MITIE SÔNIA SADAHIRA

Effect of polysaccharide addition on the foaming properties of egg white protein in aqueous and high sugar content systems

Efeito da adição de polissacarídeos nas propriedades espumantes de proteínas da clara de ovo em sistemas aquoso e com alto teor de açúcares

CAMPINAS

2014

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

Mitie Sônia Sadahira

Effect of polysaccharide addition on the foaming properties of egg white protein in aqueous and high sugar content systems

Efeito da adição de polissacarídeos nas propriedades espumantes de proteínas da clara de ovo em sistemas aquoso e com alto teor de açúcares

Thesis presented to the School of Food Engineering of University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food and Nutrition, concentration area Consumption and Food Quality.

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Alimentos e Nutrição, na área de concentração Consumo e Qualidade de Alimentos

Orientadora: Profa. Dra. Flavia Maria Netto

Este exemplar corresponde à versão final da tese defendida pela aluna Mitie Sônia Sadahira e orientada pela Profa. Dra. Flavia Maria Netto

_____
Assinatura da Orientadora

Campinas

2014

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Helena Joana Flipsen - CRB 8/5283

Sa15e

Sadahira, Mitie Sônia, 1964-
Effect of polysaccharide addition on the foaming properties of egg white protein in aqueous and high sugar contente systems / Mitie Sônia Sadahira. – Campinas, SP : [s.n.], 2014.

Orientador: Flavia Maria Netto.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Confeitos aerados. 2. Interação de biopolímeros. 3. Interação eletrostática. 4. Microestrutura. 5. Estabilidade. I. Netto, Flavia Maria,1957-. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Efeito da adição de polissacarídeos nas propriedades espumantes de proteínas da clara de ovo em sistemas aquoso e com alto teor de açúcares
Palavras-chave em inglês:
Aerated confectionery
Biopolymer interaction
Electrostatic interaction
Microstructure
Stability
Área de concentração: Consumo e Qualidade de Alimentos
Titulação: Doutora em Alimentos e Nutrição
Banca examinadora:
Flavia Maria Netto [Orientador]
Carlos Raimundo Ferreira Grosso
Denise Calil Pereira Jardim
Rosiane Lopes da Cunha
Suzana Caetano da Silva Lannes
Data de defesa: 28-08-2014
Programa de Pós-Graduação: Alimentos e Nutrição

Banca Examinadora

Profa. Dra. Flavia Maria Netto
Faculdade de Engenharia de Alimentos – UNICAMP
Orientador

Prof. Dr. Carlos Raimundo Ferreira Grosso
Faculdade de Engenharia de Alimentos – UNICAMP
Membro Titular

Dra. Denise Calil Pereira Jardim
Instituto de Tecnologia de Alimentos – ITAL
Membro Titular

Profa. Dra. Rosiane Lopes da Cunha
Faculdade de Engenharia de Alimentos – UNICAMP
Membro Titular

Profa. Dra. Suzana Caetano da Silva Lannes
Faculdade de Ciências Farmacêuticas – USP
Membro Titular

Profa. Dra. Maria Isabel Rodrigues
Faculdade de Engenharia de Alimentos – UNICAMP
Membro Suplente

Profa. Dra. Samantha Cristina de Pinho
Faculdade de Zootecnia e Engenharia de Alimentos - USP
Membro Suplente

Prof. Dr. Ângelo Luiz Fazani Cavallieri
Universidade Federal de São Carlos
Membro Suplente

PhD thesis

AUTHOR: Mitie Sônia Sadahira

TITLE: Effect of polysaccharide addition on the foaming properties of egg white protein in aqueous and high sugar content systems

SUPERVISOR: Profa. Dra. Flavia Maria Netto
Department of Food and Nutrition – FEA - UNICAMP

ABSTRACT

In aerated confectionery (marshmallow and nougat), foam is produced by aeration of sugar syrups and stabilized by proteins such as egg white protein (EW). Pectin, an anionic polysaccharide, may form electrostatic complexes with protein at pH values bellow the isoeletric point (pI) of the protein. Hydroxypropylmethylcellulose (HPMC) is a neutral polysaccharide with emulsifying properties. The study aimed at studying the foaming properties (foaming capacity and foam stability) of EW in the presence of these polysaccharides in aqueous solution and high sugar system. Firstly, the effects of EW/polysaccharide interaction on the foaming properties in aqueous solution were evaluated. The effects of biopolymer concentration (2.0-4.0% w/w), EW:pectin ratio (15:1-55:1) and temperature (70-80 ℃) were evaluated at pH 3.0, using a central composite design. At EW:pectin ratio 15:1, the complexes were close to electroneutrality and with an average size of 95.91$\pm$8.19 μm, leading to greater stability related to disproportionatin. At ratio 55:1, the complexes were not electrically neutral and with an average size of 45.92$\pm$3.47 μm, resulting in a low drainage of liquid and coalescence. The effects of biopolymer concentration (2.0-5.0% w/w), EW:HPMC ratio (2:1-18:1) and pH (3.0-6.0) at 75 ℃ were evaluated using central composite rotata ble design (CCRD) and the behavior of biopolymer in aqueous solution on the foaming properties at different pH. At pH 3.0, EW and HPMC were compatible leading to better foaming properties whereas at pH 4.5 and 6.0, EW and HPMC were incompatible resulting in lower stability related to disproportionation. In the second part of the study, the effects of EW/polysaccharide interactions on a model system of sugar with characteristics of marshmallow (density<0.50 g/mL; water activity<0.75) were evaluated. For that, a sugar solution composition (42.5% of sucrose, 42.5% of glucose syrup and 15.0% of invert sugar) was defined by a mixture experimental design. The effects of biopolymer concentration (1.40-5.60% w/w) and EW:pectin ratio (7:1-63:1) on the reponses were evaluated using CCRD, at pH 3.0. The responses were apparent viscosity of sugar/EW/pectin mixture before whipping, overrun, foam density and, rheological parameters of fresh foam and foam aged for 24 h (elastic modulus G', viscous modulus G" and phase angle δ). At EW:pectin ratio 7:1, the mixture

showed low foaming capacity and a foam with less solid character and low stability. At ratio 49:1, the mixture presented greater foaming capacity and elastic behavior of foam. The effects of biopolymer concentration (1.4-5.6% w/w) and EW:HPMC ratio (2:1-18:1) on the responses of sugar/EW/HPMC mixtures were evaluated using CCRD at pH 3.0 and the same responses evaluated in the study of sugar/EW/pectin mixtures. At biopolymer concentration 5.0% w/w and EW:HPMC ratio 14:1, experiments were carried out at different pH. At pH 3.0, the higher foaming capacity and elastic behavior were obtained. At pH 4.5, foam showed better stability than foam at pH 3.0. At pH 6.0, foam presented the poorest foaming properties and viscous behavior. Thus, the control of protein/polysaccharide interactions is a key factor for the aerated products developing with higher stability.

Keywords: Aerated confectionery, biopolymer interaction, electrostatic complexes, microstructure, stability

TESE DE DOUTORADO

AUTORA: Mitie Sônia Sadahira
Título: Efeito da adição de polissacarídeos nas propriedades espumantes de proteínas da clara de ovo em sistema aquoso e com alto teor de açúcares
Orientadora: Flavia Maria Netto
Departamento de Alimentos e Nutrição – FEA - UNICAMP

RESUMO GERAL

Nos confeitos aerados (*marshmallow* e *nougat),* a espuma é produzida pela aeração de xaropes de açúcares, estabilizada por proteínas tais como proteínas da clara de ovo (PCO). A pectina, polissacarídeo aniônico, pode formar complexos eletrostáticos com proteína em pH abaixo do ponto isoelétrico da proteína. A hidroxipropilmetilcelulose (HPMC) é um polissacarídeo neutro com propriedades emulsificantes. O trabalho visou estudar as propriedades espumantes (capacidade de aeração e estabilidade da espuma) da PCO na presença destes polissacarídeos em solução aquosa e sistema modelo de açúcares. Na primeira etapa, foram avaliados os efeitos das interações PCO/polissacarídeo nas propriedades espumantes em solução aquosa. Os efeitos da concentração de biopolímeros (2,0-4,0% p/p), proporção PCO:pectina (15:1-55:1) e temperatura (70-80 ℃) nas propriedades espumantes no pH 3,0 foram avaliados, utilizando delineamento composto central. Na proporção PCO:pectina 15:1, os complexos eram próximos da eletroneutralidade e com tamanho médio de 95,91$\pm$8,19 μm, conduzindo para maior estabilidade da espuma quanto à desproporção. Na proporção 55:1, os complexos não eram eletricamente neutros e com tamanho médio de 45,92$\pm$3,47 μm, resultando em espumas com menor drenagem de líquido e coalescência. Foram avaliados os efeitos de concentração de biopolímeros (2,0-5,0% p/p), proporção PCO:HPMC (2:1-18:1) e pH (3,0-6,0) a 75 ℃ utilizando delineamento composto central rotacional (DCCR) e do comportamento dos biopolímeros na solução aquosa em diferentes pH nas propriedades espumantes. No pH 3,0, os biopolímeros eram compatíveis, conduzindo a melhores propriedades espumantes enquanto nos pH 4,5 e 6,0, os biopolímeros eram incompatíveis, resultando em menor estabilidade com relação a desproporção. Na segunda etapa do trabalho, foram avaliados os efeitos das interações PCO/polissacarídeo em sistema modelo de açúcares com características de *marshmallow (*densidade<0,50 g/mL; atividade de água<0,75). A composição da solução de açúcares (42,5% sacarose, 42,5% xarope de glicose e 15% de açúcar invertido) foi definida utilizando delineamento experimental de mistura. Os efeitos da concentração de biopolímeros (1,40–5,60% p/p) e proporção PCO:pectina (7:1–63:1) nas respostas foram avaliadas utilizando um DCCR, no pH 3,0. As respostas foram viscosidade aparente da

mistura açúcares/PCO/pectina antes do batimento e densidade, *overrun,* parâmetros reológicos da amostra aerada recém-processada e após 24 horas (módulo elástico G', módulo viscoso G" e δ). Na proporção PCO:pectina 7:1, a mistura apresentou baixa capacidade de aeração e uma espuma com característica menos sólida e baixa estabilidade. Na proporção 49:1, a mistura apresentou maior capacidade de aeração e comportamento elástico da espuma. Os efeitos da concentração de biopolímeros (1,4-5,6% p/p) e proporção clara de ovo:HPMC (2:1-18:1) nas respostas das misturas açúcar/PCO/HPMC foram avaliados, utilizando um DCCR no pH 3,0 e as mesmas respostas avaliadas no estudo com misturas açúcar/PCO/pectina. Na concentração de biopolímeros 5,0% p/p e proporção PCO:HPMC 14:1 foram realizados experimentos em diferentes pH. No pH 3,0, foram obtidos maior capacidade de aeração e comportamento elástico. No pH 4,5, a espuma apresentou melhor estabilidade comparada a espuma no pH 3,0. No pH 6,0, a espuma apresentou propriedades espumantes ruins e comportamento viscoso. Portanto, o controle das interações proteína/polissacarídeo é um fator chave para o desenvolvimento de produtos aerados com maior estabilidade física.

Palavra-chave: Confeitos aerados, interação de biopolímeros, complexos eletrostáticos,microestrutura, estabilidade

ÍNDICE GERAL

Dedico esta tese ao Deus de amor e misericórdia, a Jesus Cristo, a Espiritualidade Maior, aos meus antepassados, meu pai Hiroshi, minha mãezinha Sayoko. Eterna gratidão!

Agradecimentos

Obrigada Deus por guiar e iluminar a realização desta tese de doutorado.

Agradeço a minha orientadora Profa. Flavia Maria Netto pelo grande incentivo para eu realizar o doutorado direto e o doutorado sanduiche no Reino Unido. Além do grande aprendizado em como pensar e escrever cientificamente. Ah! E das correções fantásticas dos artigos e da tese deixando os textos mais claros e organizados.

Gratidão à Denise Jardim pelo encorajamento e por acreditar em mim.

À Diretoria do CEREAL CHOCOTEC/ITAL (Valdecir Luccas, Fernanda Zaratini Vissotto e Carla Léa de Camargo Vianna Cruz) pelo grande apoiou na etapa final da tese.

Aos membros da banca examinadora que colaboraram com as sugestões e correções da tese, permitindo o melhoramento deste trabalho.

Profa. Maria Isabel Rodrigues pela paciência e dedicação em me ajudar nas dúvidas e discussões sobre os planejamentos experimentais.

Ao Prof. Carlos Grosso por permitir utilizar o equipamento para as medidas de potencial zeta das soluções de biopolímeros.

Yara por auxiliar no uso do equipamento Malvern Zetasizer Nano Z instrument.

Eliana por realizar a eletroforese das proteínas da clara de ovo.

Angelica que ajudou na limpeza das vidrarias, entre preparo de amostras e análises a serem realizadas na UNICAMP.

Isabela obrigada pelas dicas do equipamento Turbiscan e por ensinar a utilizar o medidor de tamanho de partícula (Horiba), e Rafael pela grande ajuda para resolver os problemas da formatação da tese.

Vania que sempre me incentivou para eu conseguir realizar a tese de doutorado. Grande amiga!

A minha família que sempre me apoiou, compreendendo as minhas ausências.

À FAPESP pelo auxílio financeiro (FAPESP 2011/50067/9) e à EMBRAPA pela bolsa de doutorado concedida.

I am very grateful to the staff of School of Food Science and Nutrittion/University of Leeds (UK), (Catherine Roberts, Ian Hardy, Angela Morrison, Dr. Mahmood Akhtar), and especially to my supervisor, Prof. Brent S. Murray, for all the support and guidance.

I would like to thank to George for correcting my abstracts and my friends Iriani, Joana, Regiane, Tugba and Woroud for all the wonderful memories throughout my time in UK.

Meu muito obrigada a todos!

ÍNDICE DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

CAPÍTULO 4

CAPÍTULO 5

CAPÍTULO 6

ANEXO A

ÍNDICE DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

CAPÍTULO 6

LISTA DE ABREVIATURAS E SIGLAS

Aa	Atividade de água
ANOVA	Analysis of variance
BS	Backscattering values
CCD	Central composite design
CCRD	Central composite rotatable design
CLSM	Confocal scanning laser microscope
DR	Drainage (% drained liquid)
EW	Egg white protein
HPMC	Hydroxypropylmethylcellulose
Hz	Hertz
$\mathbf{m_f}$	Mass of the resulting foam with the same volume of mi
$\mathbf{m_i}$	Mass of the initial solution (before whipping)
pI	Isoelectric point
pKa	Apparent dissociation constant
$\mathbf{R^2}$	Percentage of variance explained
SDS-PAGE	(Polyacrylamide gel electrophoresis) eletroforese em gel de poliacrilamida
$\mathbf{V_{bubble}}$	Bubble growth rate (% BS/min)

LISTA DE SÍMBOLOS

d_{32}	Average bubble diameter
d_{43}	Mean diameter in volume
δ	Phase angle
G'	Storage modulus
G''	Loss modulus
η	Apparent viscosity
ρ	Density

INTRODUÇÃO GERAL

Introdução geral

Uma ampla variedade de produtos alimentícios que são consumidos diariamente tem como base um componente que não consta na lista do rótulo da embalagem: o ar (DECKER; ZIEGLER, 2002). Além disso, as bolhas de ar dispersas em uma matriz são elementos importantes para as indústrias que almejam introduzir inovações em seus produtos (GERMAIN; AGUILERA, 2014).

Na indústria de confeitos, a aeração é utilizada para produzir uma variedade de produtos (balas mastigáveis, *marshmallow, nougat*, merengue e recheios), dependendo da densidade, que pode variar de 0,2 a 1,0 g/cm^3 (JACKSON, 1995).

A espuma é uma estrutura que consiste de uma fase contínua líquida ou sólida e de uma fase gasosa (ar) dispersa. Na maioria dos alimentos com características de espuma, as proteínas são os agentes tensoativos que ajudam na formação e na estabilização da fase gasosa dispersa. Geralmente, as espumas estabilizadas por proteínas são formadas por borbulhamento, batimento ou agitação de uma solução de proteínas. A propriedade espumante de uma proteína refere-se à sua habilidade em formar um filme resistente e fino na interface ar-líquido para que uma grande quantidade de bolhas de ar possa ser incorporada e estabilizada (DAMODARAN, 2008). Nos confeitos aerados, a espuma pode ser produzida pela aeração de xaropes de açúcares e estabilizada pela adição de proteínas (JACKSON, 1995). As proteínas da clara de ovo são amplamente utilizadas para estabilizar produtos aerados como *marshmallow* e *nougat*.

Por meio da gelificação e agregação, as proteínas e polissacarídeos contribuem para a estrutura e textura dos alimentos (DICKINSON, 2003; FOEGEDING; DAVIS, 2011). Além disso, as proteínas são conhecidas por suas propriedades emulsificantes e espumantes e os polissacarídeos pelas propriedades espessantes e de retenção de água (DICKINSON, 2003). As interações proteína-polissacarídeo têm um papel importante na estrutura e estabilidade de alimentos processados, onde o controle destas interações é um fator chave no desenvolvimento de novos produtos (BENICHOU et.al., 2007). Estudos têm mostrado que as interações proteína-polissacarídeo têm efeito nas propriedades espumantes (IBANOGLU; ERÇELEBI, 2007; NARCHI; VIAL; DJELVEH, 2009; SCHMIDT et al., 2010).

Espumas formadas a partir de proteínas da clara de ovo na presença de pectina, um polissacarídeo aniônico, apresentam maior estabilidade que na presença de goma

guar, um polissacarídeo neutro. Este aumento na estabilidade da espuma pode estar associado à maior espessura da camada do filme interfacial que está relacionado com aumento na resistência ao colapso da espuma (IBANOGLU; ERCEBELI, 2007).

Derivados de celulose, como a hidroxipropilmetilcelulose (HPMC), apresentam propriedades emulsificantes significativas (SARKAR, 1984) e estabilizantes de espumas e emulsões além da com capacidade de retenção de água e de aumento da viscosidade do sistema (DICKINSON, 2003; SARKAR; WALKER, 1995). A introdução de grupos hidrofóbicos (substituições metila) permite que o HPMC comporte-se como um surfactante (PEREZ et al., 2007).

Tendo em vista que as características destes dois polissacarídeos podem ser importantes na formação de espumas e nas características de produtos aerados, torna-se importante o estudo dos efeitos de interações de proteínas e polissacarídeo, da concentração de proteínas da clara de ovo e polissacarídeo, proporção proteína:polissacarídeo e pH, assim como combinações destes parâmetros nas propriedades espumantes (capacidade de aeração e estabilidade) em solução aquosa e mistura multicomponentes de açúcares.

Como os confeitos aerados são termodinamicamente instáveis, o presente trabalho tem como objetivo contribuir para o desenvolvimento de produtos, utilização de ingredientes e aditivos, otimização de processo e manutenção deste tipo de produto aerado com maior estabilidade física.

Objetivos

O objetivo geral deste trabalho foi estudar o efeito nas propriedades espumantes das interações proteínas da clara de ovo e pectina e proteínas da clara de ovo e HPMC em solução aquosa e sistema com alto teor de açúcares.

Os objetivos específicos foram:

- Avaliação dos efeitos das interações das proteínas da clara de ovo e HPMC, em solução aquosa, nas propriedades espumantes;
- Avaliação dos efeitos das interações das proteínas da clara de ovo e pectina, em solução aquosa, nas propriedades espumantes;

- Definição da mistura de açúcares a ser utilizada na avaliação das variáveis que influenciam as propriedades espumantes de sistemas proteínas da clara de ovo e pectina e proteínas da clara de ovo e HPMC em alto teor de açúcares;
- Avaliação dos efeitos das interações das proteínas da clara de ovo e HPMC, em mistura multicomponente de açúcares, nas propriedades espumantes ;
- Avaliação dos efeitos das interações das proteínas da clara de ovo e pectina, em mistura multicomponente de açúcares, nas propriedades espumantes.

Estrutura do trabalho

Este trabalho está apresentado em 7 capítulos descritos a seguir.

Capítulo 1: *Revisão Bibliográfica*

Neste capítulo está apresentada a revisão bibliográfica referente aos tópicos estudados.

Capítulo 2: *Influence of protein pectin electrostatic interaction on the foam stability mechanism*

Neste capítulo apresenta-se um estudo sobre o efeito da interação eletrostática proteina-pectina nos mecanismos da estabilidade da espuma. A estabilidade foi avaliada por velocidade do aumento do tamanho da bolha de ar e quantidade de líquido drenado (drenagem do líquido).

Capítulo 3: *Effect of pH and interaction between egg white protein and hydroxypropymethylcellulose in bulk aqueous medium on the foaming properties*

Neste capítulo foi avaliado o efeito da interação entre as proteínas da clara de ovo e hidroxipropilmeticelulose em solução nas propriedades espumantes. Foi estudada a influência do pH nas interações proteina-polissacarídeo e nas propriedades espumantes.

Capítulo 4: *Definição de um sistema modelo de açúcar para produtos aerados tipo marshmallow utilizando delineamento experimental de mistura*

Nesta etapa foi definida a mistura de açúcares para ser utilizada como modelo para o estudo do efeito das interações proteína-pectina e proteína-HPMC nas propriedades espumantes em sistemas com alto teor de açúcares.

Capítulo 5: *Effect of egg white protein-pectin electrostatic interaction in a high sugar content system on foaming and foam rheological properties*

Neste estudo foi avaliado o efeito da interação eletrostática proteína-pectina em um sistema com alto teor de açúcar nas propriedades espumantes e reológicas das suas espumas.

Capítulo 6: *Foaming and rheological properties of aerated high sugar system with egg white protein and hydroxypropymethylcellulose*

Neste estudo foi avaliado o efeito da concentração total de biopolímeros e a proporção proteína:HPMC nas propriedades espumantes e reológicas das amostras aeradas. O efeito do pH (3,0, 4,5 e 6,0) nas propriedades espumantes e reológicas foi também avaliado.

Capítulo 7: *Conclusões Gerais*

Neste capítulo são apresentadas as principais conclusões obtidas a partir dos resultados dos demais capítulos.

Referências bibliográficas

BENICHOU, A.; ASERIN, A.; LUTZ, R.; GARTI, N. Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. **Food Hydrocolloids**, v. 21, p. 379–391, 2007.

DAMODARAN, S. (2008). Amino Acids, Peptides, and Proteins. In DAMODARAN, S.; PARKIN, K. L.; FENNEMA, O.R. (Ed.), **Food Chemistry**. Boca Raton: CRC Press. p. 217–329.

DECKER, N.R.; ZIEGLER. G;R. The structure of aerated confectionery. **The Manufacturing Confectioner,** v.82, n.9, p. 101-108, 2002.

DICKINSON, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. **Food Hydrocolloids,** v. 17. p. 25-39, 2003.

FOEGEDING, E.A.; DAVIS, J.P. Food protein functionality: A comprehensive approach. **Food Hydrocolloids**, v. 25, p. 1853-1864, 2011.

GERMAIN, J.C.; AGUILERA, J.M. Multi-scale properties of protein-stabilized foams. **Food Structure** , v.1, p. 55 – 70, 2014.

IBANOGLU, E; ERÇEBELI, E.A. Thermal denaturation and functional properties of egg proteins in the presence of hydrocolloid gums. **Food Chemistry,** v. 101, n. 2, p. 626-633, 2007.

JACKSON, E.B. Liquorice paste, cream paste and aerated confectionery. In JACKSON, E. B. (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional. 1995. p. 218–235

NARCHI, I.; VIAL, CH.; DJELVEH, G. Effect of protein-polysaccharide mixtures on the continouous manufacturin of foamed food products. **Food Hydrocolloids,** n. 23, n. 1, p. 188-201, 2009.

PEREZ, O.E.et al.. Adsorption dynamics and surfgace activity at equilibrium of whey proteins and hydroxypropy-methyl-cellulose mixtures at the air-water interface. **Food Hydrocolloids,** v.21, n. 5-6, p. 794-803, 2007.

SARKAR, N. Structural interpretation of the interfacail properties of aqueous-solutions of methylcellulose and hydroxypropyl methylcellulose. **Polymer,** v. 25, n. 4, p.481-486, 1984.

SARKAR, N.; WALKER, L.C. Hydration-dehydration properties of methylcellulose and hydroxypropylmethylcellulose. **Carbohydrate Polymers**, v. 27. p.177-185, 1995.

SCHMIDT, I. et al. Foaming properties of protein/pectin electrostatic complexes and foam structure at nanoscale. **Journal of Colloid and Interface Science**, v. 345, n. 2, p.316–324, 2010.

CAPÍTULO 1. REVISÃO BIBIOGRÁFICA

Capítulo 1. Revisão bibliográfica

1 Espumas

Espumas podem ser definidas como uma dispersão de uma fase gasosa em uma fase contínua líquida ou sólida (FOEGEDING, DAVIS, 2011). Geralmente, nos produtos aerados a fase contínua é líquida. Porém, a fase líquida é transformada em sólida ao sofrer processo de cozimento ou congelamento (MURRAY; ETTELAIE, 2004). Na indústria de alimentos, as proteínas são os agentes de aeração utilizados na formação e estabilização das fases na fase gasosa dispersa (NICORESCU et. al., 2011). As espumas estabilizadas por proteínas são formadas por borbulhamento, batimento ou agitação de uma solução de proteínas (DAMODARAN, 2008).

Na indústria de confeitos, a aeração é utilizada para produzir uma variedade de produtos (balas mastigáveis, *marshmallow, nougat*, merengue e recheios), dependendo da densidade obtida que pode variar de 0,2 a 1,0 g/cm^3. Os confeitos aerados são produzidos pela aeração de xaropes de açúcares, sendo estabilizada pela adição de proteínas. A espuma é formada lentamente com a formação de grandes bolhas de ar que, com o batimento, são progressivamente quebradas em tamanhos menores até atingir uma densidade ótima para confeitos aerados (JACKSON, 1995). Os benefícios da aeração dos produtos envolvem primeiramente a textura (KINSELLA, 1981).

Segundo Campbell e Mougeot (1999) e Kinsella (1981), a aeração promove a redução da densidade do produto, alteração de sua textura e reologia, implicando em diferenças no sabor e na aparência.

2 Propriedades espumantes

As proteínas são moléculas anfifílicas que migram espontaneamente para a interface ar-água, e tal migração espontânea indica que a energia livre das proteínas é menor na interface que na solução. Portanto, quando o equilíbrio é atingido, a concentração de proteína na região da interface é sempre maior que na fase aquosa. As proteínas formam um filme viscoelástico na interface que tem a habilidade de resistir a choques mecânicos durante o armazenamento e manuseio. As espumas estabilizadas por proteínas são mais estáveis que aquelas preparadas com surfactantes de baixo peso molecular, e por isso as proteínas são bastante utilizadas para este propósito (DAMODARAN, 1996). A função da proteína é servir como “ponte” entre as duas fases

onde os grupos hidrofóbicos da proteína estão expostos para a fase gasosa e os grupos hidrofílicos para a fase aquosa, diminuindo a tensão interfacial entre as duas fases. A tensão superficial pode ser reduzida, porém não eliminada. Portanto, as espumas são termodinamicamente instáveis (WALSTRA, 2003)

As espumas são caracterizadas por duas propriedades: capacidade de aeração (*overrun*) e estabilidade da espuma. A capacidade de aeração está relacionada com o volume de ar introduzido na solução de proteínas e é determinada pelo aumento de volume da espuma. A estabilidade da espuma é importante para a vida-de-prateleira e a aparência do produto que deve ser mantida nas várias etapas do processamento tais como mistura, cozimento e formatação do produto aerado (FOEDGEDING; LUCK; DAVIS, 2006).

A estabilidade da espuma é afetada por mecanismos de instabilidade como cremeação, drenagem, desproporção e coalescência (DAMODARAN, 2005; WALSTRA, 2003). A cremeação acontece quando há uma grande diferença de densidade entre a fase dispersa e contínua. No entanto, quando a viscosidade da fase contínua for muito alta, as bolhas serão somente rompidas ou desestabilizadas por coalescência e desproporção (MURRAY, 2007).

Em espumas com fase contínua líquida, a drenagem do líquido a partir da *lamella* (fluido entre as bolhas de ar) pelo efeito da gravidade é seguida pela aproximação das superfícies das bolhas de ar e a coalescência (fusão de duas ou mais bolhas de ar) pode ocorrer, conduzindo ao colapso da espuma, perda de gás, estrutura e textura, almejadas para espumas (DAMODARAN, 2005; MURRAY; ETTELAIE, 2004). Para estabilizar uma espuma contra a coalescência é necessário diminuir a taxa de drenagem do líquido. A drenagem do líquido é reduzida pelo aumento da viscosidade da fase contínua ou aumento da repulsão entre as bolhas de ar. Ainda que a drenagem e a colescência sejam reduzidas, a difusão de gás entre as bolhas com pressões internas diferentes pode ocorrer. Este efeito é denominado desproporção e está relacionada à difusão de gás das bolhas de ar pequenas para bolhas grandes. Como resultado tem-se o desaparecimento das bolhas pequenas e a expansão das bolhas grandes. Para prevenir a desproporção, a espuma deve apresentar uma distribuição de bolhas de ar uniforme (DAMODARAN, 2005; MURRAY, 2004; WALSTRA, 2003).

