentración es una alternativa eficiente para esta finalidad.

1. Introducción

El tofu es un alimento no fermentado obtenido a partir de la coagulación de la leche de soja y su producción genera importantes cantidades de suero de tofu. Este residuo líquido contiene azúcares, proteínas y compuestos minoritarios como isoflavonas y sales minerales, representando un problema ambiental por su elevada carga orgánica (Sudyani et al., 2007). De acuerdo con Espinosa-Martos et al. (2006), la composición y las propiedades de las isoflavonas presentes en el suero de tofu lo convierten en un residuo reutilizable con aplicaciones potenciales en la industria farmacéutica y alimentaria. Las isoflavonas son fitoestrógenas de reconocidos efectos beneficiosos para la salud y se presentan en cuatro formas químicas: β-glucósidos (genistina, daidzina, glicitina), malonil glucósidos (malonil genistina, malonil daidzina, malonil glicitina), acetil glucósidos (acetil genistina, acetil daidzina, acetil glicitina) y agliconas (genisteína, daidazeína, gliciteína) (Rostagno et al., 2009). Además de la ingesta diaria de productos de soja, también es interesante obtener aislados de proteínas con altos niveles de isoflavonas y concentrados de isoflavonas de soja encapsulados a partir de la recuperación de estos compuestos de residuos industriales, le agregando valor y reduciendo el impacto ambiental. Concentrados de isoflavonas o aislados protéicos con alto nivel de isoflavonas pueden ser obtenidos a partir de técnicas de concentración a frio de residuos líquidos, como procesos de separación por membranas y crioconcentración (Noordman et al., 2003; Xu et al., 2004).

El proceso de crioconcentración es basado en la separación de fase sólido-líquido a bajas temperaturas, de manera que haya una buena retención de sabores y componentes sensibles al calor. Este proceso permite más calidad del producto concentrado y los costos totales (incluyendo el capital, la energía y de limpieza) son más pequeños que la evaporación u ósmosis inversa (Sánchez et al., 2011). Este proceso ya ha sido utilizado para concentración de azúcares, zumos de frutas, lactosuero, mosto y para el tratamiento de aguas residuales (Raventós et al., 2007; Sánchez et al., 2009; Sánchez et al., 2010; Hernández et al., 2010; Auleda et al., 2011; Sánchez et al., 2011), pero no hay informes en la literatura sobre la utilización del proceso de crioconcentración para concentrar compuestos funcionales de soja, como el propósito de este trabajo. El objetivo de esto trabajo fue analizar la composición del suero de tofu en relación a la concentración de isoflavonas para su recuperación a través del proceso de crioconcentración.

2. Material y métodos

2.1 *Crioconcentración*

El proceso de crioncentración del suero de tofu se llevó a cabo en tres etapas utilizando un equipo piloto de placas. La Figura 1 presenta el esquema del equipo de crioconcentración. Cuatro lotes de 210 kg de de suero de tofu a una concentración inicial de 1,9° Brix fueron crioconcentrados hasta 15,5 ° Brix. El flujo de suero se mantuvo constante a 1 ± 0,1 L s-1. Durante cada etapa fueron realizadas medidas de peso inicial y final, temperatura e velocidad de flujo en diferentes tempos. Además, fueron recogidas muestras del concentrado y del hielo para los análisis de isoflavonas. El hielo formado fue fundido para medir la concentración de solutos y isoflavonas para ser representativo de todo el volumen de suero de tofu procesado en la crioconcentración.

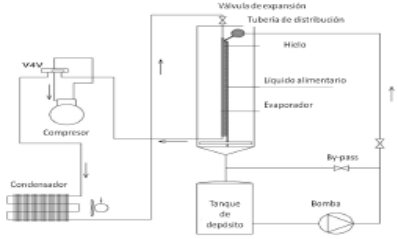


Fig. 1. Esquema básico del equipo piloto de crioconcentración

2.2 *Determinación de isoflavonas*

Las isoflavonas fueron determinadas de acuerdo con la metodología 2001.10, AOAC (2005), con adaptaciones. Para las muestras líquidas de suero de tofu, 10 g de muestra fue extraída a 65 °C durante 2 h en 40 ml de solución de metanol-agua (80 20) y los extractos fueron saponificados a temperatura ambiente con 3 ml de NaOH 2M. Los extractos se acidificaron con 1 mL de ácido acético Gracial, filtrados en filtro de papel cuantitativo y diluido con agua hasta metanol-agua (50:50). Después de este paso, los extractos fueron

centrifugados durante 5 minutos a 7000xg y analizados por cromatografía líquida de alta resolución (HPLC). Para la separación de isoflavonas, el volumen de inyección fue de 20 µL, y el sistema de gradiente binario lineal fue utilizado y las fases móviles fueron: agua / metanol, ácido acético (88:10:2) y metanol / ácido acético (98:2). El flujo de fase móvil fue de 1 mL min-1. Glucósidos de isoflavona y agliconas fueron separados en una columna de fase inversa C18 (Nova-Pack C18 150 x 3,9 mm 4µ) con la fase móvil de metanol-agua y determinadas en equipamiento Agilent 1100 DAD con detector UV en 260 nm. Para la identificación y cuantificación de los picos correspondientes a cada una de las isoflavonas, se utilizaron las curvas de calibración con regresión lineal basado en las áreas de los picos. Estas curvas de calibración fueron construidas con estándares externos de daidzina, daidzeína, genistina y genistein (Fluka 30408, 48756, 91955 y Sigma D7802). El glicitina y gliciteína se identificaron sobre la base de tiempos de retención. Los resultados se expresaron en unidades de agliconas por la suma de las concentraciones de las isoflavonas agliconas (genisteína, daidzeína y gliciteína) y los glucósidos (genistina, daidzina y glicitina) y presentados como isoflavonas mg kg^{-1} de suero de tofu.

3. Resultados y discusión

La Figura 1 presenta los resultados de cuantificación de isoflavonas en el suero de tofu del proceso industrial, en el concentrado y en el hielo obtenidos en las tres etapas de la crioconcentración. El suero de tofu contiene cantidades significativas de isoflavonas. De acuerdo con Wang y Murphy (1996), se pierde cerca de 44 % de isoflavonas en el suero durante la producción del tofu. Kao et al. (2004) evaluaron el efecto de los coagulantes en el contenido de isoflavonas y encontraron que algunos son más eficientes en rendimiento en isoflavonas, principalmente debido al facto que un coagulante es más eficiente que otro en la coagulación de proteínas y las isoflavonas son directamente enlazadas con las proteínas. Prabhakaran et al (2006) ha explicado que pierdas significativas en el suero de tofu ocurren durante la coagulación de la leche de soja para la producción de tofu, y eso puede cambiar con el tipo de coagulante utilizado. Estos resultados están de acuerdo con los de Sudiayni et al. (2007), que detectó altas concentraciones de genistina y daidzina en suero de tofu. Mantovani et al. (2011) tambien observaron altas concentraciones de isoflavonas en resíduo industrial de soja.

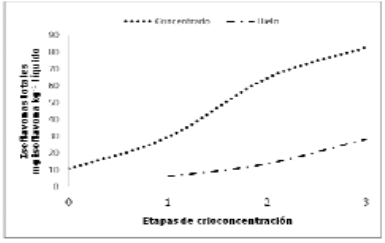


Fig. 1. Evolución del contenido de isoflavonas totales en el concentrado y el hielo.

Los resultados muestran que hay un aumento en la concentración de isoflavonas totales de 10,65 mg kg^{-1} ST a 82,95 mg kg^{-1} ST en todas las etapas de la crioconcentración del suero de tofu, principalmente agliconas, indicando su potencial de aprovechamiento para recuperación de estos compuestos funcionales. La mayoría de las isoflavonas permanece en el concentrado durante el proceso y la fracción del hielo presenta bajos valores de retención de estos compuestos. Estos resultados están de acuerdo con otras investigaciones que obtuvieron resultados satisfactorios utilizando la crioconcentración para concentrar alimentos, como zumos de frutas Sánchez et al., 2009, Sánchez et al. 2010; Auleda et al., 2011), lactosuero (Sánchez et al., 2011), azúcares (Raventós et al., 2007), mosto (Hernández et al., 2010), entre otros. Benedetti et al. (2011) también observaron un aumento de concentración de las isoflavonas utilizando procesos de concentración de extracto aquoso de soja. La retención de isoflavonas es debido a los sólidos retenidos en el hielo en las etapas de la crioconcentración. Sánchez et al (2010) afirma que eso se puede explicar por el aumento de la viscosidad de la solución con la concentración, y los solutos acumulados en la interface tienen más dificultad de moverse en la solución y son más fácilmente retenidos en el hielo que se forma.

Considerando las agliconas, se puede observar en la Figura 2 un aumento significativo en su concentración durante las tres etapas de la crioconcentración. Setchell (1998) e Izumi et al (2000) afirmaron que las agliconas constituyen un grupo de compuestos responsables por muchas actividades biológicas y son absorbidas más rápidamente en el intestino humano. Por eso, hay un interese en investigar nuevas tecnologías de concentración que conserven sus propiedades funcionales y que, además, sea posible recuperarlas a partir de resíduos alimentarios industriales.

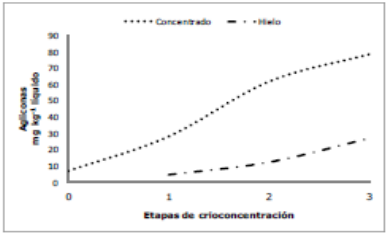


Fig. 2. Evolución del contenido de agliconas en el concentrado y el hielo.

Los conjugados β-glucósidos también presentan un aumento en su concentración en las tres etapas del proceso de crioconcentración, pero menos expresivo que las agliconas (Figura 3). De acuerdo con Burdo et al. (2008), la estructura química de las substancias puede interferir en su separación entre en concentrado y el hielo. Se sabe que los grupos isoflavonas tienen diferentes estructuras químicas y masas molares que varian entre 400-600 g mol^{-1} para glucósidos y 200-300 g mol^{-1} para agliconas (Nurmi et al, 2002).

Setchell, K. D. R. Phytoestrogens: the biochemistry, physiology and implications for human health of soy isoflavones. The American Journal of Clinical Nutrition. v.68, p.1333-1346, 1998.

Sudyani Y., Alawiyaf S., Anita, Y., Adilina I.B. Characterization of waste water from whey tofu. *International Conference on Chemical Sciences*, 2007.

Xu, L., Lamb, K., Layton, L., & Kumar, A. (2004). A membrane-based process for recovering isoflavones from a waste stream of soy processing. Food Research International, v.37(9), 867–874.