2.1 Fatores que influenciam a formação e a estabilidade das espumas

2.1.1 pH

A carga total das moléculas das proteínas e de sua conformação em função do pH têm efeito nas propriedades espumantes. No ponto isoelétrico (pI), a carga total das proteínas é próximo de zero. Devido à baixa repulsão eletrostática entre as proteínas, as forças atrativas predominam resultando na associação das proteínas. Em pH abaixo do pI, as proteínas encontram-se carregadas positivamente e em pH acima do pI, as proteínas em solução apresentam carga total negativa, conduzindo à repulsão e aumento das interações proteína-água, o que reduz a associação proteína-proteína (KUROPATWA; TOLKACH; KULOZIK, 2009; RODRÍGUEZ PATINO; PILOSOF, 2011).

Próximo ao pI, as proteínas da clara de ovo encontram-se próximas da neutralidade e a repulsão eletrostática intermolecular é baixa. Este fator pode levar à formação de um filme interfacial compacto e mais estável, aumentando a estabilidade da espuma (DAMODARAN, 1996; KUROPATWA; TOLKACH; KULOZIK, 2009). Outras proteínas, tais como as proteínas do soro de leite, apresentam boas propriedades espumantes na região próxima ao pI (DAVIS; FOEGEDING; HANSEN, 2004; PHILLIPS; SCHULMAN; KINSELLA, 1990; ZHU; DAMODARAN, 1994).

A ovalbumina, principal proteína da clara de ovo (54% do total de proteínas), apresenta melhores propriedades emulsificantes em pH ácido do que em neutro devido à maior hidrofobicidade superficial e flexibilidade das moléculas (MINE; NOUTOMI; HAGA, 1991; STEVENS, 1991). Liang e Kristinsson (2005) estudaram as propriedades espumantes das proteínas da clara de ovo parcialmente desdobradas por exposição a pH extremos (1,5; 2,5; 3,5; 10,5; 11,5 ou 12,5) e posterior retorno a valores entre 4,5 e 8,5. Os autores verificaram que a hidrofobicidade superficial aumentou após a desnaturação e teve uma boa correlação com o melhoramento das propriedades espumantes.

2.1.2 Presença de NaCl

Os íons do sal NaCl podem proteger as moléculas de proteínas com cargas elétricas, reduzindo a repulsão eletrostática entre as moléculas de proteínas adsorvida e não-adsorvidas, facilitando a adsorção das proteínas na interface ar-água (RAIKOS; CAMPBELL; EUSTON,, 2007). Davis, Foegeding e Hansen. (2004) relataram resultados

similares com respeito ao efeito do NaCl na adsorção do isolado protéico de soro de leite na interface ar-água.

2.1.3 Concentração de proteína

Estudos realizados por Lau e Dickinson (2004) com açúcar invertido, demonstraram que a capacidade de aeração e estabilidade da espuma foram influenciadas pela concentração das proteínas da clara de ovo. Na aeração de açúcar invertido, o *overrun* aumentou com o aumento da concentração de clara de ovo de 2 a 6%. Porém, a partir de 8% não houve aumento significativo do *overrun*, pois o aumento da concentração de proteína aumentou a viscosidade da mistura dificultando a incorporação de ar.

A maior concentração de proteínas leva à formação de espuma mais firme, resultado do menor tamanho das bolhas de ar formadas e do aumento da viscosidade da fase contínua. A estabilidade da espuma melhora com o aumento da concentração de proteínas, pois aumenta-se a viscosidade e facilita-se a formação de um filme de proteínas coeso na interface (DAMODARAN, 2008).

2.1.4 Presença de açúcares

Vários estudos tem mostrado o efeito dos açúcares nas propriedades espumantes (FOEGEDING; DAVIS, 2006; LAU; DICKINSON, 2005; RAIKOS; CAMPBELL; EUSTON, 2007). De acordo com Raikos, Campbell e Euston. (2007), o aumento da concentração de sacarose diminui a quantidade de ar incorporada nas amostras aeradas com clara de ovo quando comparadas com amostras sem adição de sacarose, porém a estabilidade das amostras aumentou em concentrações maiores de sacarose. A adição de açúcares aumenta a viscosidade da fase contínua (líquida), o que dificulta a incorporação de ar e a rápida difusão e desdobramento da proteína na região da interface ar-água. Os açúcares contribuem para a estabilidade da espuma pelo aumento da viscosidade da fase contínua líquida, retardando a drenagem (LAU; DICKINSON, 2005).

2.1.5 Tratamentos térmicos

Geralmente a desnaturação parcial das proteínas melhora as propriedades espumantes. Hagolle et al. (2000) constataram que o pré-tratamento térmico

(aquecimento a uma taxa de 1 ℃/min até a temperatura de 72 ℃, seguido de um resfriamento até 20 ℃) levou a melhores propriedades espumantes. A melhora da capacidade de aeração foi atribuída pelos autores à maior hidrofobicidade superficial e ao aumento da flexibilidade da molécula de proteína. De acordo com Ibanoglu e Erçebeli (2007), o tratamento térmico da clara de ovo entre 65 e 80 ℃ diminuiu a capacidade de aeração, porém a estabilidade da espuma aumentou.

O tratamento térmico de isolado proteico de soro de leite por 1 min a 70 ℃ apresentou uma melhor capacidade de aeração e estabilidade da espuma do que a proteína na forma nativa ou intensamente desnaturada (ZHU; DAMODARAN, 1994).

2.2 Microestrutura: distribuição de tamanho de bolhas de ar

A percepção da textura é determinada em grande parte pela estrutura microscópica do produto. A estrutura e, consequentemente, a textura são influenciadas por fatores tais como ingredientes e parâmetros de processo (DECKER; ZIEGLER, 2002). O tamanho, forma e distribuição do tamanho das bolhas de ar são parâmetros importantes para a produção de confeitos aerados de alta qualidade (DECKER; ZIEGLER, 2002).

Atualmente, as técnicas de imagem e microscopia estão disponíveis para pesquisar a estrutura dos alimentos e desenvolver processos que melhorem a qualidade dos produtos. A análise de imagens fornece dados quantitativos para avaliação da microestrutura do alimento (AGUILERA, 2005). A microscopia confocal permite a visualização da mudança da densidade e distribuição de tamanho de bolhas de ar durante o processo de batimento e o grau de associação das proteínas com as bolhas de ar (LAU; DICKINSON,2004).

As espumas em geral apresentam variação do tamanho de bolhas de ar, não sendo suficiente a caracterização apenas em termos de tamanho médio de bolha. A distribuição de tamanho de bolhas de ar tem sido correlacionada com a estabilidade da espuma (YANG, 2008). A coalescência e a desproporção alteram a distribuição de tamanho de bolhas de ar. Uma espuma com uma distribuição de tamanho de bolhas de ar mais uniforme e menos polidispersa apresenta instabilidade menor por desproporção (SARMA; KHILAR, 1988; UPADHYAY; GHOSAL; MEHRA, 2012).

A microscopia confocal tem sido utilizada para determinar o tamanho e a distribuição de tamanho de bolhas de ar em espumas (PERNELL et al., 2002; LAU; DICKINSON, 2004; UPADHYAY; GHOSAL; MEHRA, 2012). Esta técnica é adequada para avaliar espumas com bolhas de ar pequenas e com aumento lento das bolhas, pois o laser pode capturar imagens de várias camadas de bolhas de ar como, por exemplo, de sistemas com alto teor de açúcar (MURRAY, 2007).

3 Proteínas da clara de ovo

Para serem bons agentes de aeração, as proteínas devem apresentar: (i) habilidade para adsorver rapidamente na interface ar-água; (ii) boa estabilização estérica, bem como boa capacidade de estabilização eletrostática; (iii) formação de filme viscoelástico devido a interação entre as moléculas adsorvidas (DAMODARAN, 2008; DICKINSON, 2011; MINE, 1995).

As propriedades espumantes das proteínas são afetadas pela flexibilidade, densidade e distribuição de cargas e hidrofobicidade molecular. As proteínas, anfifílicas por natureza, são capazes de constituir uma monocamada devido à sua capacidade de orientar segmentos hidrofóbicos para a fase hidrofóbica (ar ou óleo) e os segmentos hidrofílicos são orientados para a fase aquosa. O número de segmentos ou monômeros que fazem contato com a interface dependerá, entretanto, da flexibilidade molecular (DAMODARAN, 1996; DICKINSON, 1992).

As proteínas da clara de ovo tem grande utilização em confeitos aerados, tanto em *marshmallow* como em *nougat* (JACKSON, 1995). Sua excelente capacidade de aeração se deve ao fato de ser uma mistura de proteínas composta de globulinas, ovomucóides e lisozima. As globulinas são tensoativas e contribuem para a formação de espuma. Ovomucóides e globulinas retardam a drenagem (perda da estabilidade da espuma), pois possuem alta viscosidade. A lisozima forma um complexo interfacial com a ovomucina e outras proteínas resultando em aumento da resistência do filme (DICKINSON, 1989; MINE, 1995). A hierarquia das proteínas da clara de ovo em termos da importância para formação de espuma é a seguinte: globulinas, ovalbumina, ovotransferina, lisozima, ovomucoide e ovomucina (MINE, 1995). Algumas características físico químicas das principais proteínas da clara de ovo estão apresentadas na Tabela 1.

Tabela 1. Composição e características físico químcas das principais proteínas da clara de ovo[a]

Proteína	Porcentagem do total de proteína (% em base seca)	Massa molecular (kDa)	Ponto isoelétrico (pI)
Ovalbumina	54,0	45,0	4,5
G2 globulina	4,0?	49,0	5,5
G3 globulina	4,0?	49,0	5,8
Lizosima	3,4	14,3	10,7

[a]Fonte: (Li-Chan; Nakai, 1989)

?: Dado incerto

4 Polissacarídeos

Alguns polissacarídeos possuem propriedades interfaciais como é o caso de derivados de celulose (hidroxipropilmetilcelulose) e pectina (DICKINSON, 2003; DICKINSON, 2009). Metilcelulose (MC) e hidroxipropilmetilcelulose (HPMC) são compostos por cadeias de glicose com ligações β–1,4. As aplicações do HPMC são baseadas nas substituições metil que constituem as zonas hidrofóbicas da celulose enquanto os grupos hidroxipropil são mais hidrofílicos (Figura 1). A introdução destes grupos hidrofóbicos permite que o HPMC comporte-se como um surfactante (PÉREZ et al., 2007). MC e HPMC apresentam propriedades emulsificantes (SARKAR, 1984) e estabilizantes de espumas e emulsões juntamente com capacidade de retenção de água e de aumento da viscosidade (DICKINSON, 2003). HPMC é utilizado para melhorar a qualidade de produtos de panificação, na indústria farmacêutica e na tecnologia de impressão devido às suas propriedades mecânicas e não tóxicas (PÉREZ et al., 2007).

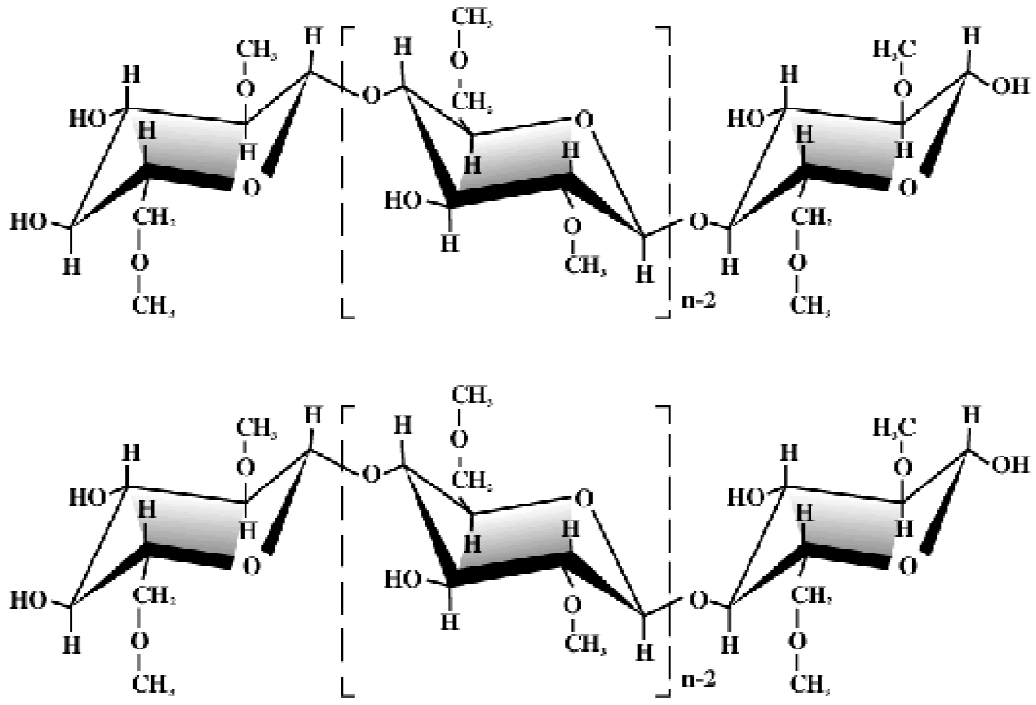


Figura 1. Estrutura da molécula de hidroxipropilmetilcelulose (Fonte: Dow Chemical Company, 2009).

A pectina, polissacarídeo aniônico carboxilado de alto peso molecular, é obtida das células da parede de vegetais, especialmente de frutas cítricas e de maçã (Figura 2). O valor de pKa da pectina é em torno de 2,9 a 3,5, apresentando carga negativa e neutra em pH acima e abaixo deste valor, respectivamente. (RALET et al., 2001; SURH, DECKER, MCCLEMENTS, 2006). É utilizada como agente de gelificação e espessante em alimentos. As propriedades funcionais dependem do grau de esterificação (DE). A pectina com alto grau de metoxilação ($\geq$ 0,50 DE) forma géis sob condições ácidas e com alto teor de açúcar e a pectina com baixo grau de metoxilação forma géis na presença de cálcio (DICKINSON, 2003; AKHTAR et.al., 2002).

A pectina de frutas cítricas e maçã, também pode apresentar boa capacidade emulsificante caso a massa molecular for reduzida para abaixo de 80 kDa por hidrólise ácida (despolimerização) (MAZOYER, LEROUX; Bruneau, 1999). Porém, de acordo com Akhtar et. al. (2002), a proteina presente na pectina encontra-se associada com a camada

adsorvida na interface óleo-água, melhorando as propriedades emulsificantes da pectina após a despolimerização (AKHTAR et.al., 2002).

A pectina de beterraba possui maior capacidade emulsificante do que pectina de frutas cítricas ou de maçã com alto e baixo grau de metoxilação devido ao maior conteúdo de proteína e de grupo acetila que apresenta característica hidrofóbica (DEA; MADDEN, 1986; LEROUX et al., 2003).

A pectina tem uma estrutura complexa que depende da fonte e do processo de extração. Basicamente, é um polímero de ácido α-D-galacturônico com ligações 1-4 (WALTER, 1991) (Figura 2).

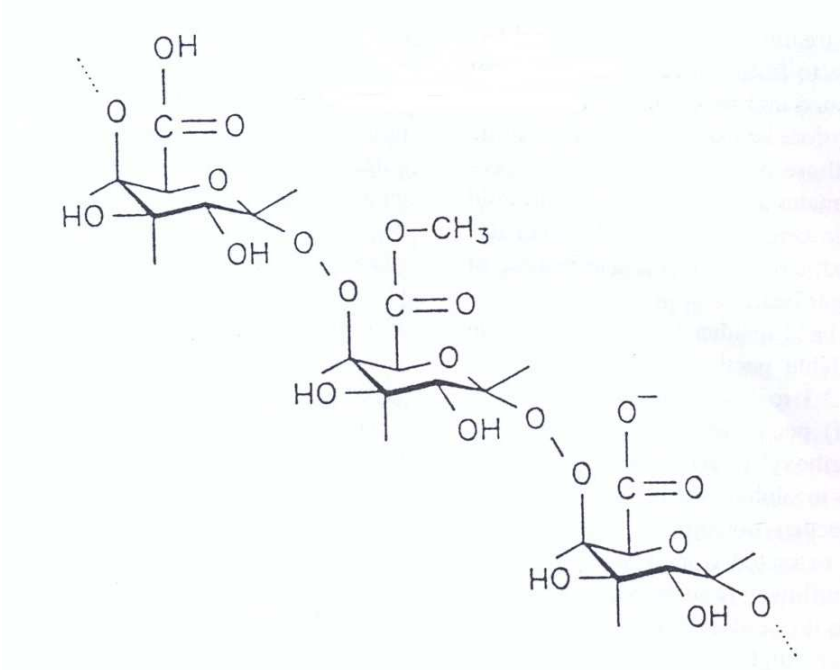


Figura 2. Estrutura da molécula de pectina (Fonte: WALTER, 1991).

A ANVISA através da Resolução da Diretoria Colegiada – RDC n. 45, de 03 de novembro de 2010 (Regulamento Técnico sobre aditivos utilizados segundo as Boas Práticas de Fabricação e suas Funções) estabelece que HPMC pode ser utilizado na função de emulsificante, espessante e estabilizante e a pectina como espessante, estabilizante, gelificante e emulficante.

5 Açúcares

A indústria de confeitos utiliza três açúcares básicos: sacarose, xarope de glicose e açúcar invertido (LEES, 1995). Para evitar o crescimento de microrganismos, os confeitos devem apresentar o teor de sólidos solúveis acima de 76% e manter a atividade de água abaixo de 0,80 à temperatura ambiente (JACKSON, 1995). O controle da atividade de água é um dos métodos para manter o alimento seguro contra o crescimento microbiológico, aumentando a vida de prateleira do produto (LABUZA; ALTUNAKAR, 2007).

A sacarose, um dos principais carboidratos utilizados na indústria de confeitos, é um dissacarídeo formado por α-D-glucose e β-D-frutose (LEES, 1995). A solução de sacarose 67,1% p/p encontra-se no estado de saturação a 20 ℃. Portanto, parte da sacarose deve ser substituída por açúcares que aumentem a solubilidade do sistema, mantendo a concentração final, sem a formação de cristais, tais como o xarope de glicose e o açúcar invertido (STANSELL, 1995).

O xarope de glicose é composto por glicose, maltose, maltotriose e oligossacarídeos, e é obtido pela conversão ácida ou enzimática do amido (CHINACHOTI, 1995). A alta viscosidade do xarope de glicose diminui a velocidade de migração da sacarose, inibindo a cristalização (JACKSON & HOWLING, 1995)

O açúcar invertido é produzido por hidrólise ácida ou enzimática da sacarose (dissacarídeo) resultando em uma mistura de glicose, frutose e sacarose. A adição do açúcar invertido contribui para diminuir a cristalização e a atividade de água devido a sua afinidade com a água (CHINACHOTI, 1995; ERGUN *et al*, 2010; JAMES, 1995).

A proporção de sacarose / xarope de glicose/ açúcar invertido influencia na textura de confeitos aerados (JACKSON, 1995). A sacarose fornece uma textura seca e firme, enquanto o xarope de glicose, uma textura pegajosa e mastigável (GROVES, 1997).

6 Interações proteína-polissacarídeo

As proteínas e os polissacarídeos controlam a estrutura, textura e estabilidade dos alimentos através das propriedades de gelificação e agregação (DICKINSON, 2003). A estrutura da proteína e a natureza da interação proteína-polissacarídeo são suscetíveis às condições do meio, tais como temperatura, força iônica e pH (DICKINSON; GALASKA, 1992).

A natureza e as forças das interações proteína-polissacarídeo em solução e em interfaces têm uma grande influência na estabilidade das dispersões e emulsões (DICKINSON, 1993; DICKINSON; EUSTON, 1991). Em soluções aquosas, uma mistura binária de proteína e polissacarídeo pode apresentar uma das três diferentes situações de equilíbrio: a) miscibilidade: ocorre normalmente a concentrações baixas de biopolímero; b) incompatibilidade: a interação proteína-polissacarideo é repulsiva e há separação em duas fases aquosas distintas, onde uma fase é rica em proteína outra em polissacarídeo, e c) coacervação: a interação proteína-polissacarídeo é de atração levando à formação de complexos solúveis sem separação de fases ou insolúveis com duas fases distintas onde uma delas é rica nos dois biopolímeros e outra fase sem biopolímeros (DICKINSON, 2003; RODRIGUEZ-PATINO; PILOSOF, 2011; TOLSTOGUZOV, 2006).

As propriedades de estabilidade físico-química das misturas de biopolímeros são determinadas em grande parte pela natureza das interações dos biopolímeros na superfície das partículas dispersas (bolhas ou gotículas). Em uma emulsão do tipo óleo em água, o agente estabilizante primário é a mistura de proteínas adsorvida na camada fina da interface óleo/água. Os polissacarídeos, geralmente, estão presentes como espessantes ou gelificantes e podem ocorrer interações diretas entre os polissacarídeos e as proteínas adsorvidas, possivelmente conduzindo à formação de uma camada estabilizante secundária (DICKINSON; EUSTON, 1991).

A incompatibilidade da proteína e polissacarídeo aumenta em regiões próximas ao pI devido à auto-associação das moléculas da proteína. Geralmente, a incompatibilidade termodinâmica acontece em pH acima do pI da proteína (GRINBERG; TOLSTOGUZOV, 1997; RODRÍGUEZ PATINO; PILOSOF, 2011). Para as misturas proteína –polissacarídeo aniônicos (carboxilados ou sulfatados, pI < 3,0), coacervação complexa é observada em $pKa_{polissacarídeo} < pH < pI_{proteína}$, região onde os dois biopolímeros possuem cargas opostas. A carga líquida das proteínas e polissacarídeos é reduzida pela interação com os íons, resultando em decréscimo das atrações eletrostáticas entre as macromoléculas a altas concentrações de sal. A baixa força iônica, o número de cargas presentes na proteína e polissacarídeo são suficientes para permitir a interação eletrostática (WEINBRECK et al., 2003; RODRIGUEZ-PATINO; PILOSOF, 2011; YE, 2008).

De acordo com Miquelim, Lannes e Mezzenga (2010), a espuma formada por proteínas da clara de ovo e carragena abaixo do pI da proteína apresentou maior

estabilidade das interfaces ar/água devido a coacervação complexa entre os biopolímeros.

Os complexos formados entre a pectina (polissacarídeo aniônico) e proteínas apresentam, de forma geral, melhores propriedades funcionais (gelificação, emulsificação e aeração) que a proteínas e polissacarídeo, quando separados (SCHMITT et al., 1998; BENICHOU et al., 2007).

Narchi, Vial e Djelveh. (2009) estudaram os efeitos das misturas de proteína e polissacarídeo na produção de produtos aerados utilizando xarope de glicose como modelo. O *overrun* das misturas proteína isolada de soro de leite e goma xantana foi reduzido, porém a mistura da proteína com pectina resultou em uma maior capacidade de aeração e estabilidade da espuma.

A estabilidade da espuma de uma solução de clara de ovo (0,05%) aumentou na presença de pectina (0,01 a 0,5%). O aumento na estabilidade estaria relacionado com o aumento da viscosidade da fase aquosa e a formação de um filme elástico forte como conseqüência de uma interação proteína-polissacarídeo na interface ar-água (IBANOGLU; ERÇEBELI, 2007). Leroux et al. (2003) avaliaram que a pectina tem a habilidade de produzir emulsões estáveis da mesma maneira que a goma arábica, mas em concentrações menores.

A formação de complexos solúveis entre a proteína de canola 2S denominada *napin* (proteína globular) e pectina aumentou a viscosidade da fase líquida, diminuindo a drenagem e melhorando a estabilidade da espuma (SCHMIDT et al., 2010).

Tratamentos térmicos, além de efeitos marcantes na estrutura e nas propriedades funcionais das proteínas, alteram também a natureza da interação proteína-polissacarídeo. A desnaturação de proteínas globulares e a mudança conformacional de polissacarídeos aumentam a exposição de mais sítios reativos e, portanto favorecem a interação entre os grupos laterais dos biopolímeros (YE, 2008). A análise térmica por Calorimetria Diferencial de Varredura (DSC) tem sido utilizada para estudar a desnaturação térmica das proteínas do ovo na presença de polissacarídeos (IBANOGLU, 2005; IBANOGLU; ERCEBELI, 2007).

A adição de hidroxipropilmetilcelulose melhorou a textura e as propriedades de batimento de creme (ZHAO et al., 2009). De acordo com Arboleya e Wilde (2005), MC e HPMC formam interfaces altamente elásticas comparadas com a β-lactoglobulina. Os

polissacarídeos MC e HPMC têm mais características tenso-ativas que a β-lactoglobulina e a β-caseina. Portanto, em altas concentrações, os polissacarídeos passam a predominar as propriedades de interface. As características elásticas destes polissacarídeos melhoraram a força da interface, o que potencialmente resultou em espumas mais estáveis.

De acordo com Jara et al. (2014), o tipo de interação entre HPMC e β-lactoglobulin (β-lg), α-lactalbumin (α-la) ou albumina de soro bovino (BSA) na interface depende do pH. No pH 6,0, a presença de HPMC resultou em maior tensão superficial e elasticidade dilatacional, indicando que a β-lg foi facilmente deslocada da interface pelo HPMC. A BSA quando co-adsorvida com o HPMC pode competir pela interface, pois a tensão superficial da mistura BSA/HPMC estava próximo do valor da tensão superficial do BSA. A α-la apresentou sinergismo quando coadsorvida com o HPMC. No pH 3,0, as três proteínas poderiam competir com o HPMC pela interface.

7 Referências bibliográficas

AGUILERA, J.M. Why food microstructure? **Journal of Food Engineering,** v. 67, p. 3-11, 2005.

AKHTAR, M.; DICKINSON, E.; MAZOYER, J.; LANGENDORFF, V. Emulsion stabilizing properties of depolymerized pectin. **Food hydrocolloids,** v.16, n. 16, p. 249-256, 2002.

ARBOLEYA, J.C.; WILDE, P.J. Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. **Food Hydrocolloids,** v. 19, n. 3, p. 485-491, 2005.

BENICHOU, A.; ASERIN, A.; LUTZ, R.; GARTI, N. Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. **Food Hydrocolloids**, v. 21, p. 379–391, 2007.

CAMPBELL, M.G.; MOUGEOT, E. Creation and characterization of aerated food products. **Trends in Food Science & Technology,** v.10, p. 283-296,1999.

CHINACHOTI, P. Carbohydrates: functionality in foods. **The American Journal of Clinical Nutrition.** v. 61,. p. 922S-929S, 1995.

DAMODARAN, S. Amino Acids, Peptides, and Proteins. In FENNEMA, O.R. (ed.). **Food Chemistry**. New York: Marcel Dekker, 1996. p. 321–429.

DAMODARAN, S. Protein stabilization of emulsions and foams. **Journal of Food Science,** v. 70, n.3, p.R54-R66, 2005.

DAMODARAN, S. Amino Acids, Peptides, and Proteins. In DAMODARAN, S.; PARKIN, K. L.; FENNEMA, O.R. (Ed.), **Food Chemistry**. Boca Raton: CRC Press, 2008. p. 217–329.

DAVIS, J. P.; FOEGEDING, E. A.; HANSEN, F. K. Electrostatic effects on the yield stress of whey protein isolate foams. **Colloids and Surfaces B: Biointerfaces**, v.34, 13–23, 2004.

DEA, I.C.M; MADDEN, J.K. Acetylated pectic polysaccharides of sugar beet. Food Hydrocolloids, v. 1, p.71-88, 1986.

DECKER, N.R.; ZIEGLER. G;R. The structure of aerated confectionery. **The Manufacturing Confectioner,** v.82, n.9, p. 101-108, 2002.

DICKINSON, E. Protein adsorption at liquid interfaces and the relationship to foam stability. In WILSON, A.J. (Ed). **Foams: Physics, chemistry and structure.** London::Springer, 1989. p. 39-53.

DICKINSON, E. **An introduction in food colloids.** Oxford**:**Oxford Univ. Press, 1992.

DICKINSON, E. Protein-polysaccharide ineractions in food colloids. In DICKINSON, E.; WASTRA, P. (Ed). **Food colloids and polymers: stability and mechanical properties.** 1993.

DICKINSON, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. **Food Hydrocolloids,** v. 17: 25-39, 2003.

DICKINSON, E. Hydrocolloids as emulsifiers and emulsion stabilizers. **Food Hydrocolloids,** v. 23, n.6, p. 1473-1482, 2009.

DICKINSON, E. Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. **Food Hydrocolloids**, v.25, n.8, 1966–1983, 2011.

DICKINSON, E.; EUSTON, S.R. Stability of food emulsions containing both protein and polysaccharide. In DICKINSON, E. (Ed.)**. Food polymers, gels and colloids.** Cambridge: Royal Society of Chemistry, 1991. p. 132-136.

DICKINSON, E.; GALAZKA, V.B. Emulsion stabilization by protein/polysaccharide complexes. In Phillips, G.O.; WEDLOCK, D.J.; WILLIAM P.A. (Ed). **Gums and stabilizers for the food industry.** Vol. 6. Oxford: IRL Press, 1992. p. 351-362

Dow Chemical Company. Chemistry: Typical Chemical Structures of Methocel. Disponível em <http://www.dow.com/ucc/amerchol/prod/spec cell/chem.htm>, acesso em 10 de junho de 2009.

ERGUN, R.; LIETHA, R.; HARTEL, R.W. Moisture and Shelf Life in Sugar Confections. **Critical Reviews in Food Science and Nutrition**, v. 50, n. 2,p. 162-192, 2010.