Wang, H. J., & Murphy, P. A. (1996). Mass balance study of isoflavones during soybean processing. Journal of Agricultural and Food Chemistry, v.44, 2377–2383.

VII Congreso Español Ingeniería de Alimentos

Ciudad Real, España 7-9 Noviembre 2012

Dª Ana Isabel Briones y Dª Desamparados Salvador Moya, co- Presidentas del VII Congreso Español de Ingeniería de Alimentos, celebrado en Ciudad Real, Campus Universidad de Castilla-La Mancha del 7 al 9 de Noviembre de 2012

CERTIFICA QUE:

MERCÈ RAVENTÓS

ha asistido y participado en este Congreso con la comunicación titulada:

RECUPERACIÓN DE ISOFLAVONAS A PARTIR DE LA CRIOCONCENTRACIÓN DE RESÍDUO LÍQUIDO DE LA PRODUCCIÓN DE TOFU

Comunicación Oral/ Poster referencia SOS-C02

Ciudad Real, a 9 de Noviembre de 2012

A.I Briones y MªD. Salvador

Co-Presidentas CESIA 2012

www.cesia.es

Trabalho apresentado no II Simpósio de Processos de Separação com Membranas- II SIMPAM, Rio de Janeiro-RJ, 2013:

Recovery and Concentration of Isoflavones of Tofu Whey Using the Nanofiltration

Silvia Benedetti[1*], Lara Alexandre Fogaça[1], Guilherme Zin[1], ElaneSchwinden Prudêncio[2], Rodrigo Santos Leite[3], José Marcos Gontijo Mandarino[3], José Carlos Cunha Petrus[1]
[1]Department of Chemistry and Food Engineering, Technology Center, Federal University of Santa Catarina, Florianópolis, SC.
[2]Department of Food Science and Technology, Agricultural Sciences Center, University of Santa Catarina, Florianópolis, SC.
[3] Embrapa Soja, Londrina, PR.
*silviabene@gmail.com

The tofu is a nutritious food produced from the hydro soluble extract of soybean. Among the production steps, a large volume of whey is eliminated during the pressing step. The tofu whey is characterized by high values of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), considerable protein content, substantial sugars and minerals (coagulants) concentrations , besides presenting low molecular weight molecules as peptides, lipids, and some functional compounds such as isoflavones and oligosaccharides.

The objective of this study was the use of tofu whey, using nanofiltration (NF) for concentration of isoflavones. Initially, the influence of pressure, temperature and tangential velocity during the process was evaluated. The experiments were carried out in duplicate at 6 bar, 28 °C and 0.3 ms^{-1} until reaching a volume reduction factor (VRF) of 4.5. The VRF was calculated as the ratio between the initial volume (L) of the tofu whey used in the feed and the final volume (L) of the concentrate after NF. The quantification of isoflavones was performed using High Performance Liquid Chromatography (HPLC) with photo diode array detector (Model 996) and automatic sample injector (Model 717 Plus) of WATERS ® (Milford, USA). It was used a reverse phase column (YMC Pack ODS-AM ®, 250 mm x 0.4 mm diameter) [1].

Throughout the nanofiltration process of tofu whey, a reduction in the permeate flux with time was observed, typical of membrane separation processes. Concerning the concentration of isoflavones, it can be observed through Figure 1 a significant increase ($p < 0.05$) in the

concentration of the β-glycosides and malonyl glycosides isomers. This behavior was also observed by other authors [2] in the concentration of tofu using a nanofiltration membrane. Regarding aglycones, no increase of its concentration in the retentate was noticed. Overall, there was high retention of isoflavones, showing that nanofiltration was effective for concentration of these bioactive compounds. Therefore, the tofu whey has a potential use on the recovery of isoflavones, and that the nanofiltration process is an effective alternative for this purpose.

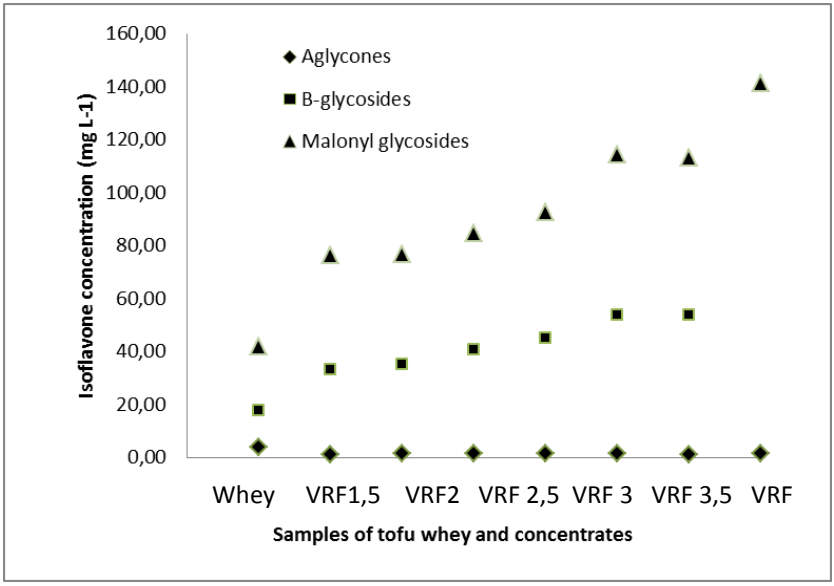


Figure 1- Results of isoflavones concentration in the tofu whey and concentrates obtained by nanofiltration process.

[1] M.A. BERHOW (2002). Modern analytical techiniques for flavonoid determination. In: BUSLIG, B.S.; MANTHEY, J.A. Flavonoids in the Living Cell. New York: Klusher, 2002. 505 p.
[2] W.J. KIM; H. H. KIM; S.H.,YOO (2005), Food Sci Biotechnol, 14 (3), 380-386.

COPPE 50 UFRJ

PAM

SIMPAM 2013

II Simpósio de Processos de Separação com Membranas

29 de julho a 02 de agosto de 2013
Rio de Janeiro, RJ - Brasil

Certificado

Certificamos que o trabalho

"Recovery and Concentration of Isoflavones of Tofu Whey Using the Nanofiltration."

de autoria de Silvia Benedetti, Lara Alexandre Fogaça, Guilherme Zin, Elane Schwinden Prudêncio, Rodrigo Santos Leite, José Marcos Gontijo Mandarino e José Carlos Cunha Petrus foi apresentado no **II Simpósio de Processos de Separação com Membranas,** realizado no período de 29 de Julho a 02 de Agosto de 2013.

Prof. Cristiano Piacsek Borges

1) Trabalho complete apresentado no 9º Congreso Iberamericano de Ingeniería de Alimentos (9º CIBIA), Valência, Espanha, 2014:

AVALIAÇÃO DO TEOR DE ISOFLAVONAS E ATIVIDADE ANTIOXIDANTE DO SORO DE TOFU CONCENTRADO POR CRIOCONCENTRAÇÃO

MSc. Silvia Benedetti[1]; Eng.Lara Alexandre Fogaça[1]; Dra.Elane Schwinden Prudêncio[2]; MSc. José Marcos Gontijo Mandarino[3]; Quim. Rodrigo Santos Leite[3]; Dr.José Carlos Cunha Petrus[1]

[1] Departamento de Engenharia Química e Engenharia de Alimentos, Universidade Federal de Santa Catarina, Florianópolis, Florianópolis, SC, Brasil. silviabene@gmail.com
[2] Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciência Agrárias, Universidade Federal de Santa Catarina, Florianópolis, Florianópolis, SC, Brasil.
[3] Embrapa Soja, Londrina-PR, Brasil.

1 Introdução

O aproveitamento e a recuperação de compostos a partir de resíduos da indústria de alimentos constitui uma alternativa à obtenção de compostos funcionais de alto valor agregado, que podem ser utilizados como insumos e/ou ingredientes na indústria de alimentos e farmacêutica, além de minimizar o impacto ambiental gerado por esses resíduos.

O tofu é um alimento muito consumido nos países orientais e geralmente produzido em pequenas indústrias. Esse produto é fabricado partir da coagulação do extrato hidrossolúvel de soja. Um das suas etapas de produção é a prensagem, na qual é eliminada grande quantidade de resíduo líquido. O soro de tofu é caracterizado por elevados valores de demanda química de oxigênio (DQO) e demanda bioquímica de oxigênio (DBO), elevado teor de proteínas (CHAI et al., 1999), concentrações substanciais de açúcares e minerais (coagulantes) (BAZINET; IPPERSIEL; LAMARCHE, 1999). Além disso, contem moléculas de baixa massa molar, como peptídios, lipídios, e alguns

compostos funcionais, tais como isoflavonas e oligossacarídeos (KIM; KIM; YOO, 2005). As isoflavonas são compostos fenólicos pertencentes à classe dos fitoestrógenos, que apresentam estrutura química semelhante ao estrogênio humano e estão presentes na soja em quatro formas químicas: β-glicosídeos, malonil glicosídeos e acetil glicosídeos e na forma não-conjugada aglicona, sendo que cada forma química possui três isômeros: a genistina, a daidzina e a glicitina (CHUN; KIM; KIM, 2008).

Um grande número de pesquisadores relatam os benefícios do consumo de produtos contendo isoflavonas na saúde humana, tais como a capacidade de reduzir o risco de doenças cardiovasculares, colesterol, aterosclerose, osteoporose, apresentar atividade estrogênica amenizando sintomas relacionados à menopausa e reduzindo o risco de câncer de próstata e mama, além de seu efeito antioxidante (KWAK, LEE e PARK, 2007; SCAMBIA et al, 2000; ZHANG et al., 2003).

Para seleção de um processo a ser utilizado na indústria de alimentos e de bioprodutos, é importante avaliar se o mesmo possui baixo custo, se é uma tecnologia eficiente e sustentável e se preserva os componentes do alimento, bem como sua atividade biológica conferida pelos seus compostos fitoquímicos (KLIMCZAK et al., 2007). Nesse contexto, a crioconcentração vem sendo considerada uma técnica promissora para concentração de compostos bioativos. Essa tecnologia promove a concentração de fluidos alimentícios através do congelamento e subsequente separação da parte aquosa dos solutos por meio de descongelamentos fracionados sucessivos (BELÉN et al., 2012). Do ponto de vista da indústria de alimentos, a crioconcentração é adequada devido à sua capacidade de preservar a qualidade nutricional dos fluidos alimentícios, pois minimiza a perda de compostos termolábeis, resultando num produto final de melhor qualidade e pode ser uma alternativa promissora às técnicas de concentração convencionais utilizadas no processamento de alimentos (SÁNCHEZ et al., 2011a,b). Considerando os aspectos positivos da crioconcentração e os benefícios potenciais das isoflavonas presentes no soro de tofu, esse estudo tem como objetivo avaliar os efeitos da crioconcentração na concentração dos compostos bioativos presentes nesse resíduo bem como da atividade antioxidante, na fração concentrada e no gelo.