FOEGEDING, E.A.; LUCK, P.J.; DAVIS, J.P. Factors determining the physical properties of protein foams. **Food Hydrocolloids,** v. 20, p. 284-292, 2006.

FOEGEDING, E.A.; DAVIS, J.P. Food protein functionality: A comprehensive approach. **Food Hydrocolloids**, v. 25, p. 1853-1864, 2011.

GRINBERG, V. Y., & TOLSTOGUZOV, V. B.. Thermodynamic incompatibility of proteins and polysaccharides in solutions. **Food Hydrocolloids**, v.11, n.2, 145–158. 1997.

GROVES, R. Control of texture in nougat. **Candy Industry,** v.162, n. 4, p.30, 1997.

HAGOLLE, N. et al. Study of the stability of egg white protein-based foams: effect of heating protein solution. **Journal of the Science of Food and Agriculture**, v. 80, p. 1245-1252, 2000.

IBANOGLU, E. Effect of hydrocolloids on the thermal denaturation of proteins. **Food Chemistry**, v. 90, 621–626, 2005.

IBANOGLU, E; ERÇEBELI, E.A. Thermal denaturation and functional properties of egg proteins in the presence of hydrocolloid gums. **Food Chemistry,** v. 101, n. 2, p. 626-633, 2007.

JACKSON, E.B. Liquorice paste, cream paste and aerated confectionery. In JACKSON, E. B. (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional. 1995. p. 218–235

JACKSON, E.B; HOWLING, D. Glucose syrups and starch hydrolysates. In E. B. Jackson (Ed.). **Sugar Confectionery Manufacture.** London: Black Academic and Professional, 1995. p. 13–37.

JAMES, D. General technical aspects of industrial sugar confectionery. In: Sugar confectionery manufacture. In E. B. Jackson (Ed.). **Sugar Confectionery Manufacture.** London: Black Academic and Professional, 1995. p. 298–311.

JARA, F.L. et.al.. Competitive adsorption behavior of b-lactoglobulin, a-lactalbumin, bovin serum albumin in presence of hydroxypropylmethylcellulose. Influence of pH. **Food Hydrocolloids**, v. 35, 189-197, 2014.

KINSELLA, J.E. Functional properties of protein: possible relationships between structure and functionin foams. **Food Chemistry,** v. 7, n. 4, p. 273-288, 1981.

KUROPATWA, M.; TOLKACH, A.; KULOZIK, U. Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. **Food Hydrocolloids**, v. 23, n. 8, 2174-2181, 2009.

LABUZA, T.P.; ALTUNAKAR, B. Water activity prediction and moisture sorption isotherms. In G.V. Barbosa-Cánovas, A.J. Fontana Jr; S.J. Schmidt, T.P. Labuza (Ed.). **Water activity in foods**. Iowa: Blackwell Publishing Professional, 2007. p. 109-171.

LAU, K.; DICKINSON, E. Structural and rheological properties of aerated high sugar systems containing egg albumen. **Journal of Food Science,** v.69, n.5, p. E232-E239, 2004.

LAU, K; DICKINSON, E. Instability and structural change in an aerated system containing egg albumen and invert sugar. **Food Hydrocolloids,** v. 19, p. 111-121, 2005.

LEES, R.. Sugar. In: Sugar confectionery manufacture. In E. B. Jackson (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional. 1995. (p. 108–128).

LEROUX, J et al. Emulsion stabilizing properties of pectin. **Food Hycrocolloids,** v. 17, n. 4, p. 455-462, 2003.

LIANG, Y; KRISTINSSON. Influence of pH- induced unfolding and refolding of egg albumen on its foaming properties. **Journal of food science**, v. 70, n.3, C222-C230, 2005.

LI-CHAN, E; NAKAI, S. Biochemical Basis for the Properties of Egg White. **CRC Crit Rev Poultry Biology**, v. 2, p. 21-58, 1989.

MAZOYER, J., LEROUX, J., BRUNEAU, G. Use of depolymerized citrus fruit and apple pectins as emulsifiers and emulsion stabilizers. *U.S. Patent 5,900,268*, 1999

MINE, Y; NOUTOMI, T.; HAGA N. Emulsifying and structural properties of ovalbumin. **Journal of Agricultural Food Chemistry**, v. 39, p. 443-446, 1991.

MINE, Y. Recent advances in the understanding of egg white protein functionality. **Trends in Food Science ; Technology**, n. 6, p.225-232, 1995.

MIQUELIM, J. N.; LANNES, S. C S; MEZZENGA, R. pH Influence on the stability of foams with protein – polysaccharide complexes at their interfaces. **Food Hydrocolloids,** v 24, n. 4, p. 398-405, 2010.

MURRAY, B.S. Stabilization of bubbles and foams. **Current Opinion in Colloid & Interface Science,** v. 12, p.232-241, 2007.

MURRAY, B.S.; ETTELAIE, R. Foam stability: proteins and nanoparticles. **Current Opinion in Colloid & Interface Science,** v. 9, p.314-320, 2004.

NARCHI, I.; VIAL, Ch.; DJELVEH,G. Effect of protein-polysaccharide mixtures on the continouous manufacturin of foamed food products. **Food Hydrocolloids,** v. 23, n. 1, p.188-201, 2009.

NICORESCU, I et al. Comparative effect of thermal treatment on the physicochemical properties of whey and egg white protein foams. **Food hydrocolloids**, v. 25, n. 4, p. 797-808, 2011.

PEREZ, O.E. et al.. Adsorption dynamics and surfgace activity at equilibrium of whey proteins and hydroxypropy-methyl-cellulose mixtures at the air-water interface. **Food Hydrocolloids,** v.21, n. 5-6, p. 794-803, 2007.

PERNELL, C.W.; FOEGEDING, E.A.; LUCK, P.J. , DAVIS, J.P. Properties of whey and egg white protein foams. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 204, p. 9-21, 2002.

PHILLIPS, L. G., SCHULMAN, W., & KINSELLA, J. E. pH and heat-treatment effects on foaming of whey-protein isolate. **Journal of Food Science**, v. 55, n. 4, 1116–1119, 1990.

RAIKOS, V.; CAMPBELL, J.; EUSTON, S.R. Effects of sucrose and sodium chloride on foaming propertie of egg white proteins. **Food Research International,** v. 40, 374-355, 2007.

RALET, M.C. et al. Enzymatically and chemically de-esterified lime pectins: characterisation, polyelectrolyte behaviour and calcium binding properties. **Carbohydrate Research**, v. 336, 117-125, 2001.

RODRÍGUEZ PATINO, J. M., & PILOSOF, A. M. R. Protein–polysaccharide interactions at fluid interfaces. **Food Hydrocolloids**, v. 25, n. 8, p. 1925–1937, 2011.

SARKAR, N. Structural interpretation of the interfacail properties of aqueous-solutions of methylcellulose and hydroxypropyl methylcellulose. **Polymer,** v. 25, n. 4, p.481-486, 1984.

SARMA, D.S.HS.R.; KHILAR, C.K. Effects of Initial Gas Volume Fraction on Stability of Aqueous Air Foams. **Ind. Eng. Chem. Res**., v. 27, 892-894, 1988.

SCHMIDT, I., NOVALES, B., BOUÉ, F., & AXELOS, M.A.V.. Foaming properties of protein/pectin electrostatic complexes and foam structure at nanoscale. **Journal of Colloid and Interface Science**, v. 345, n. 2, p. 316 – 324, 2010.

SCHMITT, C. et al. Structure and Technofunctional Properties of Protein-Polysaccharide Complexes: A Review. **Critical Reviews in Food Science and Nutrition***,* v. 38, n. 8, p. 689–753, 1998.

STANSELL, D. Caramel, toffee and fudge. In: Sugar confectionery manufacture. In E. B. Jackson (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional, 1995. p. 170–188.

STEVENS, L. Egg white proteins. **Comp. Biochem. Physiol**., v. 100B, n. 1, p. 1-9, 1991.

SURH, J; DECKER, E.A., MCCLEMENTS, D.J. Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. **Food Hydrocolloids,** v. 20, n.5, p. 607–618, 2006

TOLSTOGUZOV, V. Phase Behavior in Mixed Polysaccharide Systems. In STEPHEN, A.M.; PHILLIPS,G.O.;, WILLIAMS, P.A. (Ed.). **Food polysaccharides and their applications.** Boca Ranton: CRC Press, 2006. p. 589-627.

UPADHYAY, R.; GHOSAL, D.; MEHRA, A. Characterization of bread dough: Rheological properties and microstructure. **Journal of Food Engineering,** v. 109, 104–113, 2012.

YANG, X. **Effects of Sucrose on the Foaming and Interfacial Properties of Egg White Protein and Whey Protein Isolate**. North Carolina, USA, 2008. Originalmente apresentada como tese de doutorado, Faculty of North Carolina State University. 2008.

YE, A. Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications – a review. **International Journal of Food Science and Technology,** v. 43, p. 406-415, 2008.

ZHAO, Q.; ZHAO, M.; LI, J.; YANG, B.; SU, G.; CUI, C.; JIANG, Y. Effect of hydroxypropyl methylcellulose on the textural and whipping properties of whipped cream. **Food Hidrocolloids,** v. 23, n. 8, p. 2168-2173, 2009.

WALSTRA P. **Physical Chemistry of Foods**. New York: Marcel Dekker, 2003.

WALTER, R.H. **The Chemistry and Technology of Pectin.** New York: Academic Press, 1991.

WEINBRECK, F.; de VREIS, R.; SCHROOYEN, P.; de KRUIF, C.G. Comples coacervation of whey proteins and gum arabic. **Biomacromolecules,** v.4, p. 293-303, 2003.

ZHU, H. M., & DAMODARAN, S.. Proteose peptones and physical factors affect foaming properties of whey protein isolate. **Journal of Food Science**, v. 59, n. 3, 554–560, 1994.

CAPÍTULO 2. INFLUENCE OF PROTEIN PECTIN ELECTROSTATIC INTERACTION ON THE FOAM STABILITY MECHANISM

Carbohydrate Polymers, 103, p.55-61, 2014 ISSN: 0144-8617

Capítulo 2. Influence of protein pectin electrostatic interaction on the foam stability mechanism

Mitie S. Sadahira[a,*], Fernanda C. Rezende Lopes[b], Maria I. Rodrigues[c], Flavia M. Netto[b]

[a]Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP Campinas, Brazil; E-mail: mitie@ital.sp.gov.br
[b]Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/ UNICAMP, **:** Rua Monteiro Lobato n˚ 80 - CEP: 13.083-970, Campinas, Brazil; E-mail: fernanda.rzls@gmail.com; E-mail: flavia@fea.unicamp.br
[c]Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brasil; E-mail: protimiza@protimiza.com.br
*Corresponding author. Tel.: +55 19 3743 1959; fax: +55 19 3743 1963. E-mail address: mitie@ital.sp.gov.br (M. S. Sadahira).

Abstract

This study aimed at evaluating the effect of three independent variables: biopolymer concentration (egg white proteins and pectin) (2.0 to 4.0% w/w); protein:pectin ratio (15:1 to 55:1); and temperature (70 to 80 ℃), at pH 3.0, using a central composite design on the foaming properties (overrun, drainage and bubble growth rate). Foams produced with protein:pectin ratio 15:1 showed the lowest bubble growth rate and the greatest drainage, whereas protein:pectin ratio 55:1 presented the lowest drainage. Complexes obtained with protein:pectin ratio 15:1 were close to electroneutrality and showed larger size (95.91 $\pm$ 8.19 μm) than those obtained with protein:pectin ratio 55:1 (45.92 $\pm$ 3.47 μm) not electrically neutral. Larger particles seemed to build an interfacial viscoelastic network at the air-water interface with reduced gas permeability, leading to greater stability concerning the disproportionation. Soluble complexes of smaller sizes increased viscosity leading to a low drainage of liquid and inhibiting the bubbles coalescence.

Keywords: Electrostatic Interaction, Disproportionation, Coalescence, Drainage, Stability

Abbreviations: ANOVA: analysis of variance; CCD: central composite design; DR: drainage (% drained liquid), d_{43}: mean diameter in volume; R^2: percentage of variance explained; V_{bubble}: bubble growth rate (% BS/min)

1. Introduction

Foams consist of a dispersion of a gaseous phase in a continuous aqueous or solid phase. In most foods with foaming characteristics, proteins are surface active agents that help in the formation and stabilization of the dispersed gaseous phase (Campbell & Mougeot, 1999; Nicorescu et al., 2011). Protein-stabilized foams are formed by bubbling, whipping or shaking a protein solution. The foaming capacity of a protein refers to its ability to form a resistant and thin film at the air-liquid interface in order that a large amount of gas bubbles can be incorporated and stabilized (Damodaran, 2008). Foams are thermodynamically unstable systems and their stability is affected by factors such as drainage (due to gravity), disproportionation (gas diffusion from a small to a large bubble or to the atmosphere) and coalescence (drainage of the liquid from the lamella) (Damodaran, 2005).

Egg white protein is used as a surface-active ingredient for aerated confectionery such as marshmallow and nougat (Jackson, 1995). Besides the aeration capacity, the foam stability is an important aeration property of egg white. Its excellent aeration capacity is due to the presence of globulins, ovomucoid, and lysozyme in its composition. The globulins are surface-active substances that contribute to foaming whereas ovomucoid and globulins slow drainage (loss of foam stability) due to their high viscosity. Lysozyme forms an interfacial complex with other proteins resulting in increased film strength. The hierarchy of egg white proteins regarding the importance in foaming is as follows: globulins, ovoalbumin, ovotransferin, lysozyme, ovomucoid, and ovomucin (Dickinson, 2011; Mine, 1995).

Pectin is a carboxylated anionic polysaccharide with high molecular weight used as gelling and thickening agent in foods. Its functional properties depend on the degree of esterification (DE). High-methoxyl pectins ($\geq$ 0.50% DE) require high sugar concentration and low pH to form gels, whereas low-methoxyl pectins form gels in the presence of calcium (Dickinson, 2003).

The protein-polysaccharide interaction has a significant influence on the structure and stability of dispersions and emulsions (Dickinson, 1998; Ye, 2008). In aqueous solution, a mixture of protein and polysaccharide may present one of three characteristics: 1) miscibility: usually occurring at low biopolymer concentration; 2) incompatibility: occurring due to the repulsive interaction protein-polysaccharide, leading to separation into

two distinct aqueous phases, one rich in protein and the other in polysaccharides; 3) complex coacervation: involving electrostatic attraction between polysaccharide and protein to form a two-phase system consisting of a polymer-rich phase and another phase without biopolymers (Dickinson, 2003).

For anionic polysaccharide-protein mixtures, the complex coacervation occurs at pH values above isoelectric point of the polysaccharide ($pI_{polysaccharide}$) and under the isoelectric point of the protein ($pI_{protein}$), in a region where both biopolymers have opposite charges, creating strong electrostatic complexes (Syrbe, Bauer, & Klostermeyer, 1998; Patino & Pilosof, 2011). At a pH below the pI of the protein, the negative charge of the anionic polysaccharide may interact with the positively charged residues of the protein and lead to the formation of complexes (Dickinson, 1998). The physicochemical parameters that influence the electrical charge of protein and polysaccharides play an important role in controlling the phenomenon of complex formation. The most important parameters are pH, ionic strength, temperature, protein:polysaccharide ratio and total biopolymer concentration (Schmitt & Turgeon, 2011).

Studies have shown that the electrostatic interaction between pectin and egg white protein (Ibanoglu & Erçebeli 2007), napin (globular protein) (Schmidt, Novales, Boué, & Axelos, 2010), and whey protein isolate are effective in increasing the foam stability (Narchi, Vial, & Djelveh, 2009).

The process parameters such as total biopolymer concentration (%w/w), protein:pectin (w/w) ratio and temperature influence the electrostatic interaction between the biopolymers in the pH region where they are oppositely charged. The aim of this study was to evaluate these process parameters on the foaming properties (overrun, drainage and bubble growth rate), using a central composite design (CCD).

2. Material and methods

2.1. Materials

Dried egg white provided by Saltos Alimentos LTDA (Salto, Brazil) and low methoxyl pectin (GENU Pectin type LM CG-22, degree of esterification 47.2%, molecular

weight 90 kDa) provided by CPKelco (Grossenbrode, Germany) were used to prepare the biopolymer solutions. The other reagents were of analytical grade and deionized water was used in all experiments. The egg white proteins were characterized for protein content (79.9 $\pm$1.2%, wet basis), moisture content (10.20 $\pm$ 0.02%, wet basis) and ash (5.64 ± 0.22% wet basis), according to methodologies described by AOAC (2010). In addition, the proteins were analyzed by SDS-PAGE (LAEMMLI, 1970). Eletrophoretic profile of egg white proteins showed bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively.

2.2. Central Composite Design (CCD)

The egg white and pectin were weighed in separated beakers for solubilization in water under magnetic stirring for 2 h at room temperature, and the solutions were kept under refrigeration overnight to ensure complete hydration of biopolymers. The solutions were mixed according to the proportions of protein and pectin previously defined in the experimental design study. The pH was adjusted with 1 mol L^{-1} HCl. Based on the volume of the acid solution, the ionic strength was calculated and adjusted to 0.05 with NaCl. The protein and pectin solutions were heated in a jacketed beaker connected to a thermostatic bath to reach the temperature of beating. The foams were produced using a KEC57 KitchenAid mixer (KitchenAid, Greenville, USA) under atmospheric pressure and whipping time of 15 min at the maximum speed.

The independent variables total biopolymer concentration (% w/w), protein:pectin ratio (w/w), and temperature (°C) were selected to carry out the CCD (2^3 factorial with 3 repetitions at the central point) totaling 11 trials (Tables 1 and 2) (Rodrigues & Iemma, 2009) to evaluate the effects of these variables on foaming properties (overrun, drainage and bubble growth rate) at pH 3.0. First-order models were obtained and evaluated statistically by analysis of variance (ANOVA).

Table 1. Values of the independent variables used in CCD to produce foams containing proteins and pectin at pH 3.0.

Independent Variable	-1	0	1
Total biopolymer concentration (% p/p)	2.0	3.0	4.0
Protein:pectin ratio	15:1	35:1	55:1
Temperature (℃)	70	75	80

Control tests were carried out, in which the same experimental conditions of model validation (total biopolymer concentration, pH 3.0 and 70 ℃) were used, but without pectin addition. The results were analyzed for differences between means by Tukey's test (Tukey Honest Significant Difference) ($p < 0.05$). Student t test ($p < 0.05$) was used for comparisons between the samples with and without pectin obtained under the same experimental conditions.

2.3. Foaming properties

2.3.1. Overrun

Aliquots of foam were transferred carefully and filled up into cylindrical containers (157.1 $\pm$ 1.1 ml). The top of the container was leveled with a metal spatula to achieve uniform and plane surfaces. The overrun was determined according to Equation (1) (Lau & Dickinson, 2004).

$$\text{overrun (\%)} = 100 \times [m_i - m_f] / m_f \qquad (1)$$

where m_i is the mass of the initial solution (before whipping) and m_f is the mass of the resulting foam with the same volume of m_i.

2.3.2. Foam Stability

The foam stability was evaluated by monitoring the drainage, which consists in measuring the mass of liquid drained from the lamella (Kuropatwa, Tolkach, & Kulozik, 2009). The sample was kept at 25 ± 1 ℃ for 120 min and then the drained liquid (DR) was removed with a syringe and carefully weighed. The percentage of DR was calculated by Equation (2).

$$DR\ (\%) = (100 \times m_d) / m_i \qquad (2)$$

where m_d is the mass of drained liquid after 120 min stored at 25 $\pm$ 1 ℃, and m_i is the initial mass of foam.

In addition, the foam stability was evaluated by the bubble growth rate (V_{bubble}). The coalescence and/or disproportionation result in the growth of bubble size (Rouimi, Schorsch, Valentini, & Vaslin, 2005). The analysis was performed on a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The samples were placed in a cylindrical glass cell and scanned from bottom to top to monitor backscattering. The backscattering measurement (BS) is inversely proportional to square root of λ^*. According to Mie theory, λ^*, photon transport mean free path, is inversely proportional to the volume fraction of the bubbles and proportional to their mean diameter (d). Therefore, BS is dependent on bubble size distribution (Rouimi, Schorsch, Valentini, & Vaslin, 2005; Formulaction, no date). The mean backscattering values (BS) change with increasing the air bubble size. The V_{bubble} was determined from the slope of the %BS curve versus time.

2.4. Evaluation of complexes

2.4.1. Mean diameter

The mean diameter of the pectin/protein complexes from the solutions prepared at the same conditions of the validation model was determined using a laser diffraction particle size analyzer (Horiba Laser Scattering Particle Size Analyzer, Model LA-950, Horiba Ltd, Inc., Kyoto, Japan). Particle size calculations were based on the Mie-Scattering theory. The complexes were dispersed in deionized water at pH 3.0, and added to the

sample chamber containing the same dispersion medium to achieve a range of transmittance between 90 to 98%. For particle size measurements, the following refractive index used were: water, 1.333 and biopolymers, 1.450. The mean particle size was expressed as the volume mean diameter ($D_{4,3}$). Determinations were carried out in triplicates.

2.4.2. Zeta potential

Zeta potential measurements of the protein and pectin solutions and their mixture at the total biopolymer concentration (4% w/w) and protein:pectin ratio (15:1 or 55:1) were made at pH range from 2.0 to 7.0, using a Malvern Zetasizer Nano Series instrument (Malvern Instruments, Worcestershire, UK). The Henry equation was used to convert the electrophoretic mobility measurements into zeta potential values. The electrophoretic mobility is obtained by measuring the velocity of the particles using Laser Doppler Velocimetry (LDV). Electrophoretic determinations of zeta potential are most commonly made in aqueous media. It was assumed that the viscosity of the aqueous solution was close to water, because the total biopolymer concentration was low (4% w/w).

3 Results and discussion

3.1 Central composite design (CCD)

From the results showed in Table 2, the regression coefficients were calculated and mathematical models were built for the responses drainage and V_{bubble}. ANOVA was used to evaluate the adequacy of the fitted model (Table 3).

Table 2. CCD matrix and overrun, drainage and bubble growth rate (V_{bubble}) at pH 3.0.

Experiment	Total biopolymer concentration (% w/w) x_1	Protein:pectin ratio x_2	T (°C) x_3	Overrun* (%) y_1	Drainage* (%) y_2	V_{bubble}* (%BS/min) y_3
1	-1 (2.0)	-1 (15:1)	-1 (70)	560	58.8	0.436
2	1 (4.0)	-1 (15:1)	-1 (70)	601	42.1	0.399
3	-1 (2.0)	1 (55:1)	-1 (70)	667	54.4	0.619
4	1 (4.0)	1 (55:1)	-1 (70)	622	24.5	0.538
5	-1 (2.0)	-1 (15:1)	1 (80)	621	45.8	0.612
6	1 (4.0)	-1 (15:1)	1 (80)	576	35.9	0.568
7	-1 (2.0)	1 (55:1)	1 (80)	604	54.7	0.675
8	1 (4.0)	1 (55:1)	1 (80)	580	20.4	0.620
9	0 (3.0)	0 (35:1)	0 (75)	626	41.1	0.554
10	0 (3.0)	0 (35:1)	0 (75)	627	41.0	0.573
11	0 (3.0)	0 (35:1)	0 (75)	663	41.4	0.621

* whipping time: 15 min; ( ) true values of the independent variables for each level; vbubble (%BS/min) = the slope of the % mean backscattering values (BS) curve versus time.

For the response overrun (y_1), the R^2 and calculated F values (Table 3) indicate that it is not possible to get a response surface model, since the values are in a very close range, 560 to 667%, and within the experimental variation. The total biopolymer concentration, protein:pectin ratio, and temperature did not influence overrun ($p > 0.05$). Schmidt et al., (2010) studied foam properties of napim protein and pectin and also found that foaming capacity is not affected by the presence of pectin at the pH value at which biopolymers are oppositely charged.

For the response drainage (y_2) and V_{bubble} (y_3), the results varied from 20.4 to 58.8% and from 0.399 to 0.675 %BS/min, respectively, and both responses were significantly

affected by the independent variables. The R^2 and calculated F values (Table 3) are adequate to obtaining the first-order model (Equations 3 and 4), allowing evaluating the drainage and V_{bubble} behaviour as a function of total biopolymer concentration (x_1), protein:pectin ratio (x_2), and temperature (x_3), within the range studied.

Table 3. Percentage of variance explained (R^2), calculated F value and tabulated F for the responses overrun, drainage and V_{bubble}.

Response	R^2 (%)	Calculated F	Tabulated F*
Overrun	56.5	0.87	6.16
Drainage	98.5	76.8	5.05
V_{bubble}	93.8	22.9	4.53

*at 5% significance level

The mathematical models were built using the coded variables with statistically significant parameters, according to the measured values for the response:

$$\text{Drainage (\%)} = 41.8 - 11.3x_1 - 3.6x_2 - 2.9x_3 - 4.7x_1x_2 + 1.9\,x_2x_3 \quad (3)$$

$$V_{bubble}\ (\%BS/min) = 0.565 - 0.027x_1 + 0.054x_2 + 0.060x_3 - 0.026x_2x_3 \quad (4)$$

where x_1, x_2 and x_3 are the independent variables coded for total biopolymer concentration, protein:pectin ratio and temperature, respectively.

Equations 3 and 4 were used to generate the response surfaces for the dependent variables drainage and V_{bubble} (Fig. 1). The drainage decreased with increasing biopolymer concentration and protein:pectin ratio, while V_{bubble} increased with increasing of both protein:pectin ratio and temperature and decreasing biopolymer concentration.

The conditions to obtain foams with the lowest drainage were 4.0% biopolymer concentration, and protein:pectin ratio of 55:1. The lower V_{bubble} was obtained with the protein:pectin ratio of 15:1 at 70 ℃.

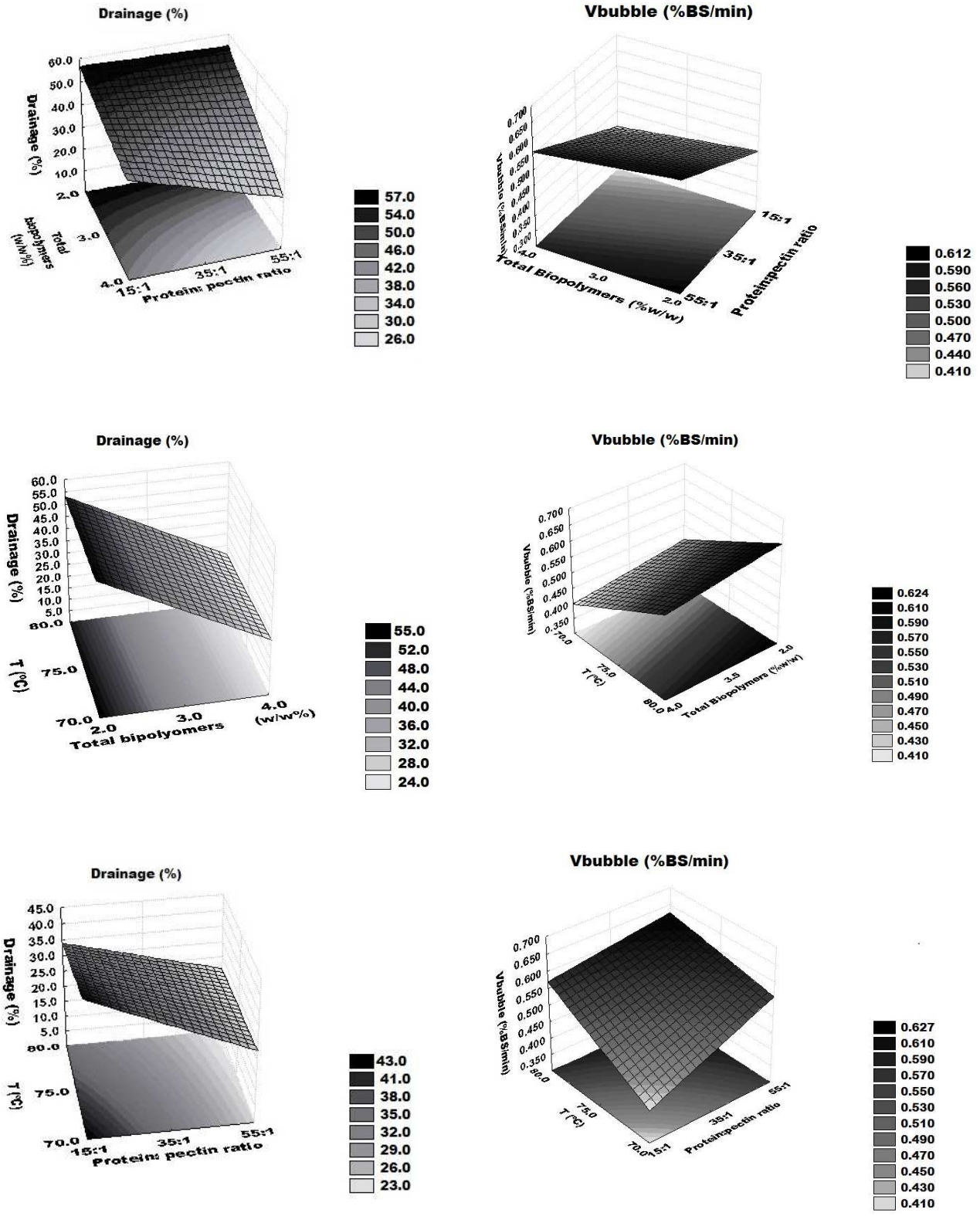


Fig. 1. Response surfaces for the dependent variables drainage and bubble growth rate (V_{bubble}). Total biopolymers: Total biopolymer concentration (% w/w)

3.2. Model validation and interaction between egg white proteins and pectin

Table 4 shows the experimental and predicted values of the coded model for drainage and V_{bubble} of the experimental validation, as well as the relative error between the experimental and predicted value for each test. In experiments A and B, for the response drainage, the variation between experimental and predicted values (relative error) was 6.9 and 10.1%, respectively, whereas the relative errors for V_{bubble} were 6.1 and 1.7%. These low relative errors show that the mathematical models are in good agreement with the experimental data in the range studied.