2 Material e métodos

2.1 Crioconcentração

O princípio deste método baseia-se no congelamento total da solução seguido por descongelamento parcial através de separação gravitacional simples. Assim, obtêm-se duas frações: o fluido concentrado (FC) e a fração de gelo (G). A crioconcentração foi realizada de acordo com metodologia proposta por Aider e Ounis (2012), com modificações. Um volume inicial de 1,5 L de soro de tofu foi dividido em 2 bateladas de 750 mL e então congelado a -20 ±2 °C. O processo de congelamento foi conduzido em um freezer por congelamento indireto. Após congelar-se completamente a solução, 50 % do seu volume inicial foi descongelado à temperatura ambiente (20±2 °C). O líquido descongelado representa o fluido concentrado da primeira etapa da crioconcentração. Esse fluido concentrado foi novamente congelado a -20 ±2 °C e então utilizado como solução de alimentação para a segunda etapa. Ao final da segunda etapa, 50 % da solução concentrada foi descongelada, coletada e congelada novamente. Esse procedimento foi repetido até a 3ª etapa de crioconcentração, sendo que em cada etapa o fluido concentrado foi congelado a -20 ±2 °C e utilizado como solução de alimentação na etapa seguinte. O gelo remanescente de cada etapa e uma alíquota de cada concentrado foi armazenado a -20 ±2 °C até o momento da realização das análises. Na Figura 6 está apresentado um diagrama exemplificando o processo de crioconcentração a ser utilizado.

O fator de concentração (FC) de cada etapa da crioconcentração foi calculado de acordo com a metodologia proposta por Aider e Ounis (2012), em função do aumento de concentração da solução em relação à quantidade de matéria seca no soro inicial de alimentação. O conteúdo total de matéria seca será determinado pela medida da perda de peso após a secagem a 80 °C e expresso como conteúdo de matéria seca/massa total (g 100 g^{-1}) (AOAC, 2005). Todas as análises do gelo e do fluido concentrado serão realizadas em triplicata. O FC, expresso em %, será calculado de acordo com a Equação 1.

$$FC = \frac{DM_n}{DM_0}.100$$

Onde DM_n é o conteúdo total de matéria seca (g) do fluido concentrado de cada etapa da crioconcentração e DM_0 é o conteúdo total de matéria seca (g) do soro de tofu inicial. O desempenho da crioconcentração (E) será determinado baseando-se no conteúdo de isoflavonas e de oligossacarídeos. A eficiência do processo é referida como o aumento do conteúdo de isoflavonas e de oligossacarídeos do fluido concentrado

em relação ao conteúdo destes mesmos compostos remanescente no gelo, calculado pela Equação 2.

$$E\ (\%) = \frac{CCC_n - CCG}{CCC_n}.100$$

Onde CCC_n é o conteúdo do composto de interesse (isoflavonas) no fluido concentrado (mg) em cada etapa da criooconcentração e CCG é conteúdo do composto de interesse no gelo (mg) em cada etapa da crioconcentração.
A matéria seca total foi determinada pela medida da perda de peso da amostras numa estufa a 80 °C e expressa com conteúdo de matéria seca/peso seco (g/100 g) (AOAC, 2005).

2.2 Quantificação de isoflavonas

A extração de isoflavonas das amostras de soro de tofu foi realizada conforme metodologia proposta Carrão-Panizzi, Góes-Favoni e Kikuchi (2002), com modificações. A separação e a quantificação das isoflavonas foram realizadas de acordo com a Berhow (2002), utilizando Cromatografia Líquida de Alta Eficiência (CLAE), com detector de arranjo de foto diodo (Modelo 996) e injetor automático de amostras (Modelo 717 Plus) da WATERS® (Milford, EUA). Foi utilizada nesta etapa uma coluna de fase reversa (YMC Pack ODS-AM®, 250 mm x 0,4 mm de diâmetro).

2.3 Determinação da atividade antioxidante pelo método FRAP (Método de redução do ferro)

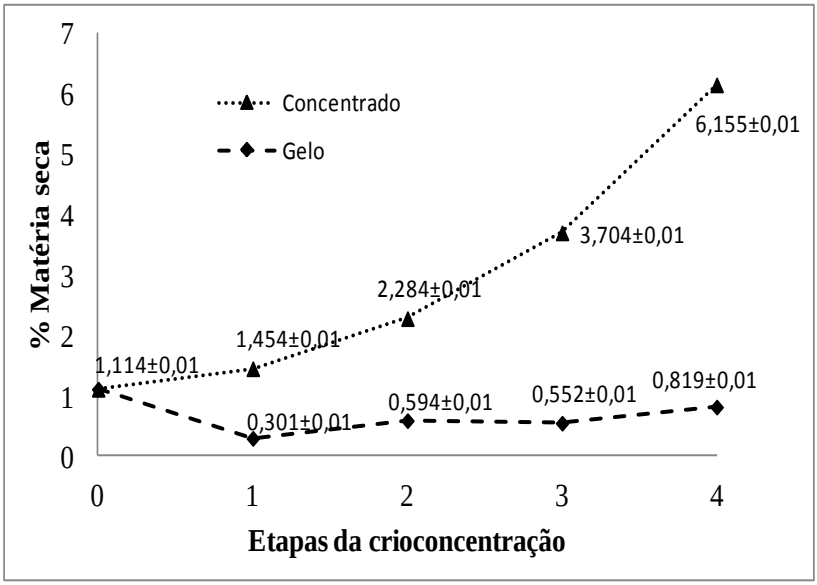
Utilizou-se o método descrito por Benzie e Strain (1996), com modificações de Arnous, Makris e Kefalas (2002). Este se baseia na medida direta da habilidade dos antioxidantes (redutores) da amostra em reduzirem, em meio ácido (pH 3,6), o complexo Fe3+ /tripiridiltriazina (TPTZ), para formar Fe2+, de intensa cor azul e absorção máxima a 593 nm. O Trolox foi usado como padrão e os resultados foram expressos como equivalente Trolox em μmol/mL.

3 Resultados e discussão

3.1 Matéria seca total e fator de concentração

A evolução do conteúdo de matéria seca total em função das etapas da crioconcentração está apresentada na Figura 1. O conteúdo de matéria seca total aumentou significativamente ($p < 0,05$) no fluido concentrado nas etapas 2 a 4 se comparado com o soro inicial. Já o fator de concentração aumentou significativamente ($p < 0,05$) em todas as etapas do processo se comparado com os valores do soro inicial de aproximadamente 130, 40 % na primeira etapa, 206, 5 % na segunda etapa, 338, 05 % na terceira etapa e 554,40 % na última etapa. O conteúdo de matéria seca total no gelo remanescente diminuiu significativamente ao longo do processo em todas as etapas se comparado ao soro inicial.

Figura 1: Conteúdo de material seca total do gelo e do fluido concentrado em função das etapas da crioconcentração. Os dados são expressos como média ± desvio padrão.



3.2 Eficiência da concentração de isoflavonas

O conteúdo de isoflavonas em ambos o fluido concentrado e o gelo, está apresentado na Tabela 1. O efeito do ciclo da crioconcentração foi

significativo no conteúdo de isoflavonas em todos os fluidos concentrados, aumentando em função da evolução do processo. Na fração de gelo, observa-se que o conteúdo de isoflavonas na primeira etapa foi significativamente maior ($p<0,05$) do que nas etapas subsequentes, sendo que no gelo das etapas 2 e 3, não houve diferença significativa na concentração desses compostos. Os compostos fenólicos apresentam um grande número de pontes de hidrogênio, o que lhes confere a capacidade de ligação com moléculas de água. Pelo aumento da concentração de isoflavonas na solução, a água intersticial torna-se menos disponível para o congelamento, resultando na retenção desses compostos no gelo durante o processo de separação. De acordo com Aider, Halleux e Akbache (2007), esse fenômeno pode reduzir a eficiência do processo. A maior eficiência na separação foi observada na terceira etapa da crioconcentração, com 89,11 %. Nas etapas anteriores a eficiência foi inferior, de 82, 23 % na segunda etapa e 79,52 % na primeira etapa. De acordo com Belén et al. (2012), normalmente ocorre perda da eficiência ao longo das etapas da crioconcentração devido ao aumento da retenção de solutos no gelo. Porém, nesse caso houve menor retenção de solutos na terceira etapa e um aumento significativo de isoflavonas no concentrado.

Tabela 1: Conteúdo de isoflavonas (mg L^{-1}) no soro de tofu na alimentação, fluidos concentrados e fração de gelo de cada etapa da crioconcentração em blocos.

Amostras	β-glicosídeos*			Malonil glicosídeos*			Agliconas*			Totais**
	G-dai	G-gli	G-gen	M-dai	M-gli	M-gen	Dai	Gli	Gen	
Soro	2,59^{d} ±0,15	1,67^{d} ±0,12	2,22^{d} ±0,24	5,64^{d} ±0,12	4,95^{d} ±0,12	10,72^{d} ±0,57	1,08^{d} ±0,13	4,07^{c} ±0,19	0,56d ±0,06	33,50^{d} ±0,17
Conc1	9,55^{c} ±0,37	7,59^{c} ±0,17	8,51^{c} ±0,16	20,50^{c} ±0,17	17,44^{c} ±0,61	33,01^{c} ±2,00	2,54^{c} ±0,07	5,95^{c} ±1,85	0,77^{c} ±0,04	105,87^{c} ±1,89
Conc2	12,02^{b} ±0,13	9,42^{b} ±0,08	11,86^{b} ±0,27	26,20^{b} ±1,30	22,56^{b} ±0,42	41,25^{b} ±2,34	3,27^{b} ±0,10	9,60^{b} ±0,38	1,03^{b} ±0,07	137,21^{b} ±3,73
Conc3	21,76^{a} ±0,16	14,91^{a} ±0,21	19,17^{a} ±0,18	42,53^{a} ±1,54	35,56^{a} ±0,53	64,55^{a} ±0,69	5,31^{a} ±0,23	12,12^{a} ±0,76	1,30^{a} ±0,10	217,21^{a} ±4,23
Gelo1	2,27^{A} ±0,06	1,76^{A} ±0,11	2,09^{A} ±0,08	5,03^{A} ±0,48	4,42^{A} ±0,29	9,30^{A} ±0,35	0,88^{A} ±0,03	2,36^{A} ±0,74	0,37^{A} ±0,02	28,47^{A} ±0,85
Gelo2	1,98^{B} ±0,06	1,44^{B} ±0,06	1,76^{B} ±0,06	4,95^{AB} ±0,31	3,87^{B} ±0,16	7,96^{B} ±0,24	0,75^{B} ±0,05	1,36^{B} ±0,04	0,31^{B} ±0,00	24,38^{B} ±0,70
Gelo3	2,06^{B} ±0,04	1,51^{B} ±0,08	1,73^{B} ±0,11	4,37^{BC} ±0,10	3,94^{B} ±0,13	8,13^{B} ±0,03	0,72^{B} ±0,03	0,95^{C} ±0,09	0,24^{C} ±0,05	23,66^{B} ±0,38