To evaluate the effect of interaction between egg white proteins and pectin on the foaming properties, control tests were carried out under the same experimental validation condition (total biopolymer concentration 4% w/w, pH 3.0 and 70 ℃), but with no pectin addition and the results are shown in Table 4. In experiment A, with protein:pectin ratio 15:1, the overrun was higher when no pectin (control) was added but there was no significant difference in drainage with the pectin addition. V_{bubble} was lower for the foams containing pectin. The foams obtained in Experiment B with protein:pectin ratio 55:1 or without pectin (control) showed no significant difference ($p > 0.05$) for overrun. However, foams from the Experiment B with pectin had lower drainage and V_{bubble} values. Under these conditions, the V_{bubble} is related to the coalescence rate, because the smaller drainage of liquid from the lamella leads to smaller coalescence of bubbles.

Table 4. Predicted values (prev., y_p), experimental values (exp., y_e) and relative error (RE = (y_e - y_p) / y_e*100)) for drainage (DR) and Vbubble and experimental values of overrun of foams obtained under the conditions for the validation of the mathematical models with and without addition of pectin (control).

Experiment	***Overrun* (%)**		**Drainage (%)**				**V_{bubble} (%BS/min)**			
	Control (without pectin)	With pectin	DR Control (without pectin)	DR (y_e) exp. with pectin	DR (y_p) prev. with pectin	RE (%)	V_{bubble} Control (Without pectin)	V_{bubble} (y_e) exp. with pectin	V_{bubble} (y_p) prev. with pectin	RE (%)
A	705.3 ± 18.9^{a,A}	588.1 ± 40.5^{b,B}	46.0 ± 3.1^{a,A}	40.8 ± 3.4^{a,A}	43.6	-6.9	0.703 ± 0.031^{a,A}	0.374 ± 0.024^{c,B}	0.397	-6.1
B	705.3 ± 18.9^{a,A}	666.5 ± 35.0^{a,b,A}	46.0 ± 3.1^{a,A}	25.8 ± 1.6^{b,B}	23.2	10.1	0.703 ± 0.031^{a,A}	0.587 ± 0.025^{b,B}	0.597	-1.7

Values are mean ± SD of triplicates. For the same response, means with different small letters in the same column differ significantly ($p < 0.05$) by Tukey's test, and means with different capital letters in the same row differ significantly ($p < 0.05$) by Student's t test. Overrun, drainage and Vbubble at the validation conditions (pH 3.0, total biopolymer concentration = 4.0% w/w, temperature 70 ℃) where A and C ontrol B (without pectin) and A with pectin (protein: pectin ratio 15:1) and B with pectin (protein: pectin ratio 55:1). RE = Relative error (%).

In order to better understand these results, zeta-potential curves, appearance of the mixtures of protein /pectin, and the mean diameter of the complexes at pH 3.0 were evaluated (Fig. 2). The mixture of egg white and pectin under conditions of the Experiment A (protein:pectin ratio 15:1) is close to electrical neutrality at pH 3.0 (Fig. 2a) leading to the formation of insoluble complexes with 95.91 $\pm$ 8.19 µm (Fig. 2b). Whereas under conditions of the Experiment B (protein:pectin ratio 55:1), the mixture is not electrically neutral at pH 3.0 with repulsive interaction between the complexes, increasing the solubility and formation of smaller complexes (45.92 $\pm$ 3.47 µm, Fig. 2d).

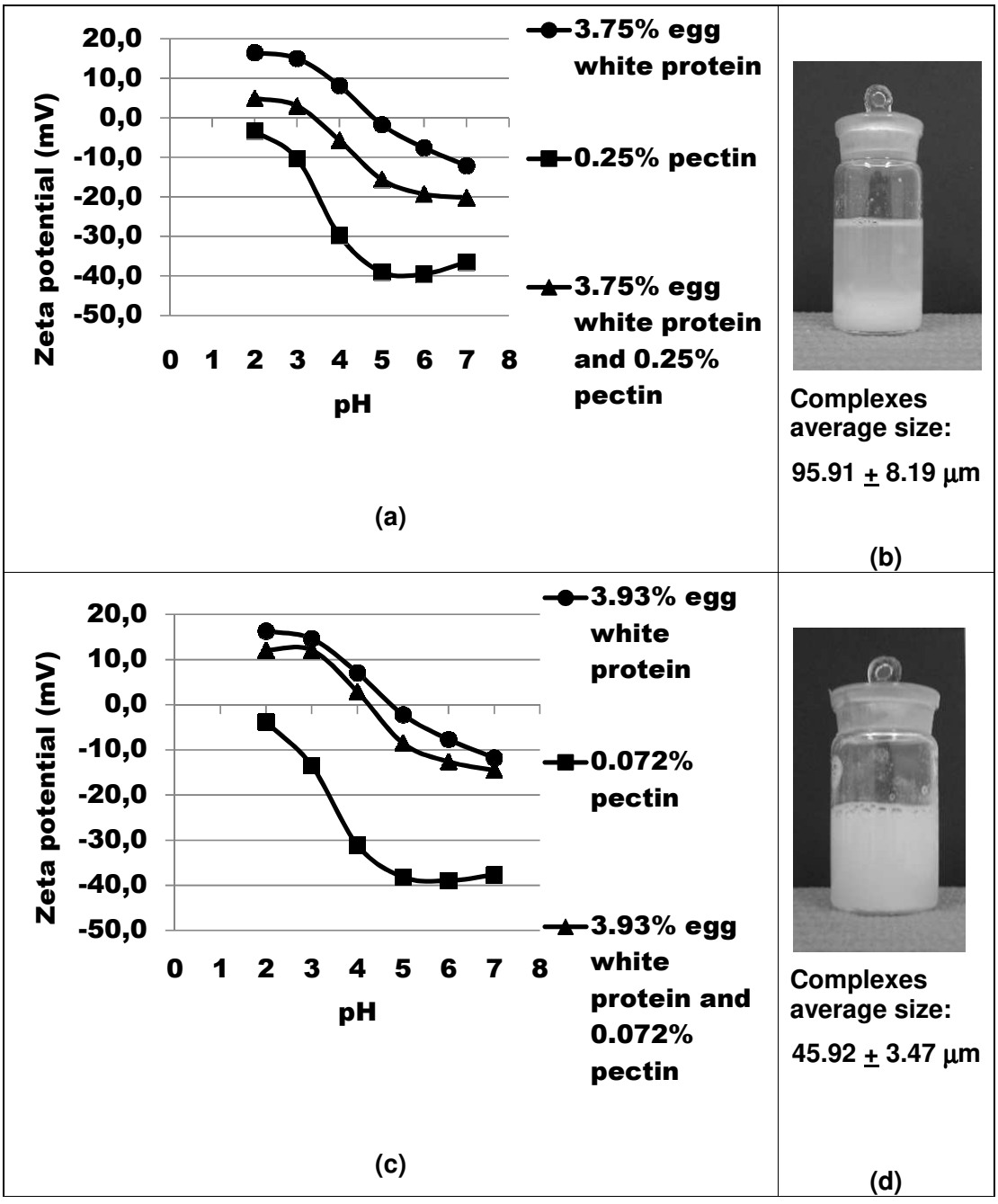


Fig. 2. Zeta potential as a function of pH (a, c); Appearance of the mixture of white egg/ pectin (b, d), solutions of egg white protein, pectin and mixture of protein/pectin at total biopolymer concentration and protein:pectin ratio for Experiment A (total biopolymer concentration 4.0% w/w; protein: pectin ratio of 15:1, at 70 ℃ and pH 3.0), and for Experiment B (total biopolymer concentration 4.0% w/w; protein: pectin ratio of 55:1, at 70 ℃ and pH 3.0, respectively.

In general, the results suggest that electrostatic interactions between the biopolymers lead to formation of soluble and insoluble complexes, which influenced foam stability since the mechanisms for stabilizing foams are related to the biopolymer proportion and electrical neutrality (Turgeon, Schmitt, & Sanchez, 2007). The formation of insoluble complexes under neutral conditions possibly led to a decrease of protein available to contribute to the aeration capacity (Schmidt et al., 2010). The electrically neutral complexes are more likely to build up a dense interfacial viscoelastic network at the air-water interface with low gas permeability, since the complexes rearrange and form a microgel at the air-water interface (Turgeon et al., 2007; Schmitt & Turgeon, 2011), leading to reduced air diffusion from a small bubble into a large bubble (disproportionation). In this condition, possibly the smaller V_{bubble} is related to greater stability with respect to disproportionation, as there was no significant different on drainage of the foams from experiment A control and experiment A with pectin. The liquid drainage from the lamella may lead to bubble coalescence (Damodaran, 2005).

Therefore, we propose the mechanism presented in Fig. 3. The egg white forms the film at the air-water interface (Fig. 3A). When the pectin is added at protein:pectin ratio of 15:1 (Fig. 3B), the charge neutrality is reached, which leads to a decrease of protein concentration available to the film formation at the air-water interface. The neutral complexes build a viscoelastic interfacial network at the air-water interface with low gas permeability leading to greater stability to disproportionation.

When protein:pectin ratio of 55:1 was used (Fig. 3C), the electrical neutrality was not reached, leading to a higher concentration of non complexed protein. Thus, the soluble complexes build a secondary layer, which contributed to the formation of a stable film at the air-water interface, inhibiting the bubbles coalescence. In this condition, the liquid drainage is smaller when compared to the foam formed only with the egg white proteins, as the soluble complexes migrate to the lamella, increasing the viscosity (Schmidt et al., 2010). The increase in viscosity causes a lower liquid drainage from the foam. The lowest drainage leads to reduced bubble coalescence and these factors together result in higher foam stability.

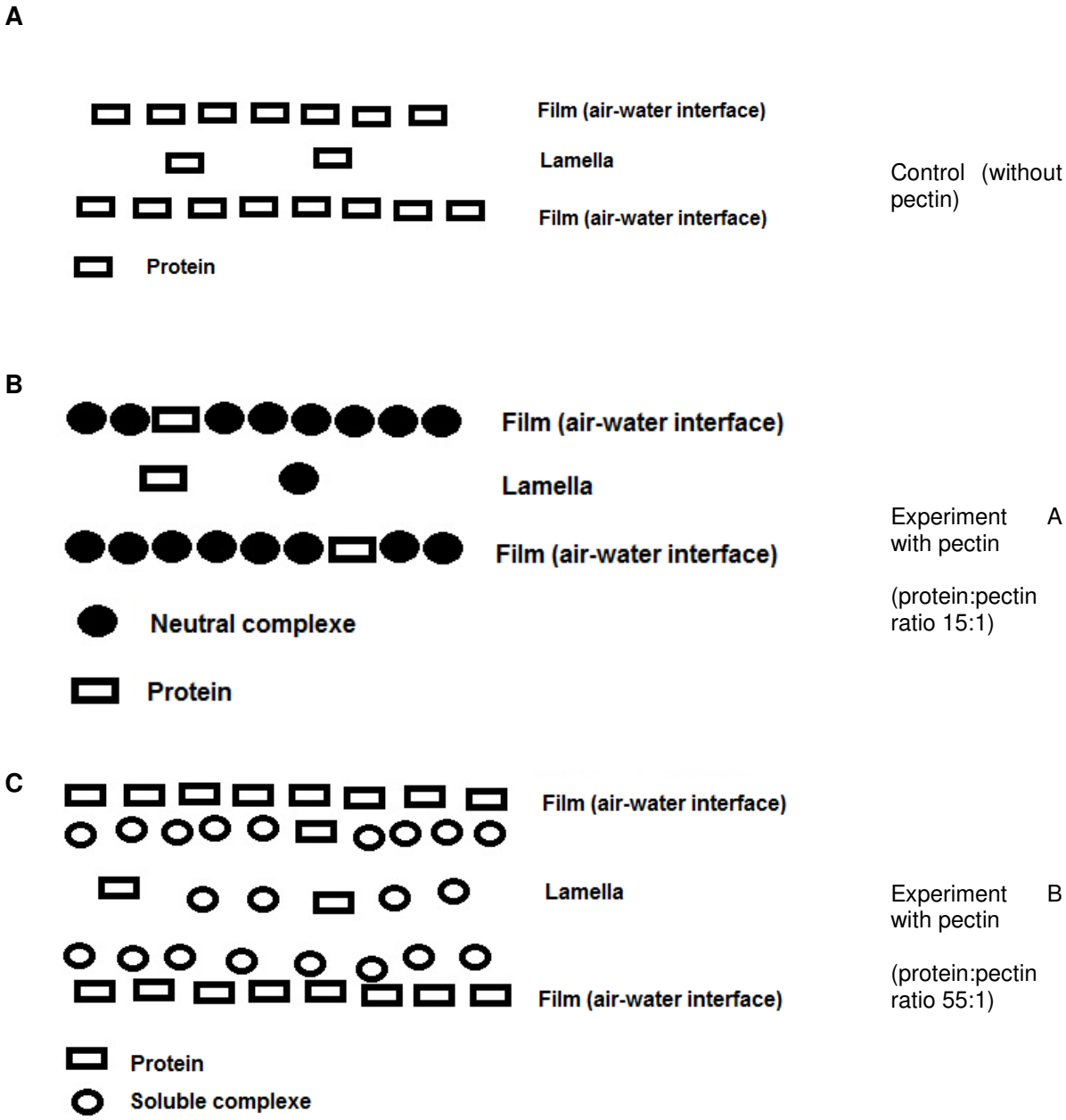


Fig. 3. Interaction mechanism of egg white proteins and pectin at the air-water interface.

4. Conclusion

In this CCD study, it was found that the protein:pectin ratio is a statistically significant influence ($p < 0.05$) on the foam stability at pH 3.0. The electrical neutrality and the size of the electrostatic complexes formed depend on the protein:pectin ratio. The

complexes close to electrical neutrality conditions (protein:pectin ratio of 15:1) had larger size and built a viscoelastic interfacial network at the air-water interface with low gas permeability leading to greater stability to disproportionation (air diffusion from a small bubble to a big bubble or to the atmosphere). At protein:pectin ratio of 55:1 the electrical neutrality was not reached, resulting in the formation of soluble complexes of smaller size. The presence of soluble complexes in the lamella increases the viscosity, leading to low liquid drainage from the foam; the soluble complexes form a secondary layer that contribute to the formation of a stable film at the air-water interface, inhibiting the bubbles coalescence. In this context pectin may be considered to improve the stability of foam and is a promising alternative for aerated products processing.

Acknowledgements

The authors thank FAPESP for financial support (FAPESP 2011/50067/9) and EMBRAPA for the PhD scholarship granted to the author Sadahira MS.

5. References

AOAC. (2010). Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, USA: Association of Official Methods Analytical Chemists.

Campbell, G., & Mougeot, E. (1999). Creation and characterization of aerated food products. *Trend in Food Science & Technology*, 10, 283-296.

Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science*, 70, R54-R66.

Damodaran, S. (2008). Amino Acids, Peptides, and Proteins. In S. Damodaran, K. Parkin, & O. R. Fennema (Eds.), *Food Chemistry* (pp. 217-329). New York: Marcel Dekker.

Dickinson, E. (1998). Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food Science & Technology*, 9, 347-354.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, 17, 25-39.

Dickinson, E. (2011). Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids*, 25(8), 1966-1983.

Formulaction. (no date). Turbiscan Classic MA 2000 – User Guide Formulaction. L'Union, France, Formulaction.

Ibanoglu, E., & Erçebeli, E.A. (2007). Thermal denaturation and functional properties of egg proteins in the presence of hydrocolloid gums. *Food Chemistry*, 101, 626-633.

Kuropatwa, M., Tolkach, A., & Kulozik, U. (2009). Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. *Food Hydrocolloids*, 23 (8), 2174-2181.

Jackson, E.B. (1995). Liquorice paste, cream paste and aerated confectionery. In E.B. Jackson (Ed.), *Sugar Confectionery Manufacture* (pp. 218-235), London: Black Academic and Professional.

Lau, K., & Dickinson, E. (2004). Structural and rheological properties of aerated high sugar systems containing egg albumen. *Journal of Food Science*, 69. (5), E232-E239.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of thehead of bacteriophage T4. *Nature*, *227*, 680–685.

Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. Trends in Food Science & Technology, 6, 225-232.

Narchi, I., Vial,Ch., & Djelveh, G. (2009). Effect of protein-polysaccharide mixtures on the continous manufacturing of foamed food products. *Food Hydrocolloids*, 23, 188–201

Nicorescu, I., Vial, C., Talansier, E., Lechevalier, V., Loisel, C., & Della Valle, D., (2011). Comparative effect of thermal treatment on the physicochemical properties of whey and egg white protein foams. *Food Hydrocolloids*, 25, 797-808.

Patino, J.M.R., & Pilosof, A.M.R. (2011). Protein-polysaccharide interactions at fluid interfaces. Food Hydrocolloids, 25, 1925 – 1937.

Rodrigues, M.I., & Iemma, A.F. (2009). *Planejamento de Experimentos e Otimização de Processos*. (2nd ed.). Campinas: Casa do Pão Editora, (Chapter 5).

Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial properties of milk protein–surfactant systems. *Food Hydrocolloids*, 19, 467–478

Schmidt, I., Novales, B., Boué, F., & Axelos, M.A.V. (2010). Foaming properties of protein/pectin electrostatic complexes and foam structure at nanoscale. *Journal of Colloid and Interface Science*, 345, 316 – 324.

Schmitt, C., & Turgeon, S.L. (2011). Protein/polysaccharide complexes and coacervates in food systems. Advances in Colloid and Interface Science, 167, 63–70.

Syrbe, A., Bauer, W.J., & Klostermeyer, H. (1998) Polymer science concepts in dairy systems – an overview of milk protein and food hydrocolloid interaction. *International Dairy Journal*, 8 (3), 179-193.

Turgeon, S.L., Schmitt, C., & Sanchez, C. (2007). Protein-polysaccharide complexes and coacervates. *Current Opinion in Colloids & Interface Science*, 12, 166-178.

Ye, A. (2008). Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications – a review. *International Journal of Food Science and Technology,* 43, 406–415.

CAPÍTULO 3. EFFECT OF pH AND INTERACTION BETWEEN EGG WHITE PROTEIN AND HYDROXYPROPYMETHYLCELLULOSE IN BULK AQUEOUS MEDIUM ON FOAMING PROPERTIES

Submitted to Carbohydrate Polymers, ISSN: 0144-8617

Capítulo 3. Effect of pH and interaction between egg white protein and hydroxypropymethylcellulose in bulk aqueous medium on foaming properties

Mitie S. Sadahira[a,*], Fernanda C. Rezende Lopes[b], Maria I. Rodrigues[c], Aureo T. Yamada[d], Flavia M. Netto[b**]

[a]Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP Campinas, Brazil; E-mail: mitie@ital.sp.gov.br

[b]Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/ UNICAMP, Rua Monteiro Lobato n° 80 - CEP: 13.083-970, Campinas, Brazil; E-mail: fernanda.rzls@gmail.com; E-mail: flavia@fea.unicamp.br

[c]Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brasil; E-mail: protimiza@protimiza.com.br

[d]Instituto de Biologia, Universidade Estadual de Campinas/UNICAMP, Rua Monteiro Lobato, nº 255 – CEP: 13083-862, Campinas, Brazil; E-mail: yamadat@unicamp.br

*Corresponding author. Tel.: +55 19 3743 1959; fax: +55 19 3743 1963. E-mail address: mitie@ital.sp.gov.br (M. S. Sadahira);

** Corresponding author:Tel.: +55 19 3521 4080; fax: +55 19 3521 4060. E-mail address: flavia@fea.unicamp.br (F.M. Netto)

Abstract

Egg white protein (EW) is used as surface-active ingredient in aerated food and hydroxypropylmethylcellulose (HPMC) is a polysaccharide that behaves as a surfactant. This study aimed at investiganting the effects of process parameters biopolymer concentration (2.0-5.0% w/w), EW:HPMC ratio (2:1-18:1), pH (3.0-6.0), and the influence of biopolymers behavior in aqueous solution at different pH on the foaming properties (overrun, drainage and bubble growth rate). Process parameters had effect on foaming properties. The pH was the major factor influencing the type of EW/HPMC interaction and affected the foaming properties of biopolymer mixture. At pH 3.0, EW and HPMC showed thermodynamic compatibility leading to better foaming properties, higher foaming capacity and stability than without HPMC addition whereas at pH 4.5 and 6.0, EW and HPMC are incompatible that causes lower stability concerning the disproportionation comparing to foam without HPMC. At pH between 3.0 and 4.5, HPMC improves foaming properties of aerated products.

Keywords: Thermodynamic incompatibility; Disproportionation; Coalescence; Drainage; Stability.

Abbreviations: Egg white protein, EW; Hydroxypropylmethylcellulose, HPMC; ANOVA, analysis of variance; CCRD, central composite rotatable design; DR, drainage (% drained liquid); R^2, percentage of variance explained; V_{bubble}, bubble growth rate (% BS/min)

1. Introduction

Food foam is a dispersion with two immiscible phases, water and air, stabilized by amphiphilic molecules such as proteins. Foam is an unstable thermodynamic system whose instability is associated to: 1) drainage of liquid from lamella; 2) coalescence due to bubble deformability and drainage of fluid between bubbles, and 3) disproportionation due to gas diffusion from smaller bubble to large one (Damodaran, 2005; Murray, Dickinson, & Wang, 2009; Murray & Ettelaie, 2004).

Proteins and polysaccharides are usually used in combination in food foam because both contribute to structure, stability and texture through their aggregation, thickening, gelling and surface properties (Dickinson, 2003; Doublier, Garnier, Renard, & Sanchez, 2000). Proteins have the ability to form an interfacial monolayer between air and water by orientation of their hydrophobic segments to hydrophobic phase (air) and their hydrophilic segments to aqueous phase. Egg white protein (EW) is widely used in aerated food such as cake, nougat, marshmallow due to its excellent foaming properties (Dickinson, 2011; Jackson, 1995; Yang & Foegeding, 2010). Polysaccharides show water holding capacity and thickening properties (Dickinson, 2003). Some polysaccharides such as hydroxypropylmethylcellulose (HPMC) also have surface active properties. HPMC is a cellulose derivative with hydroxypropyl and methyl groups added to anhydroglucose backbone. Due to these hydrophobic zones (methyl groups), HPMC behaves as surfactant, then a competitive adsorption for air-water interface between HPMC and protein occurs (Perez et al., 2007; Pérez, Wargon, & Pilosof, 2006).

Studies have shown that the effect of protein and polysaccharide interaction on the properties of air-water interface is influenced by the biopolymers behavior in the bulk phase (Baeza, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2005; Sadahira, Lopes, Rodrigues, & Netto, 2014; Schimitt, Kolodziejczyk, & Leser, 2005). Interaction between protein and polysaccharide can occur and depends on pH, ionic strength, biopolymer concentration and proportion of each biopolymer in aqueous solution. Attractive interaction between protein and polysaccharide can form insoluble or soluble complexes. Thermodynamic incompatibility takes places due to repulsive interaction between biopolymers leading the system to separate into two phases. Miscibility occurs when the biopolymers are co-soluble (Dickinson, 2003, 2008; Rodríguez Patino & Pilosof, 2011).

The process parameters - biopolymer concentration, protein:polysaccharide ratio and pH- may influence the interaction between protein and polysaccharide in aqueous solutions (Grinberg & Tolstoguzov, 1997; Rodríguez Patino & Pilosof, 2011). Therefore, to formulate stable foam based on mixture of biopolymers, it is critical to understand the impact of their interaction in bulk aqueous medium on the structure and composition of biopolymers interface, and the effect on the foaming properties of the colloidal system (Dickinson, 2011). The objectives of this work were to study the effects of these process parameters on the foaming properties (overrun, drainage and bubble growth rate), using a Central Composite Rotatable Design (CCRD) and the influence of biopolymers behavior in aqueous solution on the foaming properties.

2. Materials and methods

2.1. Materials

Dried egg white protein (EW) was donated by Saltos Alimentos LTDA (Salto, Brazil). EW presented, in wet basis (wb), 79.9 $\pm$1.2% of protein, 10.20 $\pm$ 0.02% of moisture and 5.64 ± 0.22% of ash, determined according to methodologies described by AOAC (2010). The EW eletrophoretic profile obtained by SDS-PAGE (Laemmli, 1970) showed bands of 77.7, 44.5 and 14.3 kDa corresponding to conalbumin, ovalbumin and lysozyme, respectively. Hidroxypropylmethylcellulose (HPMC, METHOCEL F50, methyl 27.00 – 30.00%, hydroxypropyl 4.00 – 7.75%) was supplied by Down S.A. (Midland, USA). The chemicals were analytical grade and deionized water was used in all experiments.

2.2. Effect of the process parameters on the foaming properties

First, a fractional factorial design 2^{4-1} with 3 replicates at central point was applied to evaluate the influence of the process parameters- total biopolymer concentration (2.0-5.0% w/w), EW:HPMC ratio (8:1-8:4), whipping temperature (70-80 ℃) and pH (3.0-6.0) - on the foaming properties. The range of process parameters were defined according to previous studies (Lau & Dickinson, 2004; Martínez, Sánchez, Ruíz-Henestrosa, Rodríguez Patino, & Pilosof, 2007). This experimental design was used to adjust the levels of independent variables (process parameters) and select the significant variables to carry

out a central composite rotational design (CCRD). From the analysis of the results of the fractional factorial design 2^{4-1}, the independent variables total biopolymers concentration (% w/w), EW:HPMC ratio (w/w), and pH were selected to carry out the CCRD. The CCRD (2^3 factorial design with 6 experiments under the axial conditions and 3 repetitions at the central point) totaling 17 trials was performed to evaluate the effects of those independent variables on foaming properties. The whipping temperature and ionic strength were maintained at 75 ℃ and 0.05, respectively. Second- order models were obtained and evaluated statistically by analysis of variance (ANOVA) (Rodrigues & Iemma, 2012).

The model validation was conducted under the following experimental conditions: 3.5% w/w of total biopolymer concentration, EW:HPMC ratio 10:1, at pH 3.0 and 4.5; 4.4% w/w of biopolymer concentration, EW:HPMC ratio 10:1, at pH 6.0, in order to evaluate the role of HPMC on foaming properties of the systems. Control tests under the same conditions but without HPMC addition were also carried out.

2.3. Preparation of EW and HPMC solutions and foams

EW and HPMC aqueous solutions were prepared under stirring in separated beakers for 2 h at room temperature and then kept under refrigeration overnight. For each trial, the solutions were prepared and mixed according to fractional factorial design 2^{4-1} or CCRD conditions. The pH was adjusted with 1 mol L^{-1} HCl. In order to maintain 0.05 ionic strength in all trials, the ionic strength was adjusted with NaCl. Then, the EW and HPMC mixed solutions were heated in a jacketed beaker connected to a thermostatic bath to reach the whipping temperature. The foams were produced using a KEC57 KitchenAid mixer (KitchenAid, Greenville, USA) under atmospheric pressure and whipping time of 15 min at the speed setting 10.

2.4. Foaming properties

2.4.1. Foaming capacity

The foaming capacity was evaluated by the overrun determination. The overrun was measured by filling cylindrical containers (157.1 $\pm$ 1.1 mL) with foam. In order to achieve uniform and plane surface, a metal spatula was used to level the top of the container. The overrun was calculated according to Eq. (1) (Lau & Dickinson, 2004).

$$\text{overrun (\%)} = 100 \times [\, m_i - m_f] / m_f \quad (1)$$

where m_i is the mass of unwhipped solution and m_f is the mass of the resulting whipped solution (foam) with the same volume of m_i.

2.4.2. Foam Stability: drainage and bubble growth rate

The drainage (DR) was obtained by measuring the mass of drained liquid from the lamella after the sample was stored at 25 ± 1 ℃ for 60 min. For that, the drained liquid was removed carefully with a syringe and weighed (Kuropatwa et al., 2009). The Eq. (2) was used to calculate the percentage of DR:

$$\text{DR (\%)} = (100 \times m_d) / m_i \quad (2)$$

where m_d is the mass of drained liquid, and m_i is the initial mass of foam.

Coalescence and/or disproportionation result in the growth of bubble size (Rouimi, Schorsch, Valentini, & Vaslin, 2005). The analysis of bubble growth rate (V_{bubble}) was carried out in a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The foams were placed into cylindrical glass tubes and then scanned in order to monitor backscattering. The backscattering level (BS) is related to square root of λ^* (photon transport mean). According to Mie theory, λ^* is inversely proportional to the gas phase volume and proportional to bubble mean diameter. Thus, the backscattering values (BS) change with increasing the air bubble size. The V_{bubble} was calculated from the slope of the %BS curve versus time (Sadahira et al., 2014).

2.5. Characterization of EW and HPMC solutions

2.5.1. Zeta potential

Zeta potential measurements of the EW and HPMC solutions and their mixture at the total biopolymer concentrations 3.5 and 4.4% (w/w) and EW:HPMC ratio of 10:1 were carried out using a Malvern Zetasizer Nano Z instrument (Malvern Instruments, Worcestershire, UK), at pH range from 2.0 to 7.0. The electrophoretic mobility is obtained by measuring the velocity of the particles using Laser Doppler Velocimetry (LDV), and then the Henry equation was used to convert the electrophoretic mobility measurements into zeta potential values.

2.5.2. Fluorescence microscopy

The microstructures of EW/HPMC mixtures were analyzed using a bright field and fluorescence microscopy (Nikon Eclipse E800, Nikon Corp., Japan) with excitation/emission filter: (520/570 nm) and 60 x objective lenses. In order to label the protein, 500 µL of sample was placed into 1.5 mL microtubes and mixed with 10 µL of 0.02% (w/v) Rhodamine B (Sigma Aldrich, USA) aqueous solution.

2.6. Statistical analysis

The results were analyzed statistically for difference among means by Tukey's test (Tukey Honest Significant Difference) ($p < 0.05$). Student t test ($p < 0.05$) was used for comparison between samples of the model validation (with HPMC) and the control test (without HPMC) under the same conditions.

3. Results and discussions

3.1. Effects of process parameters on the foaming properties

From the fractional factorial design 2^{4-1} results (Table 1), the effects of the independent variables total biopolymer concentration, EW:HPMC ratio, temperature and pH on the overrun and liquid drainage responses were calculated (Table 2). For overrun response, only total biopolymer concentration showed statistically significant effect ($p < 0.1$) whereas for liquid drainage the total biopolymer concentration and pH had statistically significant effect ($p < 0.1$). Temperature had no statistically significant effect ($p > 0.1$) for both responses. Therefore, the intermediate whipping temperature, 75 ℃, was defined to conduct the CCRD. Despite EW:HPMC ratio was not a statistically significant effect for both responses, this variable was included in the CCRD in a larger range (2:1 – 18:1) since range of EW:HPMC ratio (8:4 - 8:1) used in the first experimental design was too narrow to affect the foaming properties.

Table 1. Fractional factorial design 2^{4-1} matrix and results for overrun and drainage.

Trial	Total biopolymer concentration (% w/w)	EW:HPMC ratio	Whipping T (℃)	pH	Overrun* (%)	Drainage* (%)
1	-1 (2.0)	-1 (8:1)	-1 (70)	-1 (3.0)	900	51.1
2	1 (5.0)	-1 (8:1)	-1 (70)	1 (6.0)	823	57.6
3	-1 (2.0)	1 (8:4)	-1 (70)	1 (6.0)	880	88.7
4	1 (5.0)	1 (8:4)	-1 (70)	-1 (3.0)	458	0.0
5	-1 (2.0)	-1 (8:1)	1 (80)	1 (6.0)	900	75.2
6	1 (5.0)	-1 (8:1)	1 (80)	-1 (3.0)	388.9	0.0
7	-1 (2.0)	1 (8:4)	1 (80)	-1 (3.0)	853	52.0
8	1 (5.0)	1 (8:4)	1 (80)	1 (6.0)	442	7.0
9	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	779	53.9
10	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	772	60.5
11	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	776	56.4

* whipping time: 15 min; ( ) true values of the independent variables for each level.

Table 2. Estimate of the effects on dependent variables overrun and drainage for fractional factorial design 2^{4-1}.

	Overrun (%)				Drainage (%)			
Factors	Effect	Standard error	t (6)	p - value	Effect	Standard error	t (6)	p - value
Mean	725.03	30.09	24.09	0.00	45.67	4.36	10.47	0.00
Total bio. conc.	-355.62	70.57	-5.04	0.10	-50.60	10.23	-4.95	0.00
EW:HPMC ratio	-94.55	70.57	-1.14	0.23	-9.05	10.23	-0.88	0.41
Whipping T (℃)	-119.15	70.57	-1.60	0.14	-15.80	10.23	-1.54	0.17
pH	111.15	70.57	1.57	0.17	31.35	10.23	3.06	0.02

Total bio. conc.: total biopolymer concentration

Thus, total biopolymer concentration, EW:HPMC ratio and pH were the parameters selected to evaluate the effect on the foaming properties (overrun, drainage and bubble growth rate), using a CCRD (Table 3). The regression coefficients were calculated and mathematical models were built for the responses overrun, drainage and V_{bubble} from the foaming properties results (Table 3). ANOVA was used to evaluate the adequacy of the fitted model showed in Table 4.

Table 3. CCRD matrix and results for overrun, drainage and bubble growth rate (V_{bubble}) at 75 ℃.

Trial	Total Biopolymer concentration (% w/w) x1	EW:HPMC ratio x2	pH x3	Overrun* (%) y1	Drainage (%) y2	V_{bubble} (% BS/min) y3
1	-1 (2.6)	-1(5:1)	-1(3.6)	936	0.0	0.495
2	1 (4.4)	-1(5:1)	-1(3.6)	713	0.0	0.525
3	-1 (2.6)	1(15:1)	-1(3.6)	587	0.0	0.423
4	1 (4.4)	1(15:1)	-1(3.6)	733	0.0	0.495
5	-1 (2.6)	-1(5:1)	1(5.4)	860	58.8	0.818
6	1 (4.4)	-1(5:1)	1(5.4)	736	44.6	0.655
7	-1(2.6)	1(15:1)	1(5.4)	613	77.0	0.804
8	1 (4.4)	1(15:1)	1(5.4)	605	61.3	0.607
9	-1.68 (2.0)	0 (10:1)	0 (4.5)	591	65.6	0.621
10	1.68 (5.0)	0 (10:1)	0 (4.5)	391	27.4	0.342
11	0 (3.5)	-1.68 (2:1)	0 (4.5)	454	43.0	0.467
12	0 (3.5)	1.68 (18:1)	0 (4.5)	474	46.2	0.239
13	0 (3.5)	0 (10:1)	-1.68 (3.0)	956	0.0	0.664
14	0 (3.5)	0 (10:1)	1.68 (6.0)	825	75.3	0.981
15	0 (3.5)	0 (10:1)	0 (4.5)	524	37.5	0.332
16	0 (3.5)	0 (10:1)	0 (4.5)	564	44.4	0.370
17	0 (3.5)	0 (10:1)	0 (4.5)	522	44.7	0.303

* whipping time: 15 min; ( ) true values of the independent variables for each level; Vbubble (%BS/min) = the slope of the % mean backscattering values (BS) curve versus time.

The responses overrun (y_1), drainage (y_2) and V_{bubble} (y_3) were significantly affected by the independent variables total biopolymer concentration, EW:HPMC ratio, and pH. The R^2 and calculated F values (Table 4) indicate that the second-order model (Eq. 3 and 5) and first-order model (Eq. 4) describe the overrun, drainage and V_{bubble} behavior as a function of total biopolymer concentration (x_1), EW:HPMC ratio (x_2), and pH (x_3), within the range studied.

Table 4. Percentage of variance explained (R^2), calculated F value and tabulated F for the responses overrun, drainage and V_{bubble}.

Response	R^2 (%)	Calculated F	Tabulated F*
Overrun	80.5	12.37	3.26
Drainage	86.9	46.38	3.74
V_{bubble}	95.8	38.42	3.22

*at 5% significance level

The mathematical models for overrun, drainage and V_{bubble} as a function of coded independent variables with statistically significant parameters ($p < 0.1$) were obtained (Eq. 3, 4 and 5):

$$\text{Overrun (\%)} = 533.7 - 39.9\, x_1 - 49.3x_2 + 147.4x_3^2 + 60.6\, x_1\, x_2 \quad (3)$$

$$\text{Drainage (\%)} = 36.76 - 7.00x_1 + 26.97x_3 \quad (4)$$

$$V_{bubble}\ (\%BS/min) = 0.351 - 0.053x_1 + 0.0558x_1^2 - 0.040x_2 + 0.108x_3 + 0.177x_3^2 - 0.0578x_1x_3 \quad (5)$$

where x_1, x_2 and x_3 are the coded independent variables for total biopolymer concentration, EW:HPMC ratio and pH, respectively.

The responses surfaces obtained from Eq. 3, 4 and 5 are presented in Fig. 1 in order to show the behavior of each response overrun, drainage and V_{bubble}. Overrun increased with reducing biopolymer concentration and EW:HPMC ratio and their interaction. Drainage decreased with increasing biopolymer concentration and decreasing

pH but was not influenced by EW:HPMC ratio. V_{bubble} value decreased with increasing EW:HPMC ratio.

Pareto diagrams (Fig. 2) show significant effects of the variables (linear, quadratic and interactions between variables). The bars are proportional to the absolute values of the estimated effects and the dashed lines line represents the minimum of statistically significant effects (90% of the confidence interval) with respect to the response. Overrun was affected linearly by total biopolymer concentration, EW:HPMC ratio and their interaction and by quadratic pH. Drainage was affected only linearly by total polymers concentration and pH. V_{bubble} was affected by pH (linear and quadratic), total biopolymer concentration (linear and quadratic), EW:HPMC ratio (linear), and by interaction between pH and total biopolymer concentration.

From the Pareto diagram (Fig. 2) pH was the most significant independent variable for all responses. The lowest overrun was obtained at pH 4.5 and the highest at pH 3.0 and 6.0. The lowest and highest liquid drainage were obtained at pH 3.0 and 6.0, respectively while the lowest and highest V_{bubble} were obtained at pH 4.5 and pH 6.0, respectively. Thus, at pH 6.0, the foam presented good foaming capacity but poor stability; at pH 4.5 the foam showed poor foaming capacity but good stability concerning the V_{bubble} and at pH 3.0 the foam exhibited good foaming capacity and stability related to liquid drainage. Therefore foaming capacity and stability mechanisms are influenced mainly by pH.

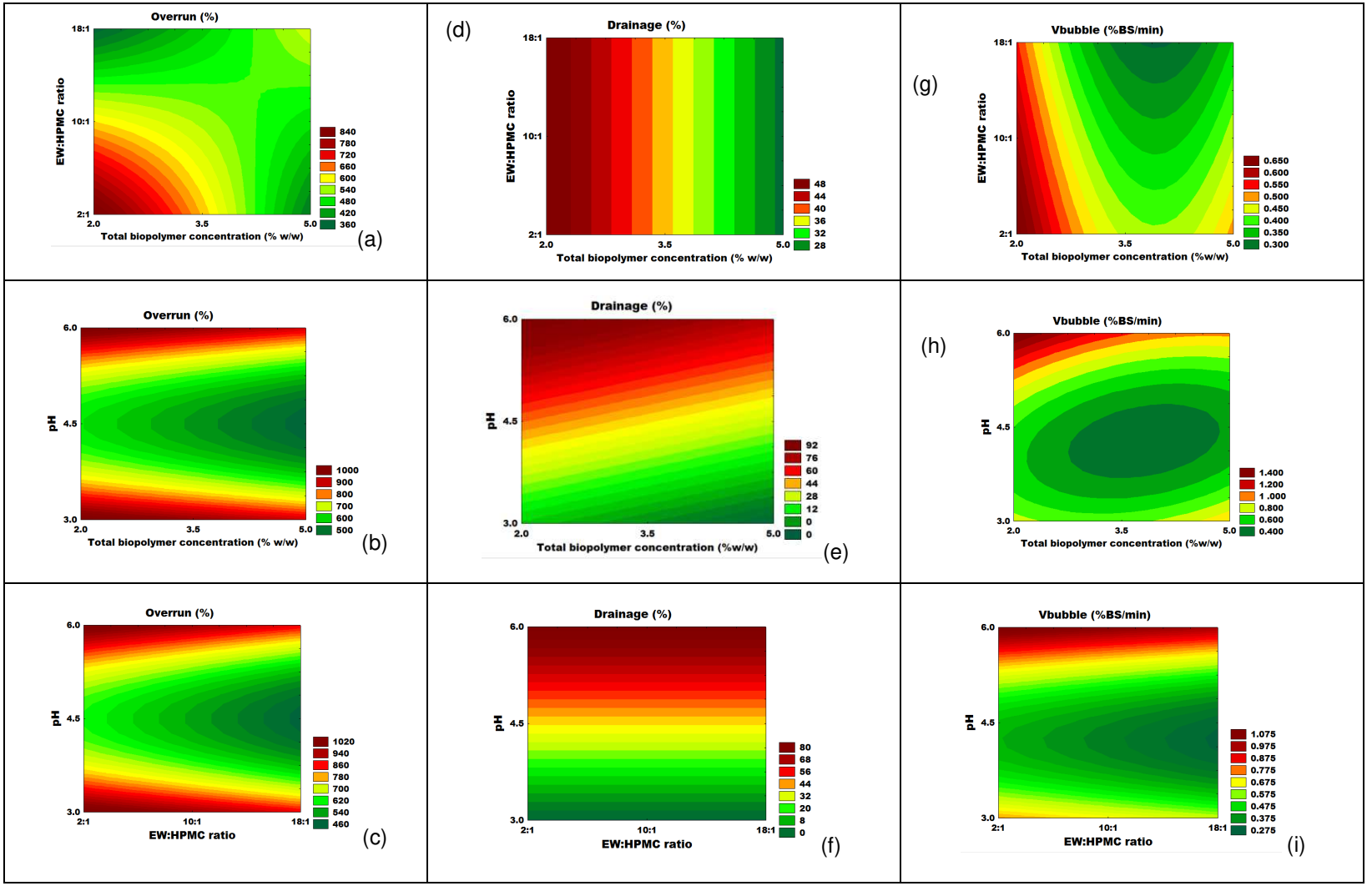
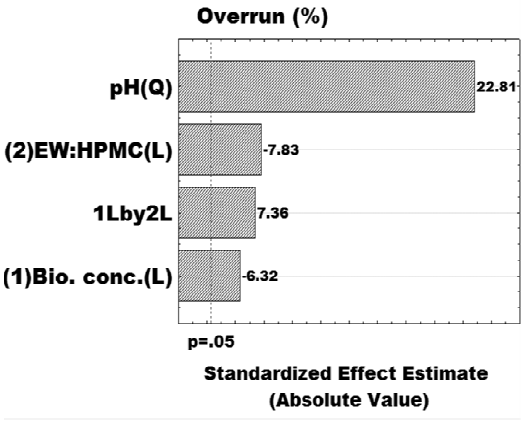
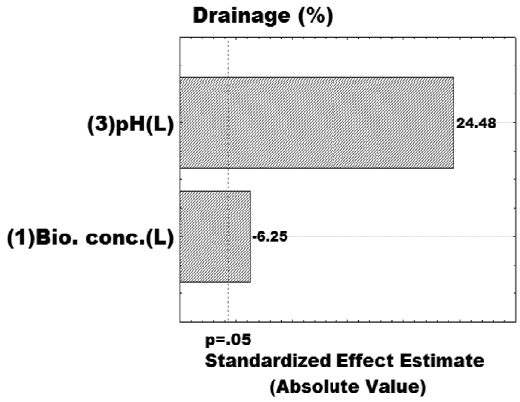


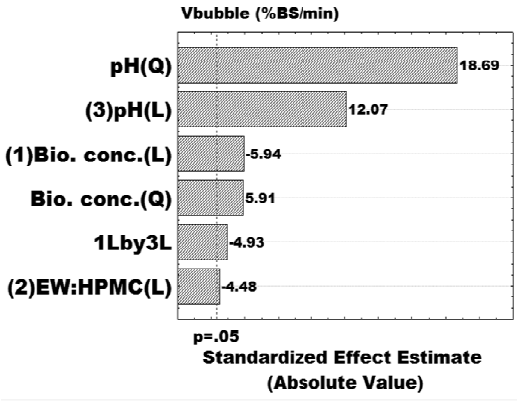
Fig. 1. Contour curves for the dependent variables overrun, drainage and bubble growth rate (V_{bubble}).



(a)



(b)



(c)

Bio. conc.: Total biopolymer concentration; EW:HPMC: EW:HPMC ratio

Fig. 2. Pareto diagram for (a) overrun, drainage (b) and V_{bubble} (c) responses.

3.2. Model validation and effect of interaction between EW and HPMC on foaming properties

Since the pH was the most important independent variable, model validation was carried out at pH 3.0, 4.5 and 6.0 under the best conditions for each pH. Thereby, at pH 3.0 (Trial A) and 4.5 (Trial B), the conditions to obtain good foam properties - high overrun, low drainage and V_{bubble} value - were 3.5% (w/w) of biopolymer concentration and EW:HPMC ratio 10:1, whereas at pH 6.0 (Trial C) the condition was 4.4% (w/w) of biopolymer concentration and EW:HPMC ratio 10:1 (Fig. 1).

The results of experimental tests, predicted values by the coded model for overrun, drainage and V_{bubble}, the relative error between the experimental, and predicted value for each test are presented in Table 5. To evaluate the role of HPMC on the foaming properties, control tests with no HPMC addition were carried out and the results are shown in Table 5.

In general, all experimental results were close to the predicted values. The exceptions were the experimental drainage and V_{bubble} obtained under the conditions of Trial C (pH 6.0), which were lower by 30 to 32% than the predicted values, respectively. This difference is possibly because the foams prepared at pH 6.0 were very unstable. Despite this deviation, the results from validation experiments were satisfactory.

Under Trial A conditions, the overrun was higher and the drainage and V_{bubble} were lower when HPMC was in the system. For Trial B and C, the overrun and V_{bubble} showed higher values while no significant difference in drainage was observed when HPMC was added. The lower V_{bubble} of the foam with HPMC than whitout HPMC obtained under the trial A conditions is related to the increased stability concerning to coalescence rate. Coalescence and disproportionation lead to growth of bubble size while coalescence may occur due to drainage of the liquid from the lamella (Damodaran, 2005). By the other hand, for Trials B and C, the higher V_{bubble} of the foams with HPMC is related to lower stability with reference to disproportionation since there was no significant difference in drainage between foams with and without HPMC.

Table 5. Predicted values (y_p), experimental values (y_e) and relative error (RE = (y_e - y_p) / y_e*100)) for overrun (OV), drainage (DR) and V_{bubble} and experimental values of overrun of foams obtained under the conditions for the validation of the mathematical models with and without addition of HPMC (control).

Trial	Overrun (%)				Drainage (%)				V_{bubble} (%BS/min)			
	Control (without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)	Control (without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)	Control (Without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)
A pH 3.0	727.0 ± 8.0^{b,A}	877.9 ± 8.9^{a,B}	950.0	-8.2	37.1 ± 5.1^{b,A}	0.0 ± 0.0^{c,B}	-8.5	0.0	0.948 ± 0.108^{a,A}	0.620 ± 0.068^{b,B}	0.667	-5.7
B pH 4.5	134.7 ± 41.1^{e,A}	576.7 ± 10.3^{c,B}	534.0	7.5	45.4 ± 3.5^{b,A}	36.7 ± 5.1^{b,A}	36.8	-0.3	0.130 ± 0.027^{d,A}	0.397 ± 0.066^{c,B}	0.351	11.6
C pH 6.0	219.1 ± 34.4^{d,A}	864.8 ± 18.8^{a,B}	910.0	-5.2	56.8 ± 1.5^{a,A}	57.6 ± 2.4^{a,A}	75.3	-30.6	0.138 ± 0.026^{d,A}	0.707 ± 0.074^{b,B}	0.937	-32.6

A (biopolymer concentration = 3.5% w/w, protein:HPMC ratio 10:1); B (biopolymer concentration = 3.5% w/w, protein:HPMC ratio 10:1); C (biopolymer concentration = 4.4% w/w, protein:HPMC ratio = 10:1). Overrun, drainage and Vbubble at the validation conditions . Values are mean ± SD of triplicates, except to overrun values of B and Control C that are 6 repetitions. For the same response, means with different small letters in the same column differ significantly ($p < 0.05$) by Tukey's test, and means with different capital letters in the same row differ significantly ($p < 0.05$) by Student's t test. RE = Relative error (%).

To better understand the foaming properties of the EW/HPMC mixtures used for the validation model, their zeta-potential curves, microstructures, and appearance were analyzed and the results are shown in Fig. 3.

Under Trial A conditions (pH 3.0), EW and HPMC solutions presented net positive charge (pH < pI) and electrical neutrality, respectively. However, the EW/HPMC mixture showed lower zeta potential value than the protein solution, suggesting hydrogen bond or hydrophobic interactions between them occurred (Fig. 3a) (Rodríguez Patino & Pilosof, 2011). The microstructure of this mixture (Fig. 3b) showed homogenous pattern, without separated domain, indicating thermodynamic compatibility of the biopolymers (Jara, Pérez, & Pilosof, 2010). The thermodynamic compatibility led to better foaming properties (higher foaming capacity and stability) than without HPMC (control). At this pH, EW is partially unfolded, exposing more hydrophobic groups and increasing flexibility and amphiphilic nature, thus improving the foaming properties (Mleko, Kristinsson, Liang, & Gustaw, 2007). In addition, possibly because the biopolymers compatibility, a secondary layer was built up around air bubbles, contributing to formation of a stable film at the air-water interface, inhibiting the bubbles coalescence and increases the viscosity of liquid from lamella leading a lower drainage value.

EW, HPMC and their mixture are close to electrical neutrality under Trial B conditions (pH 4.5), which is accordance with the EW pI (Fig. 3d). The microstructure of the EW/HPMC mixture presented phase separation (Fig. 3e) revealed by red and black areas which are protein-enriched domains and HPMC-enriched domains, respectively. Thermodynamic compatibility between proteins and neutral polysaccharides decreases with approaching of the proteins pI (Grinberg & Tolstoguzov, 1997; Polyakov, Grinberg, Antonov, & Tolstoguzov, 1979; Samant, Singhal, Kulkarni, & Rege, 1993). Under Trial C conditions (pH 6.0), EW, HPMC and their mixture carry net negative charge and the EW/HPMC mixture showed zeta potential value close to the protein solution. Under this condition, phase separation occurred between protein (red area) and HPMC (black area) as is shown in Fig. 3h. Repulsive interactions between two biopolymers in solution cause mutual exclusion of each biopolymer from the local vicinity of the other, leading to phase separation. Generally, thermodynamic incompatibility takes place at pH higher than protein isoelectric point (Grinberg & Tolstoguzov, 1997; Rodríguez Patino & Pilosof, 2011).

At pH 4.5 and 6.0 (Trial B and C conditions, respectively), the EW/HPMC mixture showed incompatibility that causes lower stability concerning the disproportionation

comparing to foam without HPMC (Table 5). However, foam prepared without HPMC at pH 4.5 showed lower overrun because at this pH, which is in EW pI vicinity, the protein solubility is low, diminishing foaming capacity whereas at pH 6.0, the electrostatic repulsion of EW increased, resulting in a lower ability of protein to interact at the interface and to build a stable protein film (Kuropatwa et al., 2009). Due to HPMC surfactant properties (Arboleya & Wilde, 2005), a competitive adsorption could take place between protein and HPMC and if thermodynamic incompatibility of protein/polysaccharide mixture occurs at film interface, the repulsion between both biopolymers reduces the stability (Damodaran & Razumovsky, 2003; Sengupta & Damodaran, 2000).

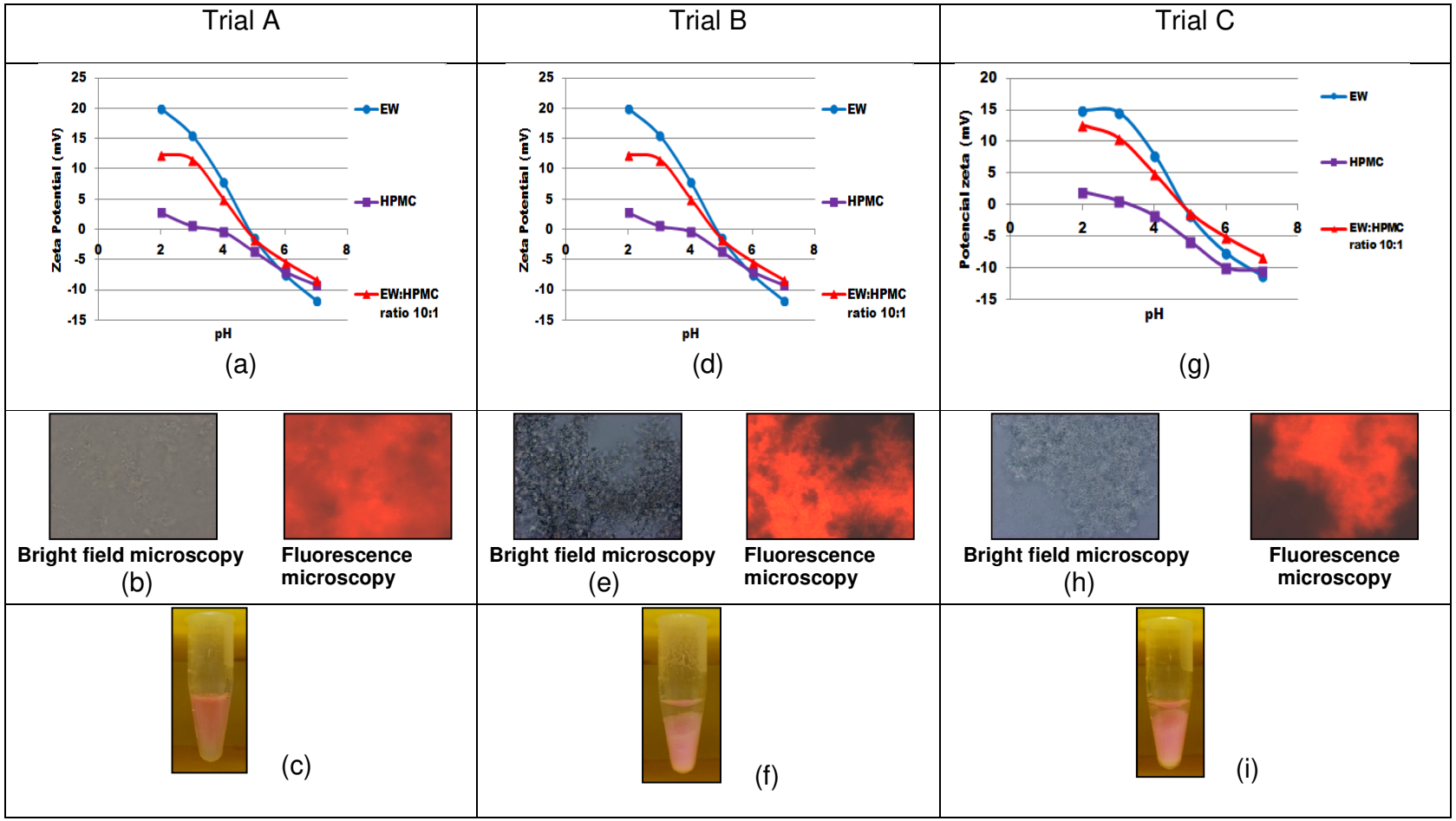


Fig. 3. Zeta potential as a function of pH (a, d and g); its microstructure in bright and fluorescence microscopy(b, e and h) and appearance of the EW/HPMC phases after Rhodamine B conjugation and centrifugation at 2655 g (c, f and i) for Trial A (total biopolymer concentration 3.5% w/w; protein: HPMC ratio of 10:1, pH 3.0), for Trial B (total biopolymer concentration 3.5% w/w; protein: HPMC ratio of 10:1, pH 4.5) and for Trial C (total biopolymer concentration 4.4% w/w; EW:HPMC ratio of 10:1; at pH 6.0), respectively. Note the bright red fluorescence of EW fraction precipitated in Trial B (pH4.5) and C (pH6.0), while the EW remains a homogeneous pattern in A (pH3.0).

4. Conclusion

The foaming properties depend on biopolymer concentration, EW:HPMC, pH and interaction between biopolymers in aqueous solution, in the studied range of CCRD. The pH was the major factor influencing on foaming properties and interaction between EW and HPMC in aqueous solution. At pH 3.0, EW and HPMC showed thermodynamic compatibility leading to better foaming properties (higher foaming capacity and stability) than without HPMC. Whereas at pH 4.5 and 6.0, the incompatibility between EW and HPMC causes lower stability concerning the disproportionation comparing to foam without HPMC. Due to HPMC surfactant properties, competitive adsorption could take place between EW and HPMC and the thermodynamic incompatibility of EW/HPMC mixture at film interface affects the stability. HPMC improved the foaming capacity of samples at pH 4.5 and 6.0, however it reduced the stability related to disproportionation comparing the foam without HPMC. Therefore, HPMC could be used to obtain aerated product with good foaming properties in pH between 3.0 and 4.5.

Acknowledgements

The authors are grateful to FAPESP for financial support (FAPESP 2011/50067/9) and EMBRAPA for the PhD scholarship granted to the author Sadahira MS.

5. References

AOAC. (2010). Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, USA: Association of Official Methods Analytical Chemists.

Arboleya, J., & Wilde, P. (2005). Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. *Food Hydrocolloids*, *19*(3), 485–491.

Baeza, R. I., Carrera Sánchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2005). Interactions between b-lactoglobulin and polysaccharides at the air-water interface and the influence on foam properties. In E. Dickinson (Ed.), *Food Colloids: Interactions, Microstructure and Processing* (pp. 301–316). Cambridge: Royal Society of Chemistry.

Damodaran, S. (2005). Protein Stabilization of Emulsions and Foams. *Journal of Food Science*, *70*(3), R54–R66.