*Dai=daizeína; Gli=gliciteína; Gen=genisteína; G-gen=genistina; G-gli=glicitina; G-dai: daidzina; M-dai: malonil daidzina; M-gli: malonil glicitina; M-gen: Malonil genistina. ** Isoflavonas totais: agliconas + β-glicosídeos + Malonil glicosídeos.

^{a,b} Letras minúsculas sobrescritas na mesma coluna indicam diferenças significativas ($p < 0,05$) entre as amostras de soro de tofu e concentrados. ^{A,B} Letras maiúsculas indicam diferenças significativas ($p < 0,05$) entre as amostras de gelo.

3.3 Avaliação da atividade antioxidante

A atividade antioxidante do soro inicial e do fluido concentrado de cada etapa da crioconcentração, medido através do método FRAP, está apresentada na Tabela 2.

Tabela 2: Atividade antioxidante do soro inicial e dos fluidos concentratos de cada etapa da crioconcentração.

	Soro inicial	**Conc 1**	**Conc 2**	**Conc 3**	**Conc 4**
FRAP (µmol/mL)	120,64±5,18^{b}	72,2±1,76^{c}	141,53±1,76^{a}	113,98±4,02^{b}	145,76±2,04^{a}

Os valores de FRAP aumentaram significativamente ($p < 0,05$) no fluido concentrado na etapa 4, de aproximadamente 120 µmol/mL para 146 µmol/mL, embora as etapas intermediárias tenham apresentando alguns resultados insatisfatórios. A atividade antioxidante de produtos da soja também foi relatada em outros estudos, na inibição da auto-oxidação do ascorbato em produtos fermentados e também na soja in natura (WANG; YU; CHOU, 2006; CHAIYASUT et al., 2010; NIAMNUY et al., 2011).

4 Conclusão

O presente estudo mostrou que é possível aumentar o conteúdo de compostos bioativos e a atividade antioxidante do soro de tofu através da crioconcentração em blocos. Esse processo mostrou-se uma possível alternativa para preservar as características funcionais de produtos da soja. A crioconcentração promoveu um maior fator de concentração do conteúdo de matéria seca total no soro de tofu. O concentrado apresentou aumento na concentração de isoflavonas em todas as etapas, principalmente na etapa final. Embora o processo seja eficiente, houve retenção de parte das isoflavonas no gelo. A atividade antioxidante medida pelo método FRAP aumentou significativamente no concentrado final, sendo correlacionada ao conteúdo de isoflavonas com potencial antioxidante comprovado.

5 Bibliografia

AIDER, M., DE HALLEUX, D.; AKBACHE, A. Whey cryoconcentration and impact on its composition. **Journal of Food Engineering**, v.82, n.1, 92-102, 2007.

AIDER, M.; OUNIS, W. B. Skim milk cryoconcentration as affected by the thawing mode: Gravitational vs. microwave-assisted. **International Journal of Food Science and Technology**, 47(1), 195-202, 2012.

BAZINET, L.; IPPERSIEL, D.; LAMARCHE, F. Recovery of magnesium and protein from soy tofu whey by electrodialytic configurations. **Journal of Chemical Technology and Biotechnology,** v.74, p.663-668, 1999.

BELÉN, F., SÁNCHEZ, J., HERNÁNDEZ, E., AULEDA, J.M., RAVENTÓS, M. One option for the management of wastewater from tofu production: Freeze concentration in a falling-film system. **Journal of Food Engineering**, v.110, p.364-373, 2012.

BENZIE, I. F.; STRAIN, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. **Analytical Biochemistry**, v.239, n.1, p.70-76, 1996.

BERHOW, M.A. Modern analytical techiniques for flavonoid determination. In: BUSLIG, B.S.; MANTHEY, J.A. **Flavonoids in the Living Cell**. New York: Klusher, 2002. 505 p.

CARRÃO-PANIZZI, M. C.; GÓES-FAVONI, S.P.; KIKUCHI, A. Extraction time for isoflavone determination. **Brazilian Archives of Biology and Technology**, v.45, n.4, p.515-518, 2002.

CHAI, X.; MI, Y.; YUE, P-L.; CHEN, G. Bean curd wastewater treatment by membrane separation. **Separation and Purification Technology**, v.15, p.175-180, 1999.