Damodaran, S., & Razumovsky, L. (2003). Competitive adsorption and thermodynamic incompatibility of mixing of b -casein and gum arabic at the air – water interface, *17*, 355–363.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems q. *Food Hydrocolloids*, *17*, 25–39.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter*, *4*, 932–942.

Dickinson, E. (2011). Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids*, *25*(8), 1966–1983.

Doublier, J., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, *5*, 202–214.

Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*(2), 145–158.

Jackson, E. B. (1995). *Sugar Confectionery Manufacture* (pp. 218–235). London: Black Academic and Professional.

Jara, F. L., Carrera Sánchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2014). Competitive adsorption behavior of β-lactoglobulin, α-lactalbumin, bovin serum albumin in presence of hydroxypropylmethylcellulose. Influence of pH. *Food Hydrocolloids*, *35*, 189–197.

Jara, F., Pérez, O. E., & Pilosof, A. M. R. (2010). Impact of phase separation of whey proteins/hydroxypropylmethylcellulose mixtures on gelation dynamics and gels properties. *Food Hydrocolloids*, *24*(6-7), 641–651.

Kuropatwa, M., Tolkach, A., & Kulozik, U. (2009). Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. *Food Hydrocolloids*, *23*(8), 2174–2181. doi:10.1016/j.foodhyd.2009.05.001

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of thehead of bacteriophage T4. *Nature*, *227*, 680–685.

Lau, K., & Dickinson, E. (2004). Structural and Rheological Properties of Aerated High Sugar Systems Containing Egg Albumen. *Journal of Food Science*, *69*(5), E232–E239.

Martinez, K., Carrera Sanchez, C., Ruız-Henestrosa, V., Rodriguez Patino, J. M., Pilosof, A.M.R. (2007). Effect of limited hydrolysis of soy protein on the interactions with polysaccharides at the air–water interface. *Food Hydrocolloids*, 21, 813–822.

Mleko, S., Kristinsson, H. G., Liang, Y., & Gustaw, W. (2007). Rheological properties of foams generated from egg albumin after pH treatment. *LWT - Food Science and Technology*, *40*(5), 908–914.

Murray, B. S., Dickinson, E., & Wang, Y. (2009). Bubble stability in the presence of oil-in-water emulsion droplets: Influence of surface shear versus dilatational rheology. *Food Hydrocolloids*, *23*(4), 1198–1208.

Murray, B. S., & Ettelaie, R. (2004). Foam stability : proteins and nanoparticles. *Current Opinion in Colloid & Interface Science*, *9*, 314–320.

Perez, O. E., Carrera Sanchez, C., Rodriguez Patino, J. M., & Pilosof, A. M. . (2007). Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl–methyl–cellulose mixtures at the air-water interface. *Food Hydrocolloids*, *21*(5-6), 794–803.

Pérez, O. E., Wargon, V., & Pilosof, A. M. R. (2006). Gelation and structural characteristics of incompatible whey proteins/hydroxypropylmethylcellulose mixtures. *Food Hydrocolloids*, *20*(7), 966–974.

Polyakov, V. I., Grinberg, V. Y., Antonov, Y. A., & Tolstoguzov, V. B. (1979). Polymer Bulletin 9. *Polymer Bulletin*, *1*, 593–597.

Rodrigues, M. I., & Iemma, A. F. (2012). *Experimental Design and Process Optimization* (2 nd.). Campinas: Carita Editora.

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*(8), 1925–1937.

Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial properties of milk protein?surfactant systems. *Food Hydrocolloids*, *19*(3), 467–478.

Sadahira, M. S., Lopes, F. C. R., Rodrigues, M. I., & Netto, F. M. (2014). Influence of protein–pectin electrostatic interaction on the foam stability mechanism. *Carbohydrate Polymers*, *103*, 55–61.

Samant, S. K., Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1993). Review Protein-polysaccharide interactions : a new approach in food formulations. *International Journal of Food Science and Technology*, *28*, 547–562.

Schimitt, C., Kolodziejczyk, E., & Leser, M. E. (2005). Interfacial and foam stabilization properties of b-lactoglobulin e Acacia gum electrostatic complexes. In E. Dickinson (Ed.), *Food colloids: Interactions, microstructure and processing* (pp. 284–300). Cambridge: Royal Society of Chemistry.

Sengupta, T., & Damodaran, S. (2000). Incompatibility and Phase Separation in a Bovine Serum Albumin / β -Casein / Water Ternary Film at the Air – Water Interface. *Journal of Colloid And Interface Science*, *229*, 21–28.

Yang, X., & Foegeding, E. A. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids*, *24*(2-3), 227–238.

CAPÍTULO 4. Definição de um sistema modelo de açúcar para produtos aerados tipo *marshmallow* utilizando delineamento experimental de mistura

Sadahira, M.S, Saito, M, Rodrigues, M.I., & Netto, F.M.

A ser submetido na revista Journal of Food Science

Capítulo 4. Definição de um sistema modelo de açúcar para produtos aerados tipo *marshmallow* utilizando delineamento experimental de mistura

Sadahira, M.S.[a], Saito, M, Rodrigues[a], M.I., & Netto, F.M[c].

[a]Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP Campinas, Brazil; E-mail: mitie@ital.sp.gov.br

[b]Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brasil; E-mail: protimiza@protimiza.com.br

[c]Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/ UNICAMP, Rua Monteiro Lobato n° 80 - CEP: 13.083-970, Campinas, Brazil; E-mail: fernanda.rzls@gmail.com; E-mail: flavia@fea.unicamp.br

Resumo

Nos confeitos aerados, a espuma é produzida pela aeração de xaropes de açúcares e estabilizada pela adição de proteínas. Nestes produtos, utiliza-se, em geral, mistura de sacarose, xarope de glicose e açúcar invertido para obter soluções de açúcares com sólidos solúveis acima de 76% (p/p), o que permite manter a estabilidade microbiológica, sem favorecer a formação de cristais de açúcar. O objetivo deste estudo foi definir a composição da solução de açúcares de um sistema modelo de açúcar para produto aerado tipo *marshmallow* utilizando o delineamento experimental de mistura. Os resultados mostraram que o pH influenciou na capacidade de aeração das misturas (sacarose, xarope de glicose e açúcar invertido). Em pH 3,0 e 4,5 as espumas apresentaram menor densidade do que em pH 6,0. A atividade de água (Aa) das espumas diminuiu com o aumento da concentração de açúcar invertido e aumentou com o aumento da concentração de sacarose e xarope de glicose. Pela análise das curvas de contorno obtidas dos resultados do delineamento experimental de mistura definiu-se a mistura de açúcares em 42,5% sacarose, 42,5% xarope de glicose e 15% de açúcar invertido como sistema modelo para produtos aerados tipo *marshmallow*. Nestas condições, o sistema modelo apresentou densidade menor que 0,50 g/mL e Aa abaixo de 0,75 que são características dos *marshmallows* produzidos industrialmente.

Palavras-chave: Produtos aerados, sacarose, xarope de glicose, açúcar invertido.

1. Introdução

Espumas são encontradas na indústria de alimentos na forma de pães, bolos, *cookies*, merengues, nougats, sorvetes, etc. (CAMPBEL & MOUGEOUT, 1999). A espuma é uma dispersão de bolhas de gás em uma fase contínua de líquido ou sólido e, como acontece em muitos sistemas coloidais, é um sistema termodinamicamente instável (DICKINSON, 1992). Portanto, além da capacidade de aeração, é importante avaliar a estabilidade da espuma, que é a sua habilidade de resistir à perda de bolhas de gás ou ao colapso devido à influência da gravidade (LAU; DICKINSON, 2004). A estabilidade da espuma é analisada por fatores como drenagem (devido à gravidade), desproporção (difusão do ar de uma bolha pequena para uma bolha grande ou para atmosfera) e coalescência (quebra da bolha pela ruptura da lamela) (DAMODARAN, 2005). A capacidade de aeração está relacionada com o volume de ar introduzido na solução de proteínas e é determinada pelo aumento de volume da espuma formada.

A aeração promove redução da densidade do produto, alteração de sua textura e reologia, resultando em mudanças na aparência e possibilidade de redução da vida-de-prateleira devido à porosidade com o possível aumento das reações de oxidação (CAMPBELL; MOUGEOT, 1999; KINSELLA, 1981).

Na indústria de confeitos, a aeração é utilizada para produzir uma grande variedade de produtos (balas mastigáveis, *marshmallow*, *nougat*, merengue e recheios), caracterizados por sua densidade, que pode variar de 0,2 a 1,0 g/mL. Nos confeitos aerados, a espuma é produzida pela aeração de xaropes de açúcares, sendo estabilizada pela adição de proteínas. A composição da solução de açúcares deve ter acima de 76% de sólidos solúveis para que a atividade de água (Aa) permaneça abaixo de 0,80 e dessa forma manter os confeitos estáveis microbiologicamente à temperatura ambiente (JACKSON, 1995). O controle da Aa é um dos métodos para manter o alimento seguro contra o crescimento microbiano, aumentando a vida-de-prateleira do produto (LABUZA & ALTUNAKAR, 2007).

A sacarose é um dos principais carboidratos utilizados em confeitaria. Uma solução de sacarose encontra-se saturada na concentração de 67% de sólidos solúveis à temperatura ambiente. Para obter-se concentração acima de 76 % de sólidos solúveis, parte da sacarose deve ser substituída por outros tipos de açúcares que aumentem a

solubilidade do sistema sem a formação de cristais, como por exemplo, o xarope de glicose e o açúcar invertido (STANSELL, 1995).

O xarope de glicose é composto por glicose, maltose e oligossacarídeos e é obtido pela conversão ácida ou enzimática do amido. A indústria de confeitos utiliza o xarope de glicose para controlar a cristalização da sacarose (CHINACHOTI, 1995).

O açúcar invertido é produzido por hidrólise ácida ou enzimática da sacarose (dissacarídeo), resultando em uma mistura de glicose, frutose e sacarose. A adição do açúcar invertido nesse sistema contribui para diminuir a cristalização e atividade de água devido a sua afinidade com a água (CHINACHOTI, 1995; ERGUN, LIETHA, HARTEL, 2010; JAMES, 1995).

O principal uso das proteínas da clara de ovo em confeitos é em produtos aerados. É utilizada como agente de aeração tanto em *marshmallow* como em *nougat* (JACKSON, 1995). A força motriz da proteína para adsorção na interface ar-água é a interação hidrofóbica/hidrofílica das proteínas com a água e a intensidade desta interação depende do pH (THAKUR, R.K *et al*., 2006).

O objetivo deste estudo foi definir a composição da solução de açúcares de um sistema modelo utilizando o delineamento experimental de mistura para obtenção de um produto aerado tipo marshmallow com densidade abaixo de 50 g/mL e Aa abaixo de 0,75.

2. Materiais e métodos

2.1. Materiais

Sacarose, xarope de glicose 40DE e açúcar invertido com taxa de inversão de 54% foram fornecidos pela Guarani S.A. (Olimpia, Brasil), Cargill Agrícola S.A. (Uberlandia, Brasil) e Dulcini S.A. (Pirassununga, Brasil), respectivamente. Proteínas da clara de ovo desidratada (em base úmida: 79,9$\pm$1,2% de proteínas, 10,20 $\pm$ 0,02% de umidade, cinzas 5,64 $\pm$ 0,22) foi fornecida pela Saltos Alimentos LTDA (Salto, Brasil). Os demais reagentes foram de grau analítico e água deionizada foi utilizada em todos os experimentos.

2.2. Delineamento experimental de mistura

Com base no estudo de LAU & DICKINSON (2004), foram fixados os seguintes parâmetros de processo para a realização do delineamento experimental de mistura: teor

de sólidos de 80º Brix, temperatura de batimento da solução de açúcares de 70 ℃, velocidade de batimento máxima (posição 10) da batedeira planetária KitchenAid (modelo KEC57, Greenville, USA) concentração de agente de aeração (proteínas da clara de ovos) de 6% (p/p base sólidos solúveis totais) e tempo de batimento total de 6 minutos.

Foi realizado um delineamento experimental de mistura modelo quadrático com os três tipos de açúcares (sacarose, xarope de glicose 40DE e açúcar invertido 54% de inversão) nos pH 3,0, 4,5 e 6,0. O projeto de mistura é um método de delineamento experimental que permite investigar propriedades de um sistema multicomponentes em função da sua composição. A característica principal do projeto de mistura é que a soma de todos os componentes deve ser igual a 100% o que significa que os componentes não podem ser manipulados independentemente (BARROS NETO, SCARMINIO, BRUNS, 2002).

Para um sistema de três componentes, o espaço é um triângulo equilátero onde cada um dos lados representa a composição em proporção de um componente, variando de 0 a 1. A soma das proporções dos vários componentes de uma mistura é sempre 1.

O planejamento experimental empregado para determinar os valores dos coeficientes do modelo quadrático do projeto de mistura é o chamado *simplex lattice*, apresentado na Tabela 1, onde a porcentagem corresponde aos sólidos totais de cada açúcar na mistura. Análise de ANOVA e regressão foram aplicadas para ajustar o dado ao modelo quadrático e a obtenção das curvas de contorno utilizando o programa Statistica 7.0 (Statsoft, EUA).

A expressão geral do modelo quadrático está apresentada na equação 1.

$$y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \qquad (1)$$

As variáveis dependentes (respostas) para o delineamento experimental de mistura foram densidade aparente e atividade de água (Aa).

2.3. Preparo das espumas

As proteínas da clara de ovo foram hidratadas na proporção 1:1,5 (proteínas:água deionizada) sob agitação mecânica com agitador magnético e mantida sob refrigeração no mínimo de 12 horas para garantir a hidratação das proteínas. Para a realização do

ensaio, o pH foi ajustado para 6,0, 4,5 ou 3,0 com ácido cítrico (61,9% m/m e 38,7% m/m).

As soluções de açúcares fooram aquecidas até que fosse atingido teor de sólidos solúveis igual a 80 ºBrix. Em seguida, a solução de açúcares foi resfriada até a temperatura de batimento de 70 ºC. Alíquota de solução de açúcares foi retirada, colocada em provetas e pesada para a determinação da densidade aparente inicial da calda.

As espumas foram produzidas utilizando-se a batedeira planetária KitchenAid (modelo KEC57, Greenville, USA) em escala laboratorial com batedor tipo globo de arame e sob pressão atmosférica.

2.4. Capacidade de aeração: Densidade aparente e overrun

Para a determinação de densidade aparente e *overrun*, recipientes cilíndricos de volumes conhecidos foram preenchidos cuidadosamente para não alterar a estrutura da espuma. O excesso de espuma foi retirado com uma espátula de metal para que fossem obtidas superfícies retas e homogêneas. Os recipientes preenchidos com as amostras foram pesados para que se determinasse a massa final das espumas para o cálculo do *overrun* e densidade aparente.

A densidade da espuma foi calculada de acordo com a equação 2 e as medidas foram realizadas em duplicata. O *overrun* foi calculado conforme o Método de Lau e Dickinson (2004), utilizando a equação 3.

$$d = (m_f - m_r)/ V_r \quad (2)$$

$$\textit{overrun}\ (\%) = 100 \times (m_i - m_f)/m_f \quad (3)$$

A equação (2) pode ser escrita como:

$$\textit{overrun}\ (\%) - 100 \times (d_i - d_f)/d_f \quad (4)$$

onde, m_f: massa final do recipiente com amostra de espuma; m_r : massa do recipiente; V_r: volume ocupado pela amostra no recipiente; m_i: massa inicial da calda de açúcar; d_i: densidade aparente inicial da calda; d_f:densidade aparente final da espuma; d: densidade aparente.

2.5. Atividade de água

A atividade de água (Aa) das espumas foi medida diretamente em equipamento Aqualab Series 4 modelo TEV (Decagon Devices, Pullman, Washington, USA). As análises foram realizadas em triplicata, à temperatura de 25 ºC.

3. Resultados e discussão

3.1. Delineamento experimental de mistura

Os experimentos foram conduzidos de acordo com o delineamento experimental apresentado na Tabela 1. A partir dos resultados do delineamento experimental de mistura de açúcares (Tabela 1), modelos matemáticos foram obtidos para as respostas densidade (g/mL) e Aa das espumas obtidas em pH 3,0, 4,5 e 6,0. De acordo com a ANOVA apresentada na Tabela 2, os modelos matemáticos são adequados para a construção das curvas de contorno (Figura 1).

Tabela 1. Delineamento experimental de mistura com variáveis independentes x_1 (sacarose % p/p sólidos solúveis totais), x_2 (xarope de glicose % p/p sólidos solúveis totais) e x_3 (açúcar invertido % p/p sólidos solúveis totais) para as respostas densidade (den.), atividade de água (Aa) a 25 ℃, nos pH 3,0, 4,5 e 6 ,0.

Ensaio	Sac (x_1)	Xar. Glic. (x_2)	Aç. Inver. (x_3)	Sac. (80ºBrix) (g)	Xar. Glic. (83ºBrix) (g)	Aç.Inv (75ºBrix) (g)	pH 3,0		pH 4,5		pH 6,0	
							Den. (g/mL)	Aa	Den (g/mL)	Aa	Den (g/mL)	Aa
1	1,00	0,00	0,00	800,0	0,0	0,0	0,53	0,8347	0,55	0,8165	0,61	0,8401
2	0,00	1,00	0,00	0,0	963,9	0,0	0,45	0,8088	0,49	0,8153	0,53	0,8070
3	0,00	0,00	1,00	0,0	0,0	1066,7	0,39	0,6454	0,46	0,6506	0,55	0,6869
4	0,50	0,50	0,00	400,0	481,9	0,0	0,48	0,7442	0,46	0,7593	0,48	0,7481
5	0,50	0,00	0,50	400,0	0,0	533,3	0,41	0,6818	0,42	0,6647	0,56	0,6811
6	0,00	0,50	0,50	0,0	481,9	533,3	0,40	0,7016	0,41	0,7099	0,48	0,7097
7	0,67	0,17	0,17	533,3	160,6	177,8	0,46	0,7102	0,44	0,7149	0,51	0,7261
8	0,17	0,67	0,17	133,3	642,6	177,8	0,43	0,7533	0,44	0,7456	0,48	0,7354
9	0,17	0,17	0,67	133,3	160,6	711,1	0,39	0,6688	0,39	0,6899	0,50	0,6705
10	0,33	0,33	0,33	266,7	321,3	355,6	0,41	0,7112	0,42	0,7140	0,49	0,7061
11	0,33	0,33	0,33	266,7	321,3	355,6	0,40	0,7156	0,41	0,7081	0,49	0,7149
12	0,33	0,33	0,33	266,7	321,3	355,6	0,42	0,7122	0,42	0,7070	0,50	0,7127

Tabela 2. Porcentagem de variação explicada (R^2), $F_{calculado}$ value and $F_{tabelado}$ para as respostas densidade aparente e atividade de água (Aa) utilizando análise de variância (ANOVA).

pH	Resposta	R^2 (%)	$F_{calc.}$	$F^*_{tabel.}$	Equação
3,0	Densidade aparente	91	27,68	4,07	$y_1 = 0,52x_1 + 0,44x_2 + 0,38x_3 - 0,21x_1x_3$
	Aa	95	32,32	4,12	$y_2 = 0,82x_1 + 0,81x_2 + 0,64x_3 - 0,27x_1x_2 - 0,20x_1x_3$
4,5	Densidade aparente	96	30,90	4,39	$y_3 = 0,54x_1 + 0,50x_2 + 0,46x_3 - 0,22x_1x_2 - 0,35x_1x_3 - 0,25x_2x_3$
	Aa	96	37,77	4,12	$y_4 = 0,81x_1 + 0,81x_2 + 0,65x_3 - 0,21x_1x_2 - 0,24x_1x_3$
6,0	Densidade aparente	95	32,43	4,12	$y_5 = 0,60x_1 + 0,53x_2 + 0,54x_3 - 0,35x_1x_2 - 0,23x_2x_3$
	Aa	96	32,40	4,12	$y_6 = 0,81x_1 + 0,81x_2 + 0,68x_3 - 0,21x_1x_2 - 0,24x_1x_3$

*nível de significância 5%; x_1, x_2, x_3 : variáveis independentes para concentração de sacarose, xarope de glicose e açúcar invertido, respectivamente.

A Figura 1 mostra as curvas de contornos dos modelos quadráticos (Equações y_1, y_2, y_3, y_4, y_5 e y_6) do delineamento de misturas de açúcares para as respostas de densidade (g/mL) e de Aa das espumas obtidas em pH 3,0, 4,5 e 6,0.

Observa-se que a densidade aparente das espumas depende do pH, onde a menor densidade aparente foi obtida no pH 3,0 e 4,5 e a maior densidade aparente no pH 6,0 (Figura 1a, 1c e 1e). Em pH abaixo do pI (~ 4,5), as proteínas sofrem desnovelamento parcial, o que resulta no aumento da flexibilidade e de grupos hidrofóbicos na superfície, aumentado a natureza anfifílica das proteínas, melhorando as suas capacidade de aeração (LIANG; KRISTINSSON, 2005).

No pH 3,0 (Figura 1a), o aumento da proporção do açúcar invertido diminuiu a densidade aparente da espuma enquanto o aumento da proporção de sacarose aumentou a densidade aparente da espuma. Porém, a interação entre os dois tipos de açúcares diminuiu a densidade aparente.

O aumento da proporção de açúcar invertido diminuiu a densidade aparente da espuma e o aumento da proporção de sacarose e xarope de glicose aumentou a densidade aparente, no pH 4,5. A interação entre os três tipos de açúcares diminuiu a densidade aparente (Figura 1c).

No pH 6,0, o aumento da proporção de sacarose aumentou a densidade aparente da espuma e a interação entre sacarose e xarope de glicose e entre açúcar invertido e xarope de glicose diminuíram a densidade aparente (Figura 1e).

A capacidade de aeração depende do tipo de açúcar utilizado. O açúcar invertido apresenta viscosidade menor do que xarope de glicose, facilitando a incorporação de ar e dessa forma diminuindo a densidade da espuma.O xarope de glicose possui viscosidade maior devido a presença de oligossacarídeos (cadeias longas), formando espumas com maior densidade. O aumento da proporção de sacarose na solução de açúcares a 80 ºBrix, aumenta a possibilidade de cristalização da sacarose resultando no aumento da densidade da espuma. A interação entre sacarose, xarope de glicose e açúcar invertido aumenta a solubilidade da solução, evitando a cristalização da sacarose e facilitando a incorporação de ar.

A Aa diminuiu com o aumento da proporção de açúcar invertido e aumentou com o aumento da proporção de sacarose e xarope de glicose nos pH 3,0, 4,5 e 6,0 (Figura 1b, 1d, 1f).

O açúcar invertido é uma mistura de mono e dissacarídeos onde o maior número de moles do soluto leva a diminuição da atividade de água das espumas. Por outro lado, o xarope de glicose é uma mistura de oligossacarídeos, dissacarídeos e monossacarídeos, onde os oligossacarídeos são solutos de alta massa molecular, que aumentam a Aa das espumas. A maior massa molecular conduz a menor número de moles do soluto aumentando o valor de Aa (GRANT, 2004)

A solução de sacarose a 80° Brix encontra-se supers aturada resultando na cristalização do açúcar na temperatura ambiente. Dessa forma, ocorre a diminuição a concentração de sacarose na fase líquida, conduzindo ao aumento do valor de Aa das espumas (WILLS, 1998; ZAMORRA & CHIRIFE, 2006).

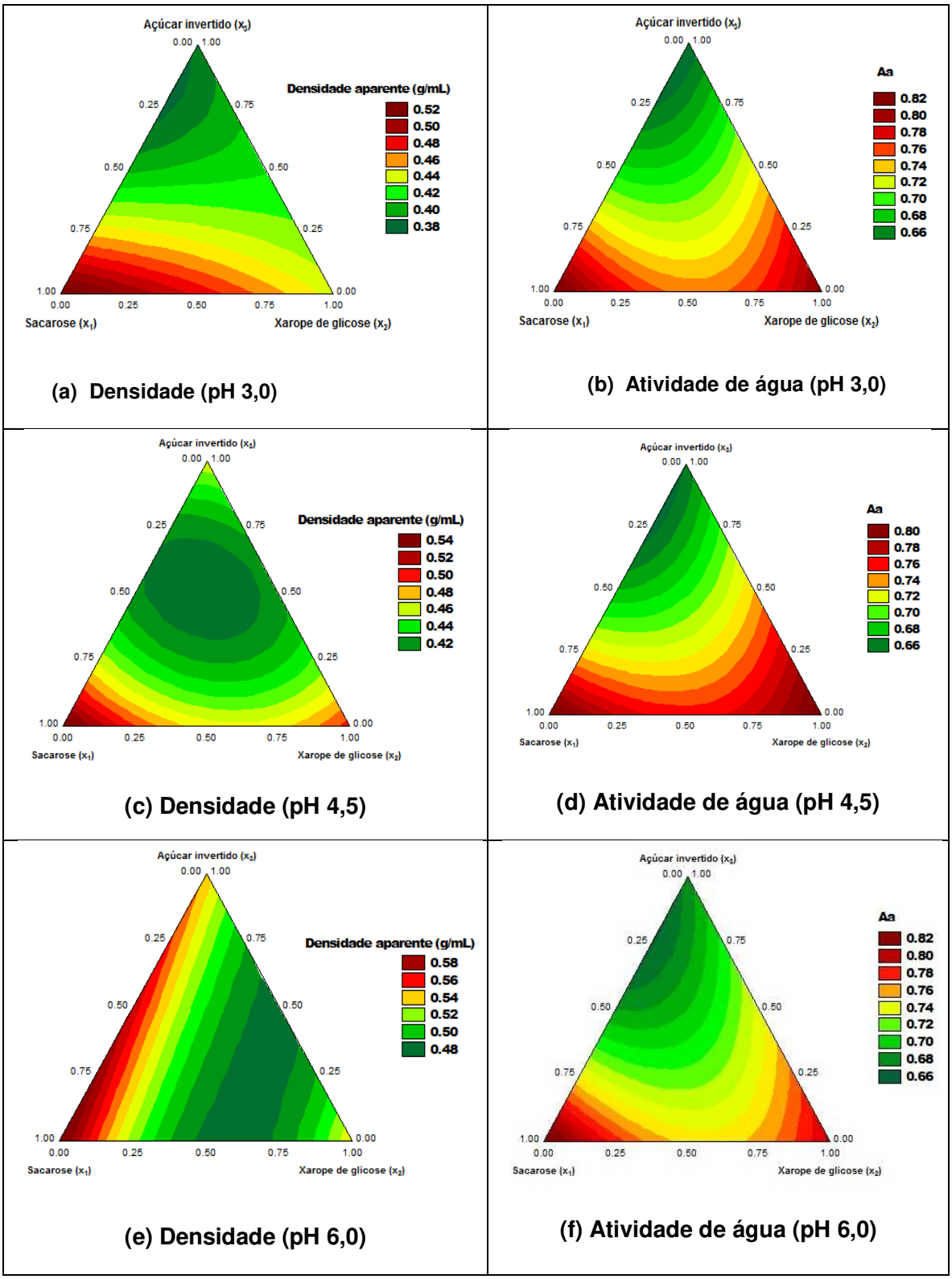


Figura 1. Curvas de contorno para as respostas densidade aparente (g/mL) e atividade de água (Aa) nos pH 3,0 (a, b), 4,5 (c, d) e 6,0 (e,f).

Para a definição da mistura de açúcares, os critérios para a obtenção de amostra aerada foram densidade menor que 0,50 g/mL (JACKSON, 1995) e Aa abaixo de 0,75, pois abaixo deste valor, a deterioração bacteriana é sujeita às bactérias halófilas, no caso de alimentos salgados e à deterioração do produto, geralmente lenta, por bolores xerófilos ou leveduras osmófilas (não patogênicos) (CHRISTIAN, 1980). A análise das curvas de contorno (Figura 2) mostra a mistura de açúcares com 42,5% sacarose, 42,5% xarope de glicose e 15% de açúcar invertido é adequada para ser utilizada como sistema modelo para produtos aerados tipo *marshmallow.*

4. Conclusão

No delineamento experimental de mistura, o pH influenciou a capacidade de aeração das misturas dos açúcares (sacarose, xarope de glicose e açúcar invertido). Menores densidades aparentes foram obtidas no pH 3,0 e 4,5 e a maior densidade aparente no pH 6,0. A Aa diminuiu com o aumento da proporção de açúcar invertido e aumentou com o aumento da proporção de sacarose e xarope de glicose nos pH 3,0, 4,5 e 6,0.

A mistura de açúcares em 42,5% sacarose, 42,5% xarope de glicose e 15% de açúcar invertido foi definida como sistema modelo de açúcares que mostrou-se adequada para obtenção de produtos aerados tipo *marshmallow*. Nestas condições, a espuma apresentou densidade menor que 0,50 g/mL e Aa abaixo de 0,75.

Agradecimentos

Os autores agradecem à FAPESP pelo auxílio financeiro (FAPESP 2011/50067/9) e a EMBRAPA pela bolsa de doutorado concedida a autora SADAHIRA MS.

5. Referências bibliográficas

BARROS NETO, B..; SCARMINIO, I.S.; BRUNS, R.E. **Como fazer experimentos. Campimas:** Editora da UNICAMP, 2001.

CAMPBELL, M.G.; MOUGEOT, E. Creation and characterization of aerated food products. **Trends in Food Science ; Technology,** v.10, p. 283-296,1999.

CHINACHOTI, P. Carbohydrates: functionality in foods. **Am. J. Clin. Nutr.**, v, 61, p. 922S-929S, 1995.

DAMODARAN, S.. Protein Stabilization of Emulsions and Foams. **Journal of Food Science**, v.*70, n.*3, R54–R66, 2005.

CHRISTIAN, J.H.B. Reduced water activity. In SILIKER, J.H. et al. (Ed.) **Microbiol Ecology of Foods, Vol. 1 – Factors affecting life and death of microorganism**.New York: Academic Press, 1980. p. 79-90.

DICKINSON, E. **An introduction in food colloids. Oxford:** Oxford Univ. Press. 1992.

ERGUN, R.; LIETHA, R.; HARTEL, R.W. Moisture and Shelf Life in Sugar Confections. **Critical Reviews in Food Science and Nutrition**, v, 50, n. 2, p. 162-192, , 2010.

ESSE, R.; SAARI, A. Shelf-life and moisture management. In Steele, R. (Ed.), **Understanding and measuring the shelf-life of food**. Cambridge: Woodhead Publishing Limited. 2004. p. 24-41.

GRANT, W.D. Life at low water activity. **Phylosophical of Transactions of Royal Society**, v. 359, p.1249–1267, 2004.

JACKSON, E.B. **Sugar Confectionery Manufacture.** London: Black Academic and Professional. 1995.

JAMES, D. Sugar. In: Sugar confectionery manufacture. In JACKSON, E. B. (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional. 1995. p. 298–311.

KINSELLA, J.E. Functional properties of protein: possible relationships between structure and functionin foams. **Food Chemistry,** v.7, p. 273-288, 1981.

LABUZA, T.P.; ALTUNAKAR, B. Water activity prediction and moisture sorption isotherms. In BARBOSA-CÁNOVAS, G.V.; FONTANA Jr; A.J.; SCHMIDT, S.J.; Labuza, T.P. (Ed.). **Water activity in foods** . Iowa: Blackwell Publishing Professional. 2007. p. 109-171.

LAU, K.; DICKINSON, E. Structural and rheological properties of aerated high sugar systems containing egg albumen. **Journal of Food Science,** v.69, n.5, p. E232-E239, 2004.

LIANG, Y.; KRISTINSSON, H. Influence of pH-Induced Unfolding and Refolding of Egg Albumen on Its Foaming Properties. **Journal of Food Science**, v.70, n. 3, C222-C230, 2005.

STANSELL, D. Caramel, toffee and fudge. In: Sugar confectionery manufacture. In JACKSON, E. B. (Ed.). **Sugar Confectionery Manufacture**. London: Black Academic and Professional. 1995. p. 170–188.

THAKUR, R. K.; VIAL, C.; DJELVEH, G. Effect of pH of food emulsions on their continuous foaming using a mechanically agitated column. **Innovative Food Science and Emerging Technologies,** v. 7, p. 203-210, 2006.

WILLS, D. Water activity and its importance in making candy. **The Manufacturing Confectioner**, v.78, n.8, p.71-74, 1998.

ZAMORA, M. C.; CHIRIFE, J. Determination of water activity change due to crystallization in honeys from Argentina. **Food Control**, v. 17, p. 59-64, 2006.

CAPÍTULO 5. EFFECT OF EGG WHITE PROTEIN-PECTIN ELECTROSTATIC INTERACTION IN A HIGH SUGAR CONTENT SYSTEM ON FOAMING AND FOAM RHEOLOGICAL PROPERTIES

To be submitted to Food Hydrocolloids, ISSN: 0268-005X

Capítulo 5. Effect of egg white protein-pectin electrostatic interaction in a high sugar content system on foaming and foam rheological properties

Mitie S. Sadahira[a,*], Maria I. Rodrigues[b], Mahmood Akhtar[c], Brent S. Murray[c], Flavia M. Netto[d,*]

[a]Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP Campinas, Brazil; E-mail: mitie@ital.sp.gov.br
[b]Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brasil; E-mail: protimiza@protimiza.com.br
[c] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK; E-mail: B.S.Murray@leeds.ac.uk; M.Akhtar@food.leeds.ac.uk
[d]Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/ UNICAMP, Rua Monteiro Lobato n° 80 - CEP: 13.083-970, Campinas, Brazil; E-mail: fernanda.rzls@gmail.com; E-mail: flavia@fea.unicamp.br

Abstract

The aim of this study was to evaluate the effect of electrostatic interaction between egg white protein (EW) and pectin in a high sugar content system (80 wt% total solid) on the foaming capacity (density and overrun) and foam rheological properties. A central composite rotatable design was carried out to study the effects of biopolymer concentration (1.40–5.60%, w/w) and EW:pectin ratio (7:1–63:1) on the apparent viscosity before whipping, foaming capacity and foam rheological properties (storage modulus G', loss modulus G" and phase angle δ) of sugar/EW/pectin mixtures at pH 3.0. The apparent viscosity increased as biopolymer concentration increase while EW:pectin ratio had no significant effect ($p>0.10$) on this response. At 7:1 EW:pectin ratio, the mixture presented low foaming capacity resulting in foam with less solid character and low stability possibly due to the pectin excess in the system. At 49:1 EW:pectin ratio, the mixture showed higher foaming capacity and foam elasticity. Possibly, the formation of soluble complexes between EW and pectin increased the continuous phase viscosity that enhanced the foam stability related to liquid drainage.

Keywords: Sucrose, glucose syrup, invert sugar, electrostatic complexes, rheology

Abbreviations: EW, egg white protein; ANOVA, analysis of variance; CCRD, central composite rotatable design; G', storage modulus; G", loss modulus; δ, phase angle; R^2, percentage of variance explained.

1. Introduction

Food foam is formed by air, liquid and surface-active agent such as proteins (Kinsella, 1981). The formation of air bubbles modifies the texture and the rheological properties of aerated food (Campbell & Mougeot, 1999).

In the confectionery industry, aeration is used to obtain products such as nougat, marshmallow, chews and pulled sugar. The density of these products varies between 0.2 and 1.0 g/mL. In aerated confectionery, foams are produced by aeration of a mixture of sugar syrup and proteins. Egg white protein (EW) is the most used surface active agent to produce marshmallow and nougat (Jackson, 1995). Polysaccharides are also be used due to their thickening and gelling properties; their addition can improve foam stability because they control the rheology and network structure of the continuous phase (Dickinson, 2003, 2008).

Pectin is a carboxylated anionic polysaccharide with high molecular weight. Its functional properties depend on the degree of esterification (DE). High-methoxyl pectins (≥0.50% DE) require high sugar concentration and low pH to form gels, whereas low-methoxyl pectins form gels in the presence of calcium (Dickinson, 2003; Akhtar *et.al.*, 2002).

Proteins and polysaccharides contribute to food structural and textural properties due to their aggregation and gelation properties (Benichou et al., 2007). Mixture of polysaccharide and protein solution can exhibit one of the three behaviors: miscibility, complex coacervation and thermodynamic incompatibility. Miscibility usually occurs at low biopolymer concentrations. Coacervation takes place due to attractive interactions between protein and polysaccharide leading to the formation of soluble and/or insoluble complexes. Thermodynamic incompatibility results in a separation into two distinct phases, due to the limited thermodynamic compatibility between proteins and polysaccharides in aqueous solution (Dickinson, 2003; Doublier et al., 2000; Rodríguez Patino & Pilosof, 2011).

Electrostatic interaction between positively charged protein ($pH < pI_{protein}$ – isoelectric point) and negatively charged polysaccharide ($pH > pKa_{polysaccharide}$) can result in soluble and/or insoluble complexes formation (Benichou et al., 2007; Dickinson, 2008). The physicochemical parameters that influence electrical charge of protein and polysaccharide and the electrostatic complexes formation are pH, ionic strength,

temperature, protein:polysaccharide ratio and total biopolymer concentration(Schmitt & Turgeon, 2011). Studies have shown that the electrostatic interaction between pectin (pKa ~ 2.9-3.5) and EW (pI ~ 4.5-4.9) (Ibanoglu & Erçelebi, 2007; Surh, Decker, & McClements, 2006; Ralet, Dronnet, Buchholt, & Thibault, 2001; Sadahira, Lopes, Rodrigues, & Netto, 2014) are effective in increasing foam stability in aqueous solution.

The aim of this study was to evaluate the effect of electrostatic interaction between EW and pectin in a high sugar content system on the foaming and foams rheological properties.

2. Materials and methods

2.1. Materials

Sucrose (Tate & Lyle, UK) was purchased from the local supermarket. Glucose syrup (40 D.E., 83 wt% total solid) and invert sugar syrup (80 wt% total solid) were kindly donated by Brenntag UK & Ireland (Leeds, UK) and by British Sugar (Peterborough, UK), respectively. These sugars were used to prepare the multicomponent models systems of sugars. Dried egg white protein (EW) was supplied by Saltos Alimentos LTDA (Salto, Brazil) and low methoxyl pectin (GENU Pectin type LM CG-22, degree of esterification 47,2%, molecular weight 90 kDa) by CPKelco (Grossenbrode, Germany) were used to prepare the biopolymer blends. EW presented, in wet basis, 79.9 $\pm$1.2% of protein 10.20 $\pm$ 0.02% of moisture and 5.64 ± 0.22% ash, determined according to methodologies described by AOAC (2010). The SDS-PAGE analysis of EW (Laemmli, 1970) showed the eletrophoretic profile with bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively. The other chemicals were of analytical grade and Milli-Q water was used in all experiments.

2.2. Preparation of solutions and foams

According to our previous study (thesis chapter 4), the composition of sugar mixture used as a model system to evaluate the foaming and rheological properties in

aerated products was sucrose (42.5 wt% total sugar solid), glucose syrup (42.5 wt% total sugar solid) and invert sugar (15 wt% total sugar solid). This sugar mixture resulted in foams with characteristics similar to aerated confectionery such as marshmallow: density between 0.25 g/mL and 0.50 g/mL and water activity range 0.778 - 0.665 (Jackson, 1995; Wills, 1998).

Sugars mixture (500 g) was heated in hot plate stirrer to reach 80 wt% total solid then was cooled to beating temperature, 70 ℃. The biopolymers, in appropriate amounts to each trial condition (Table 1), were hydrated together in 36 g of water under magnetic stirring for 1 h at room temperature. The pH was adjusted to 3.0 with 4 mol L^{-1} citric acid.

The sugars mixture (at 70 ℃) and EW/pectin blend were mixed in a Kitchen Aid 5KPM5 stand mixer (Havant, UK) with a flat beater for 1 min at speed setting 4. Then, the sugar/EW/pectin mixture was whipped using a whisk beater operating at speed setting 10 under atmospheric pressure for 6 min.

A Central Composite Rotatable Design CCRD (2^2 factorial design with 4 trials under the axial conditions and 3 repetitions at the central point) totaling 11 trials (Table 1) (Rodrigues & Iemma, 2012) was carried out to evaluate the effect of total biopolymer concentration (w/w%) and EW:pectin ratio (w/w) on apparent viscosity of sugar/EW/pectin mixture before whipping, foaming capacity (density and overrun) for fresh foam and rheological properties (G', G" and δ at 1 Hz) for fresh foam and foam aged for 24 h. From the results, second-order models were obtained and evaluated statistically by analysis of variance (ANOVA) using the software Statistica 7.0 (Statsoft, USA).

To evaluate the effect of EW:pectin ratio on foaming properties, trials were carried out under the best experimental conditions obtained from CCRD to obtain good foamability (low density, high overrun) and solid character (high G' value, low δ value) (total biopolymer concentration, 80% total solid, 70 ℃ and pH 3.0) at different EW:pectin ratio. The results were analyzed for differences between means by Student t test ($p < 0.05$). The model validation was performed under the same conditions.

2.3. Foaming properties

2.3.1. Foaming capacity: Density and Overrun

Foaming capacity was studied by measuring density and overrun. Cylindrical containers (35.43 $\pm$ 0.21 mL) were carefully filled up with foam. The top of the container was leveled with a metal spatula to achieve uniform and plane surfaces to obtain constant volume. The foam weight of foam was recorded and then the foam density was determined.

The density was determined by Equation 1:

Density (g/mL) = mf / volume of cylindrical container (1)

The overrun is defined by Equation (2) (Lau & Dickinson, 2004).

$$\text{overrun (\%)} = 100 \times [m_i - m_f] / m_f \qquad (2)$$

where m_i is the mass of the initial solution (unwhipped sample) and m_f is the mass of the whipped sample with the same volume of m_i.

2.3.2. Bubble size distribution

Microscopic observations of the EW/pectin in high sugar content system foams were obtained using a Leica confocal scanning laser microscope (model TCS SP2, Heidelberg, Germany) equipped with an Ar/HeNe laser and 10x objective lens (HC PL APO CS 20 × 0.7 DRY). The fluorescence dye Rhodamine B (0.1% in water), purchased from Aldrich (Dorset, UK), was used to stain the protein. Rhodamine B was excited at 50% of maximum absorption at 488 nm, and the detection bandwidth was set from 500 to 600 nm. Fresh foam sample was placed into well and then the dye (0.1 mL) was added. The well was covered with a cover slide and the images were recorded after 24 h.

Bubble size distributions were obtained by analyzing the CLSM images using Image J software. For each sample, 1000 bubbles were measured. Mean bubble size was characterized by average bubble diameter d_{32} defined by Eq.3.

$$d_{32} = \sum_i di^3 / \sum_i di^2 \qquad (3)$$

2.3.3. Liquid drainage

Foam samples were poured in plastic containers and kept in closed plastic box at 25 ℃. The liquid drainage was followed during 10 days by visual observation and registered by photographs.

2.4. Rheology

The rheological properties were measured using a controlled stress rheometer (Kinexus, Malvern Instruments Limited, Worcestershire, UK) and a parallel-plate geometry (65 mm flat plate) at 25 ℃. The analysis were carried out in 3 repetitions.

Apparent viscosity of sugar/EW/pectin mixture before whipping was measured over a range of shear rate (0.5 to 100 s^{-1}), using a gap of 1 mm, according to previous studies with glucose syrup and honey (Schellart, 2011).

The viscoeslatic modulus (storage modulus G', loss modulus G" and phase angle δ) of foam were measured under small oscillatory deformation and the gap was 3 mm. The gap was selected to avoid crushing or destroying of the gas bubbles (Zmudzinsk et al., 2014). Stress sweep tests were carried out at a frequency of 1Hz to determine the linear viscoelasticity region in oscillatory shear. Then, the foam samples were also subjected to a frequency sweep from 0.1 to 10 Hz at constant strain amplitude within the linear viscoelastic region of each sample. All the measurements were carried out immediately after the foam preparation and after 24 h of storage at 25 ℃. For each trial, the measurements were performed in triplicate.

3. Results and discussion

3.1. Apparent viscosity, foaming capacity and rheological properties of high sugar system/EW/pectin mixtures

In order to evaluate the foaming capacity and foam rheological properties of high sugar content system with EW and pectin, a CCRD was carried with independent variables total biopolymer concentration and EW:pectin ratio at pH 3.0. From the results showed in Table 1, the regression coefficients were calculated and mathematical models were built

for the responses apparent viscosity of sugar/EW/pectin mixtures before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties (G', G" and δ at 1 Hz) for fresh foam and foam aged for 24 h. The shear rate range is 10 – 10^3 s^{-1} for mixing process in food industry (Steffe, 1996). ANOVA was used to evaluate the adequacy of the fitted model (Table 2).

The viscosity-shear rate relationship for sugar/EW/pectin mixtures before whipping and frequency sweep data for fresh foam and foam aged for 24 h from trials of Table 1 are shown in Fig. A1 and Fig. A2, A3, A4, A5 (Annexe A), respectively.

The flow curves of sugar/EW/pectin mixtures before whipping were fitted to a power-law model, $\sigma = K D^n$. Where σ is the shear stress, *K* and *n* (power law index) are fitted parameters. The values of the power-law index, *n,* are shown in Table A1 (Annexe A). For Trials 1, 3, 5, 7, 8, 9, 10 and 11, the *n* value were close to unity. Trials 2, 4, 6 were thinning shear fluids ($n < 1$).

Table 1. Design matrix for CCRD with independent variables biopolymer concentration, EW:pectin ratio and results for responses apparent viscosity (η) of sugar/EW/pectin mixture before whipping at 10 s^{-1}, density (ρ), overrun, rheological properties (G', G" and δ, at 1 Hz) for fresh foam and foam aged for 24 h, at pH 3.0.

Trial	Total Biopol. conc. (%w/w)	EW:pectin ratio	η (Pa.s)	ρ (g/mL)	Overrun (%)	Fresh foam			Foam aged for 24 h		
						G' (Pa)	G" (Pa)	δ (°)	G' (Pa)	G" (Pa)	δ (°)
	x_1	x_2	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9
1	-1 (2.00)	-1 (15:1)	7.69	0.48	137.9	2075.5	1509.5	36.61	878.6	1365.0	57.23
2	1 (5.00)	-1 (15:1)	26.05	0.56	125.5	751.8	868.9	49.29	455.3	762.2	59.2
3	-1 (2.00)	1 (55:1)	5.69	0.38	229.5	6003.0	2053	19.04	1233.3	1033.4	39.98
4	1 (5.00)	1 (55:1)	23.81	0.44	175.1	3258.5	1646	27.49	1263.3	1072.8	40.36
5	-1.41 (1.40)	0 (35:1)	5.2	0.45	177.7	1873.5	1221	34.38	674.5	1067.3	57.74
6	1.41 (5.60)	0 (35:1)	22.34	0.44	180.5	2524.0	1473.5	30.84	1109.5	1131.0	45.59
7	0 (3.50)	-1.41 (7:1)	8.37	0.64	95.9	368.4	598.1	58.49	411.9	662.0	58.1
8	0 (3.50)	1.41 (63:1)	10.42	0.36	234.1	7105.0	2576.0	20.27	1751.7	1351.0	37.63
9	0 (3.50)	0 (35:1)	8.66	0.37	210.5	6292.5	2450.0	22.25	1801	1588.3	41.4
10	0 (3.50)	0 (35:1)	8.91	0.37	211.3	5526.5	1915.0	19.54	1500.8	1192.1	38.25
11	0 (3.50)	0 (35:1)	8.71	0.36	220.1	5686.5	2159.5	21.52	1482.3	1186.4	38.62

( ) true values of the independent variables for each level; Total Biopol. conc.: total biopolymer concentration; G': elastic modulus; G": viscous modulus; δ: phase angle.

Foams aged for 24 h presented G" values in a narrow range (1033.4 to 1588.3 Pa), within the experimental error, with exception of Trials 2 and 7, with lower values. Therefore, for this response, the values of R^2 and $F_{calculated}$ indicate that is not possible to obtain a model and contour curve (Table 2).

The wide range of values for responses apparent viscosity, density, *overrun*, G' and δ of fresh and 24 h aged foams indicate that independent variables affect significantly the responses. The R^2 and $F_{calculated}$ (Table 3) are adequate to obtaining a second order model for: apparent viscosity before whipping, foaming capacity (density and overrun) and rheological properties (G', G" and δ) for fresh foam and foam aged for 24 h as a function of total biopolymers concentration (x_1), EW:pectin ratio (x_2), within the experimental conditions studied (Equations $y_{1\text{-}4}$, $y_{6\text{-}7}$, y_9).

Table 2. Percentage of variance explained (R^2), calculated F ($F_{calc.}$) value and tabulated F ($F_{tab.}$) for the responses apparent viscosity (η) of sugar/EW/pectin mixtures before whipping, density (ρ), overrun, rheological properties (G', G" and δ) for fresh foam and foam aged for 24 h, by analysis of variance (ANOVA).

Response		R^2 (%)	$F_{calc.}$	$F^*_{tab.}$	Equation
Apparent viscosity (η) (Pa.s)		88.0	29.43	4.46	$y_1 = 10.04 + 7.60x_1 + 3.18x_1^2$
Density (ρ) (g/mL)		90.0	20.49	4.35	$y_2 = 0.37 + 0.03x_1^2 - 0.08x_2 + 0.07x_2^2$
Overrun (%)		90.0	21.34	4.35	$y_3 = 213.98 - 18.76x_1^2 + 42.13x_2 - 25.85x_2^2$
Fresh foam	G' (Pa)	89.0	18.87	4.35	$y_4 = 5834.88 - 1810.93x_1^2 + 1997.55x_2 - 1037.36x_2^2$
	G" (Pa)	81.0	10.00	4.35	$y_5 = 2174.61 - 401.96x_1^2 + 515.22x_2 - 281.36x_2^2$
	δ (°)	90.0	20.84	4.35	$y_6 = 21.11 + 5.03x_1^2 - 11.69x_2 + 8.44x_2^2$
Foam aged for 24h	G' (Pa)	87.0	15.01	4.35	$y_7 = 1594.82 - 359.97x_1^2 + 382.62x_2 - 264.50x_2^2$
	G" (Pa)	30.0	---	---	No regression coefficient was statistically significant ($p > 0.10$)
	δ (°)	90.0	20.1	4.35	$y_9 = 39.42 + 6.00x_1^2 - 8.14x_2 + 4.09x_2^2$

x_1, x_2 : coded independent variables for total biopolymer concentration and EW:pectin ratio, respectively. . ---:there is no regression coefficient.

Equations showed in Table 2 were used to generate the contour curves for the dependent variables (Figure 1). The apparent viscosity of sugar/EW/pectin mixture before whipping increased with increasing biopolymer concentration. The EW:pectin ratio had no significant effect ($p>0.10$) on this response (Fig. 1a). At biopolymer concentration between 3.0 and 4.0% (w/w) and EW:pectin ratio from 40:1 to 63:1, the mixtures showed high foaming capacity (low density and high overrun value), and the foams, elastic and solid behavior. On the other hand, increasing relative pectin concentration (EW:pectin ratio < 35:1) the foaming capacity decreased and the foam became less elastic (low value of G')

and solid (high value of δ) (Stanley, Goff, & Smith, 1996; Thakur, Vial, & Djelveh, 2008). Possibly, EW:pectin ratio lower than 35:1 leads to formation of insoluble complexes between the biopolymers resulting in reduced protein availability, which may hinder the incorporation of air (Schmidt et al., 2010). In the region of low density and high overrun (Fig 1b, Fig 1c), G' and δ values of fresh and 24 h aged foams were higher (5000–6000 Pa) and lower (20–24°), respectively, compared to the high density and low overrun regions (Fig. 1d, Fig 1e, Fig. 1f, Fig. 1g). Thus foam elastic behavior enhances air incorporation.

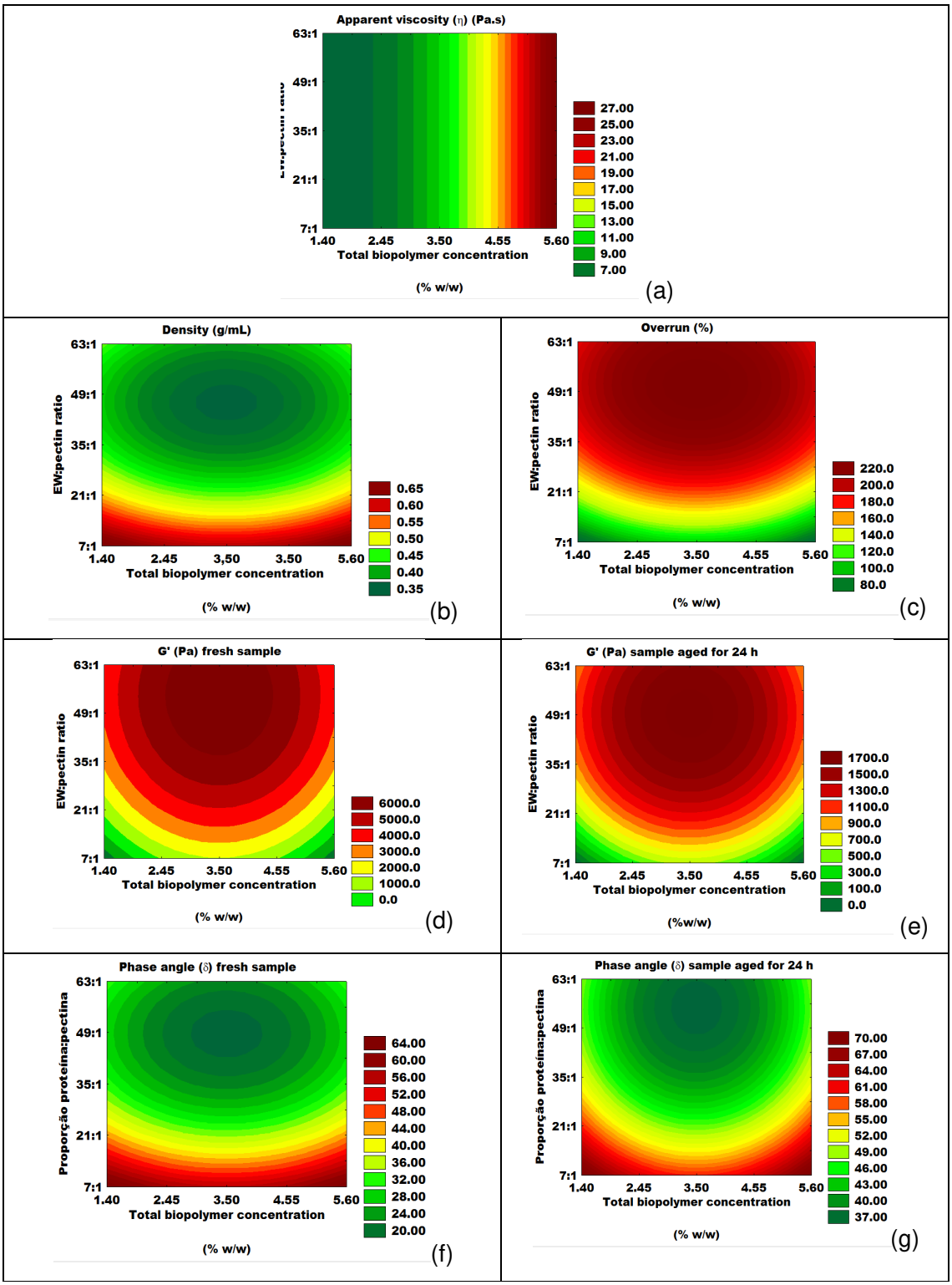


Fig. 1. Contour curves for the dependent variables apparent viscosity (η) of mixture of sugars and biopolymers (y_1) before whipping (a), density (y_2) (b), *overrun* (y_3) (c), rheological properties of fresh sample G' (y_4) (d) e δ (y_6) (f) and sample aged for 24 h G' (y_7) (e) and δ (y_9) (g).

The contour curves (Fig. 1) were analyzed and to obtain foam with good properties (low density, high overrun, high G' value, low δ value) the best conditions were total biopolymer concentration 3.5% w/w and EW:pectin ratio 49:1.

3.2. Effect of EW:pectin ratio on foaming properties

At pH 3.0, the pH is bellow the EW pI (4.5) and above the pectin pKa. Thus, at this pH, negative charge of pectin may interact with positive charge of protein leading to electrostatic complexes formation. Since protein:polysaccharide ratio is one of the parameters that influences complexes formation (Schmitt & Turgeon, 2011), to evaluate the EW:pectin ratio effect on foaming and rheological properties, experiments with total biopolymer concentration 3.5 w/w% and EW:pectin ratio of 7:1 (Trial A) or 49:1 (Trial B) were carried out. Model validation was carried out under these conditions and the results are shown in Table 3.

Most of the results were close to predicted values. However, for foam under Trial A condition (EW:pectin ratio 7:1), relative errors for G' values of fresh foam and foam aged for 24 h were 35 and 192%, respectively. The model did not fit at low G' value (327.1 and 391.8 Pa.s), possibly due to high instability of the foam, which caused difficulties to analyses performing.

The apparent viscosity of sugar/EW/pectin mixtures did not exhibit statistically significant difference ($p>0.05$) between Trial A and B. This result corroborates with a previous finding of this study, that EW:pectin ratio had no effect on the mixture viscosity (Table 2 and Figure 1a).

Table 3. Predicted values (Pred.), experimental values (Exp.) and relative error (RE = (Exp. – Pred.)/Exper.*100)) for the responses apparent viscosity at 10 s^{-1} of sugar/EW/pectin mixture (total biopolymer concentration 3.5 w/w% and EW:pectin ratio of 7:1 (Trial A) or 49:1 (Trial B)) before whipping, and foam density, *overrun,* rheological properties (G', G" e δ at 1 Hz) of fresh and aged for 24 h foam.

Trial	η (Pa.s)	Density (g/mL)	*Overrun* (%)	Fresh foam			Foam aged for 24h		
				G' (Pa)	G" (Pa)	δ (°)	G' (Pa)	G" (Pa)	δ (°)
A (Exp.)	9.15 ± 1.10[a]	0.66 ± 0.03[a]	91.6 ± 8.6[a]	327.1 ± 58.3[a]	556.8 ± 58.3[a]	60.34 ± 1.60[a]	391.8 ± 18.6[a]	583.2 ± 74.9[a]	55.98 ± 2.13[a]
A (Pred.)	10.4	0.62	103.2	956		54.37	529.5		59.03
A (RE %)	-9.7	5.8	-12.6	-192.3		9.9	-35.1		-5.4
B (Exp.)	9.40 ± 1.64[a]	0.37 ± 0.00[b]	198.9 ± 8.8[b]	6894.7 ± 509.8[b]	2323.8 ± 189.7[b]	18.53 ± 0.25[b]	1595.1 ± 259.2[b]	1089.5 ± 127.4[b]	34.41 ± 1.91[b]
B (Pred.)	10.4	0.35	230.8	6724.9		17.06	1733.1		35.73
B (RE %)	6.8	5.9	-16.0	2.5		7.9	-8.6		-3.8

Values are mean ± SD of triplicates, except to η, G' and G" of fresh sample that are mean ± SD of duplicates. For the same response, mean with different small letters in the same column differ significantly ($p < 0.05$) by Student t test; apparent viscosity of sugar/biopolymers mixture (η), Density (ρ), *overrun,* rheological properties of fresh sample and sample aged for 24 hours (elastic modulus G', viscous modulus G" and phase angle δ).RE = Relative error (%).