CHAIYASUT, C.; KUMAR, T.; TIPDUANGTA, P.; RUNGSEEVIJITPRAPA, W. Isoflavone content and antioxidant activity of Thai fermented soybean and its capsule formulation. **African Journal of Biotechnology**, v. 9, n.26, p. 4120-4126, 2010.

CHUN, J.; KIM, J.S.; KIM, J.H. Enrichment of isoflavone aglycones in soymilk by fermentation with single and mixed cultures of *Streptococcus infantarius* 12 and *Weissella sp*. **Food Chemistry**, v.109, p.278-284, 2008.

KIM, W.J.; KIM, H.H.; YOO, S.H. Ultra- and- nano-filtration process optimization of isoflavones and oligosaccharides from sunmul. **Food Science and Biotechnology**, v.14, n.3, p.380-386, 2005.

KLIMCZAK, I.; MAŁECKA, M.; SZLACHTA, M.; GLISZCZYŃSKA-ŚWIGŁO, A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. **Journal of Food Composition and Analysis**, v. 20, n. 3-4, p. 313-322, 2007.

KWAK, C.S.; LEE, M.S.; PARK, S.C. Higher antioxidant properties of hungkookjang, a fermented soybean paste, may be due to increased aglycone and malonylglycoside isoflavone during fermentation. **Nutrition Research**, v. 27, p.719-727, 2007.

NIAMNUY, C.; NACHAISIN, M.; LAOHAVANICH, J.; DEVAHASTIN, S. Evaluation of bioactive compounds and bioactivities of soybean dried by different methods and conditions. **Food Chemistry**, v.129, p. 899-906, 2011.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Freeze concentration of whey in a falling-film based pilot plant: Process and characterization. **Journal of Food Engineering**, v. 103, p.147-155, 2011a.

SÁNCHEZ, J.; HERNÁNDEZ, E.; AULEDA, J.M.; RAVENTÓS, M. Review: freeze concentration technology applied to dairy products**. Food Science and Technology International**, v.17, n.1., p.5-13, 2011b.

SCAMBIA, G.; MANGO, D.; SIGNORILE, P.G.; ANSELMI, R; PALENA, C; GALLO, D.; BOMBARDELLI, E.; MORAZZONI, P.; RIVA, A .; MANCUSO, S. Clinical effects of a standardized soy extract in postmenopausal women: a pilot study. **Menopause**, v.7, p. 105-111, 2000.

WANG, Y-C.; YU, R-C.; CHOU, C-C. Antioxidative activities of soymilkfermented with lactic acid bacteria and bifidobacteria. **Food Microbiology**, v. 23, p.128-135, 2006.

9 CIBIA
Congreso Iberoamericano
de Ingeniería de Alimentos
Valencia (España), 13 - 16 Enero 2014
UNIVERSITAT POLITÈCNICA DE VALÈNCIA

CERTIFICADO PRESENTACIÓN POSTER

D./Dª. **José Carlos Cunha Petrus, Lara Alexandre Fogaça, Silvia Benedetti, José Marcos Gontijo Mandarino, Rodrigo Santos Leite, Elane Schwinden Prudêncio,** han presentado el poster título **AVALIAÇÃO DO TEOR DE ISOFLAVONAS E ATIVIDADE ANTIOXIDANTE DO SORO DE TOFU CONCENTRADO POR CRIOCONCENTRAÇÃO** en el ***9º Congreso Iberoamericano de Ingeniería de Alimentos***, celebrado en la Universidad Politécnica de Valencia (España) entre los días 13 y 16 de Enero 2014.

Valencia, 16 de Enero de 2014

Prof. Pedro Fito Maupoey
Presidente Cibia9

ANEXO C

Assunto: FBP Manuscript: FBP-D-14-00130
Data: 20.03.2014 03:23
De: "Food and Bioproducts Processing" <fbp@elsevier.com>
Para: elane@cca.ufsc.br

Dear Elane

Food and Bioproducts Processing
Official journal of the European Federation of Chemical Engineering: Part C

FBP-D-14-00130 - **"POTENTIAL OF CONCENTRATION PROCESSES IN THE RECOVERY OF TOFU WHEY ISOFLAVONES"**

Your submission "POTENTIAL OF CONCENTRATION PROCESSES IN THE RECOVERY OF TOFU WHEY ISOFLAVONES" has been assigned manuscript number FBP-D-14-00130.

To track the status of your paper, please do the following:

1. Go to this URL: http://ees.elsevier.com/fbp/

2. Enter your login details

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to Food and Bioproducts Processing.

Kind regards

Catherine Cliffe
Food and Bioproducts Processing

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

ANEXO D
SUBMISSÃO ARTIGO ATIV. ANTIOXIDANTE

Assunto: Submission Confirmation
Data: 25.04.2014 00:37
De: "Industrial Crops and Products" <esubmissionsupport@elsevier.com>
Para: elane@cca.ufsc.br

Dear Elane,

We have received your article "**Antioxidant properties of tofu whey concentrate by freeze concentration and nanofiltration processes**" for consideration for publication in Industrial Crops and Products.

Your manuscript will be given a reference number once an editor has been assigned.

To track the status of your paper, please do the following:

1. Go to this URL: http://ees.elsevier.com/indcro/

2. Enter these login details:
Your username is: elane@cca.ufsc.br

If you need to retrieve password details, please go to:

http://ees.elsevier.com/indcro/automail_query.asp

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Industrial Crops and Products