Under Trial A conditions, the foaming capacity was lower than at Trial B. Possibly, under Trial A conditions (EW:pectin ratio 7:1) there is an excess of negative charge from pectin (thesis chapter 2), inhibiting the building of a protein layer at interface. The low overrun obtained under Trial A conditions indicates reduced air bubbles formation, which resulted in a viscous behavior (G" > G'), and low solid character (low δ value) (Table 3). On the other hand, foam from Trial B (EW:pectin 49:1) presented higher foaming capacity, elastic behavior and solid character.

The bubble size distribution of foams obtained under Trial A and B conditions are showed in Figure 2b and 2e. Foam from Trial A presented wider bubble size distribution than the foam from Trial B. As can be observed in Fig. 2c, foam obtained with lower EW:pectin ratio (Trial A) presented creaming and liquid drainage after 10 days of storage at 25 ºC. Foam prepared under Trial B conditions showed smaller bubble size variation (Fig. 3e) suggesting greater stability than under Trial A conditions and no drainage was observed after 10 days (Fig. 2f). However, foam from Trial B exhibited bimodal bubble size distribution indicating that disproportionation (gas diffusion from smaller bubble to larger bubble) occurred**.**

Sugar/EW/pectin mixtures (Trials A and B) before whipping presented phase separation (Fig. 3). Under Trial A condition (EW:pectin ratio 7:1), it is suggested that complex coacervation did not occur and two layers were formed, the top one opaque containing mainly EW and a clear bottom one containing sugar and pectin due to pectin is in excess. Under Trial B conditions, a turbid bottom layer was obtained, indicating soluble complex formation.

The greater stability of foam from Trial B is possibly because the soluble complexes are dispersed in the sugar syrup, increasing the apparent viscosity of continuous phase and enhancing the foam stability related to liquid drainage (Fig. 2f). Possibly, under the same total biopolymer concentration (3.5% w/w) of Trial A and B, EW:pectin ratio between 15:1 and 35:1 favor the insoluble complexes formation, which may facilitate the development of a viscoelastic interfacial network at the air–water interface. This network, with low gas permeability, may lead to greater stability by hampering disproportionation (thesis chapter 2).

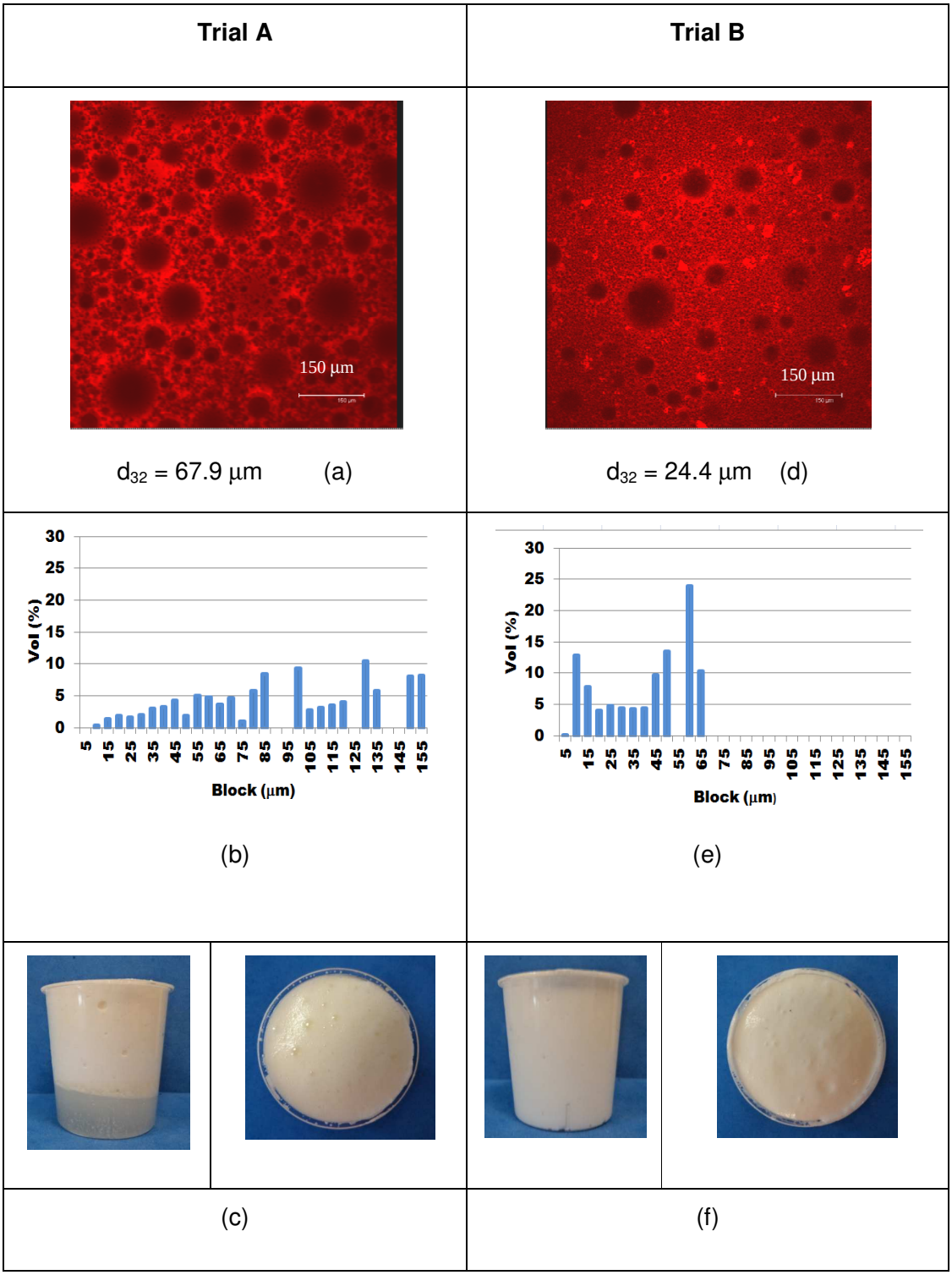


Fig. 2. Confocal microscopy images (after 24h) (a, d), bubble size distribution (b, e) and photographs (after 10 days) (c, f) of foams with total biopolymer concentration 3.5% w/w (80% total solid, 70 ºC and pH 3.0) where Trial A (EW:pectin ratio 7:1) and Trial B (EW:pectin 49:1).

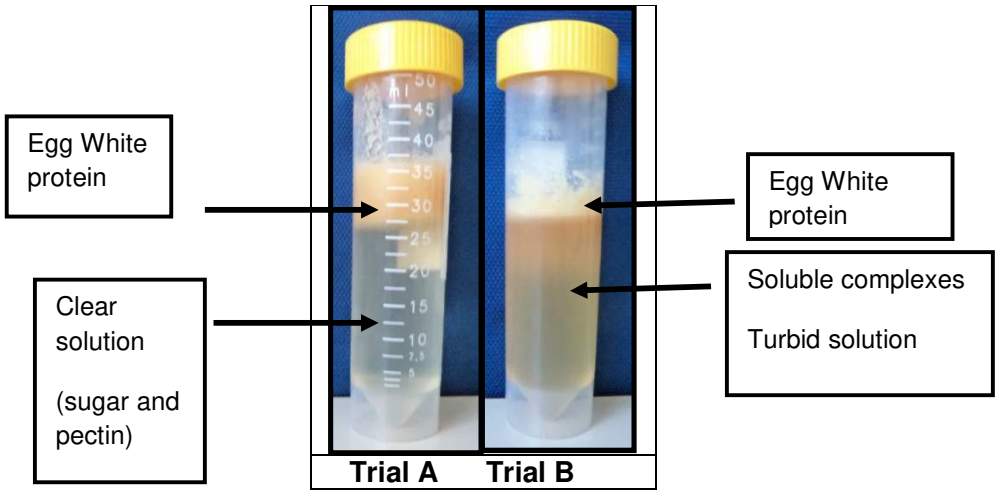


Fig. 3. Phase separation of sugar/EW/pectin mixture before whipping obtained under Trial A and Trial B conditions. Total biopolymer concentration 3.5 w/w% (80% total solid, 70 ℃ and pH 3.0) where Trial A (EW:pectin ratio 7:1) and Trial B (EW:pectin 49:1).

4. Conclusion

At pH 3.0, sugar/EW/pectin mixtures with biopolymer concentration between 3.0 and 4.0% (w/w) and EW:pectin ratio from 40:1 to 63:1 presented high foaming capacity, and the foams, elastic and solid behavior. Increasing pectin concentration (EW:pectin ratio < 35:1) the foaming capacity decreased and the foam became less elastic and solid, within the studied range. At EW:pectin ratio 7:1, the sugar/EW/pectin mixtures before whipping presented phase separation however biopolymers complexation did not occur due to excess of pectin. At this condition, the foaming capacity was low and the foams showed viscous behavior and low stability (creaming and liquid drainage). At EW:pectin ratio 49:1, higher foaming capacity, foam with elastic and solid behaviors and greater stability than at EW:pectin ratio 7:1 was obtained. Formation of soluble complexes between EW and pectin increases the apparent viscosity of foam continuous phase and enhancing its stability related to liquid drainage. Thus, the effect of electrostatic interaction between EW and pectin on foaming and rheological properties in a high sugar content system depend on EW:pectin ratio which are related to electrostatic complexes formation at pH 3.0. Further studies will be required to evaluate the types of electrostatic complexes between 7:1 and 49:1 EW:pectin ratio in a high sugar system to correlate to viscosity of each phase separated of sugar/EW/pectin mixture before whipping, foaming and rheological properties, and foam stability mechanism.

Acknowledgements

The authors thank FAPESP for financial support (FAPESP 2011/50067/9) and EMBRAPA for the doctoral scholarship granted to the author Sadahira MS.

5. References

Akhtar, M.; Dickinson, E.; Mazoyer, J.; Langendorff, V. Emulsion stabilizing properties of depolymerized pectin. *Food hydrocolloids,* 16 , 249-256, 2002.

AOAC. (2010). Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, USA: Association of Official Methods Analytical Chemists.

Benichou, A.; Aserin, A.; Lutz, R.; Garti, N. Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocolloids*, 21, 379–391, 2007.

Campbell, G. M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science & Technology*, *10*, 283–296.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems q. *Food Hydrocolloids*, *17*, 25–39.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter*, *4*, 932–942.

Doublier, J., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, *5*, 202–214.

Ibanoglu, E., & Erçelebi, E. A. (2007). Thermal denaturation and functional properties of egg proteins in the presence of hydrocolloid gums. *Food Chemistry*, *101*(2), 626–633.

Jackson, E. B. (1995). *Sugar Confectionery Manufacture* (pp. 218–235). London: Black Academic and Professional.

Kinsella, J. (1981). Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chemistry*, *7*(4), 273–288.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of thehead of bacteriophage T4. *Nature*, *227*, 680–685.

Lau, K., & Dickinson, E. (2004). Structural and Rheological Properties of Aerated High Sugar Systems Containing Egg Albumen. *Journal of Food Science*, *69*(5), E232–E239.

Ralet, M., Dronnet, V., Buchholt, C., & Thibault, J. F. (2001). Enzymatically and chemically de-esterified lime pectins : characterisation , polyelectrolyte behaviour and calcium binding properties. *Carbohydrate Research*, *336*, 117–125.

Rodrigues, M. I., & Iemma, A. F. (2012). *Experimental Design and Process Optimization* (2 nd.). Campinas: Carita Editora.

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*(8), 1925–1937.

Sadahira, M. S., Lopes, F. C. R., Rodrigues, M. I., & Netto, F. M. (2014). Influence of protein–pectin electrostatic interaction on the foam stability mechanism. *Carbohydrate Polymers*, *103*, 55–61.

Schellart, W.P. (2001). Rheology and density of glucose syrup and honey: Determining their suitability for usage in analogue and fluid dynamic models of geological processes. *Journal of Structural Geology,* 33, 1079-1088.

Schmidt, I., Novales, B., Boué, F., & Axelos, M.A.V. (2010). Foaming properties of protein/pectin electrostatic complexes and foam structure at nanoscale. *Journal of Colloid and Interface Science*, 345, 316 – 324.

Schmitt, C., & Turgeon, S.L. (2011). Protein/polysaccharide complexes and coacervates in food systems. Advances in Colloid and Interface Science, 167, 63–70.

Stanley, D. W., Goff, H. D., & Smith, a. K. (1996). Texture-structure relationships in foamed dairy emulsions. *Food Research International*, *29*(1), 1–13.

Steffe, J. F. (1996). Rheological methods in food process engineering (2nd ed.). EastLansing, MI: Freeman Press.

Surh, J., Decker, E., & Mcclements, D. (2006). Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids*, *20*(5), 607–618.

Thakur, R. K., Vial, C., & Djelveh, G. (2008). Effect of composition and process parameters on elasticity and solidity of foamed food. *Chemical Engineering and Processing*, *47*, 474–483.

Wills, D. (1998). Water activity and its importance in making candy. *The Manufacturing Confectioner*, *78*(8), 71–74.

Zmudzinsk, D., Ptaszek, P., Kruk, J., Kaczmarczyk, K., Roznowski, W., Berski, W., … Grzesik, M. (2014). The role of hydrocolloids in mechanical properties of fresh foams based on egg white proteins. *Journal of Food Engineering*, *121*, 128–134.

CAPÍTULO 6. FOAMING AND RHEOLOGICAL PROPERTIES OF AERATED HIGH SUGAR SYSTEM WITH EGG WHITE PROTEIN AND HYDROXYPROPYMETHYLCELLULOSE

To be submitted to Food Hydrocolloids, ISSN: 0268-005X

Capítulo 6. Foaming and rheological properties of aerated high sugar system with egg white protein and hydroxypropylmethylcellulose

Mitie S. Sadahira[a,*], Maria I. Rodrigues[b], Mahmood Akhtar[c], Brent S. Murray[c], Flavia M. Netto[d]

[a]Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP Campinas, Brazil; E-mail: mitie@ital.sp.gov.br
[b]Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brasil; E-mail: protimiza@protimiza.com.br
[c] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK; E-mail: B.S.Murray@leeds.ac.uk; E-mail: M.Akhtar@food.leeds.ac.uk
[d]Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/ UNICAMP, Rua Monteiro Lobato n° 80 - CEP: 13.083-970, Campinas, Brazil; E-mail: fernanda.rzls@gmail.com; E-mail: flavia@fea.unicamp.br

Abstract

In aerated confectionery, sugars, proteins, and polysaccharide may interact with each other affecting foaming and rheological properties. The objective of this study was to evaluate the effects of the total biopolymer concentration (1.4-5.6% w/w) and EW:HPMC ratio (2:1-18:1) on the apparent viscosity before whipping, foaming capacity (density and overrun) and foam rheological properties (G', G" and δ) of sugar/EW/HPMC mixtures using a central composite rotatable design. The apparent viscosity increased with increasing total biopolymer concentration and decreasing EW:HPMC ratio. Mixtures with low apparent viscosity led to foams which presented liquid drainage whereas those with high apparent viscosity led to lower foaming capacity, and foams with lower G' and higher δ values (less elastic and solid behavior). The conditions to obtain intermediate apparent viscosity, high foaming capacity, elastic and solid behavior were 5.0% w/w total biopolymer concentration and EW:HPMC ratio 14:1. Under these conditions, experiments were carried out to evaluate the effect of interactions between EW and HPMC at pH 3.0, 4.5 and 6.0 on the foaming and rheological properties. The greatest foaming capacity, elastic and solid behavior, with no foam drainage, were obtained at pH 3.0. At pH 4.5, foam presented monodisperse bubble size distribution, leading to better stability than foam at pH 3.0. At pH 6.0, foam showed the poorest foaming properties and viscous behavior. The use of HPMC could be considered to increase the stability of aerated confectionery at pH close to 4.5.

Keywords: foam, rheology, high sugar, elastic behavior, semi solid, stability

Abbreviations: ANOVA, analysis of variance; η, apparent viscosity; d_{32}, average bubble diameter; CCRD, central composite rotatable design; CSLM, Confocal scanning laser microscopy; EW, egg

white protein; pI, isoelectric point; G', elastic modulus; G", viscous modulus; HPMC, hydroxypropylmethylcellulose; δ, phase angle; R^2, explained percentage of variation.

1. Introduction

Food foam is a dispersion of air bubbles in a continuous liquid phase or solid phase, stabilized by surface-active ingredients (Damodaran, 2008). It is a thermodynamic unstable system where drainage, coalescence and disproportionation are the factors that affect its stability. Liquid drainage from lamella film due to gravity leads to coalescence of adjacent bubbles and rupture of lamella film between them. Disproportionation is the diffusion of gas from small to large bubble or to atmosphere. Even in the absence of liquid drainage and coalescence, disproportionation is difficult to prevent because the pressure in a small bubble is greater than in larger ones (Damodaran, 2005; Murray & Ettelaie, 2004; Walstra & Vliet, 2008).

Many foods such as bakery products, beverages, mousses, ice cream and confectionery are foams. The aeration process results in changes in the texture and rheology providing a different mouthfeel and appearance (Campbell & Mougeot, 1999). Aerated confectionery such as marshmallows and nougats are manufactured using high-boiled syrup and surface-active agent such as proteins, which can be combined with polysaccharides (Lees & Jackson, 1992). In confectionery industry, to prevent microbial growth at ambient temperature, the product has to be higher than 76 wt% total solid. At this level of total solid, to avoid crystals formation, part of sucrose should be replaced by others sugars such as glucose syrup and/or invert sugar to increase the system solubility (Stansell, 1995).

Sugars, proteins and polysaccharide may interact with each other, affecting foaming capacity, foam stability and rheological properties.

Sugars influence functional properties of protein such as adsorption and gelation. The interaction with sucrose decreases the ovalbumin surface activity at pH 7.0, whereas for sodium caseinate there is an increase in the protein surface activity (Antipova, Semenova, & Belyakova, 1999). Sucrose concentration influences gelation rate of whey proteins (Bryant & Mcclements, 2000) and the adsorption rate of bovine serum albumin (BSA) to air-aqueous interfaces. The difference in adsorption rate of BSA depends on the type and concentration of sugar. The process of adsorption may be attributed to an increase in aqueous phase viscosity and in protein surface hydrophilicity or to the preferential interactions of protein with solvent components (Guzey, Mcclements, & Weiss, 2003). High sugar concentration (> 60 wt% total solid) improves the stability of aerated confectionery

decreasing drainage rate by the increasing the liquid continuous phase viscosity, but decreases the foam overrun (Lau & Dickinson, 2005; Raikos, Campbell, & Euston, 2007).

In order to perform as a good foaming agent, proteins should be able to adsorb rapidly at the air-water interface, to undergo rapid conformational change and rearrangement at the interface and form a cohesive viscoelastic film via intermolecular interactions (Damodaran, 2008; Dickinson, 2011; Mine, 1995). Egg white protein (EW) is used as surface-active ingredient to produce marshmallow and nougat (Jackson, 1995). Its excellent foaming properties are due to the interaction between its protein components. Globulins contribute to foamability, ovomucoid prevents foam drainage due to high viscosity, and lysozyme forms complexes with other proteins enhancing film strength and foam stability.

Polysaccharides act as thickening, water-holding or gelling agents and their use can increase foam stability by either increasing the viscosity of the continuous phase or forming a three dimensional network (Dickinson, 2003; Walsh, Russell, & Fitzgerald, 2008). Hydroxypropylmethylcellulose (HPMC) is a polysaccharide that presents active surface property due to methyl (hydrophobic group) and hydroxypropyl (hydrophilic group) (Perez et al., 2007).

The incorporation of air bubbles modifies food texture, which then exhibit semi-solid behavior (Thakur et al., 2008). G' and G" represent the elastic and viscous behaviors of a material, respectively (Thakur et al, 2008). When G' is higher than G", a material behaves like a solid, whereas when G" is higher than G', it behaves like a liquid (Rao, 1999). The solidity of semi-solid food may also be defined either by tan δ (G"/G') or by the phase angle δ value. Tan δ = 0 (phase angle δ = 0) and tan $\delta = \infty$ ($\delta = 90^{\circ}$) characterize an ideal solid and viscous behavior, respectively. For aerated emulsions such as ice cream, the incorporation of air bubbles led to increasing elastic modulus (G'), with less effect on viscous modulus (G"), therefore, leading to reduced tan δ (Goff *et al.*, 1995).

The objective of this study was to evaluate the effect of total biopolymer concentration (w/w%) and EW:HPMC ratio on a high sugar content system on the foaming and their rheological properties. The effect of pH (3.0, 4.5, and 6.0) on foaming properties was also evaluated.

2. Materials and methods

2.1. Materials

Sucrose (Tate & Lyle, UK) was purchased from local supermarket. Glucose syrup (40 D.E.) and invert sugar syrup (80 wt% total solid) were donated by Brenntag UK & Ireland (Leeds, UK) and by British Sugar (Peterborough, UK), respectively. Dried egg white protein (EW) and hydroxypropylmethylcellulose (HPMC, METHOCEL F50, methyl 27.00 – 30.00%, hydroxypropyl 4.00 – 7.75%, 50 cPs viscosity in 2% solution) were provided by Saltos Alimentos LTDA (Salto, Brazil) and Down S.A. (Midland, USA), respectively. EW presented, in wet basis, 79.9 $\pm$1.2% of protein 10.20 $\pm$ 0.02% of moisture and 5.64 ± 0.22% ash, determined according to methodologies described by AOAC (2010). The SDS-PAGE analysis of EW (Laemmli, 1970) showed the eletrophoretic profile with bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively. The other reagents were analytical grade and Milli-Q water was used in all experiments.

2.2. Preparation of solutions and foams

The sugar mixture used as a model system to evaluate the foaming and rheological properties in aerated products was composed by sucrose (42.5 wt% total sugar solid), glucose syrup (42.5 wt% total sugar solid) and invert sugar (15 wt% total sugar solid). This composition, established in a previous study (thesis chapter 4), is adequate to obtain foams with density between 0.25 g/mL and 0.50 g/mL and water activity from 0.665 to 0.778 , which are characteristics of aerated confectionery such as marshmallow (Jackson, 1995; Wills, 1998).

Sugar mixture was heated in hot plate stirrer to reach 80 wt% total sugar solid and cooled to the whipping temperature, 70 ℃. The biopolymers, in appropriate amounts to each trial condition (Table 1), were hydrated together in 36 g of water under magnetic stirring for 1 h at room temperature. The pH was adjusted to 3.0 with 4 mol L^{-1} citric acid.

For the foams preparation, sugar mixture (500 g) and hydrated EW/HPMC blends were mixed using a Kitchen Aid 5KPM5 stand mixer (Havant, UK) at speed setting 4, for 1 min and equipped with a flat beater. Then, the foams were produced using a whisk beater, operating at speed setting 10 under atmospheric pressure and whipping time of 6 min.

A Central Composite Rotatable Design CCRD (2^2 factorial design with 4 trials under the axial conditions and 3 repetitions at the central point) totaling 11 trials (Table 1) (Rodrigues & Iemma, 2012) was carried out to evaluate the effect of total biopolymer concentration (w/w%) and EW:HPMC ratio (w/w) on apparent viscosity of sugar/biopolymer mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) for fresh foam and rheological properties (G', G" and δ at 1 Hz) for fresh foam and foam aged for 24 h were evaluated. Second-order models were obtained and analyzed statistically by analysis of variance (ANOVA).

In order to evaluate the effect of pH on foaming and rheological properties of the sugar/EW/HPMC mixture, experiments were carried out at pH 3.0, 4.5 and 6.0 under the conditions used for the model validation (total biopolymer concentration 5.0 %w/w, EW:HPMC ratio 14:1, 80 wt% total sugar solid and 70 ℃). The results were analyzed for differences among means by Tukey's test ($p < 0.05$).

2.3. Foaming properties

2.3.1. Foaming capacity: density and overrun

Foam samples were carefully filled up into cylindrical containers (35.43 $\pm$ 0.21 mL). To obtain constant volume, the top of the container was leveled with a metal spatula to achieve uniform and plane surfaces. The foam weight was recorded and then the foam density was determined. The overrun was determined according to Equation 1 (Lau & Dickinson, 2004).

$$\text{overrun (\%)} = 100 \times [m_i - m_f] / m_f \qquad (1)$$

where m_i is the mass of the initial solution (before whipping) and m_f is the mass of the resulting foam with the same volume of m_i.

The density was determined by Equation 2:

$$\text{Density (g/mL)} = m_f / \text{volume of cylindrical container} \qquad (2)$$

2.3.3. Liquid drainage

Foam samples were poured in plastic containers and kept in closed plastic box and stored at 25 ℃ . The liquid drainage was followed during 30 days by visual observation and registered by photographs.

2.3.3. Bubble size distribution

Microscopy images of the foams samples were carried out using a Leica Confocal Scanning Laser Microscope (model TCS SP2, Heidelberg, Germany) equipped with an Ar/HeNe laser and 10x objective lens (HC PL APO CS 20 × 0.7 DRY). Rhodamine B (tetraethylrhodamine; dye content approximately 95%), purchased from Aldrich (Dorset, UK), was used as the labeling dye at a level of 0.1 mL of 0.1% (w/v). The fluorescence dye, Rhodamine B, was excited at 50% of maximum absorption at 488 nm, and the detection bandwidth was set from 500 to 600 nm. Images were recorded at low magnificance and analyzed by the Image J software. Fresh foam sample was placed into a well and the dye was added. The well was covered with a cover slide, pressed down to maintain a flat surface over the well and the images were recorded after 24h.

Foam bubble size distributions were measured by analyzing the CLSM images using Image J software. For each sample, 1000 bubbles were measured. Mean bubble size was characterized by average bubble diameter d_{32} defined by the equation 3:

$$d_{32} = \sum_i di^3 / \sum_i di^2 \qquad (3)$$

2.4. Rheological properties

A stress-controlled rheometer (Kinexus, Malvern Instruments Limited, Worcestershire, UK) equipped with parallel-plate geometry (65 mm flat plate) was used to measure the rheological properties at 25 ℃. Apparent viscosity of sugar/EW /HPMC mixture before whipping was measured as a function of shear rate (0.1 to 100 s^{-1}), using a 1 mm gap, according to previous studies with glucose syrupand honey (Schellart (2011). The viscoeslatic modulus (elastic modulus G', viscous modulus G" and phase angle δ) of foam were determined at small deformation and the gap was 3 mm, which was selected to avoid crushing or destroying of the gas bubbles (Zmudzinski et al.2014).

To determine the linear viscoelasticity region in oscillatory shear, stress sweep tests were carried out at 1 Hz. Samples were also subjected to a frequency sweep from 0.1 to 10 Hz at constant strain amplitude within the linear viscoelastic region of each sample. The rheological measurements were carried out in 3 repetitions for fresh foam and foam aged for 24 h.

3. Results and discussion

3.1. Apparent viscosity, foaming and rheological properties of high sugar system/EW/HPMC mixtures

A CCRD was carried out with total biopolymer concentration and EW:HPMC ratio as independent variables to evaluate the effect of these variables on the apparent viscosity of sugar/EW/HPMC mixture before whipping, the foaming capacity and rheological properties of aerated samples. The experimental conditions as well as results are shown in Table 1.

Mathematical models were built for the responses apparent viscosity of sugar/ EW/HPMC mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties (G', G" and δ at 1 Hz) for fresh and aged for 24 h foams. For mixing process in food industry, the shear rate range is between 10 and 10^3 s^{-1} (Steffe, 1996). On the basis of ANOVA, the adequacy of the fitted model was evaluated (Table 2).

The viscosity-shear rate relationship for sugar/EW/HPMC mixtures before whipping and frequency sweep data for fresh foam and foam aged for 24 h from trials of Table 1 are shown in Fig. B1 and Fig. B2, B3, B4, B5 (Annexe B), respectively.

The flow curves of sugar/EW/HPMC mixtures before whipping were fitted to a power-law model, $\sigma = K D^n$. Where σ is the shear stress, K (consistency index (Pa.s^n)) and n (power law index) are fitted parameters. The values of the power-law index, *n,* are shown in Table B1 (Annexe B). For Trials 3 and 5, the *n* value were close to unity. Trials 1,2, 4, 6, 7, 8, 9, 10 and 11 were thinning shear fluids ($n < 1$).

Table 1. Design matrix of the CCRD with independent variables total biopolymer concentration (w/w% of total sugar solids) and EW:HPMC ratio, and the results for responses apparent viscosity of sugar/EW/HPMC mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties (G', G" and δ at 1Hz) for fresh foam and foam aged for 24 h, at pH 3.0.

Trial	Total Biopol. conc. (%w/w) x_1	EW:HPMC ratio x_2	η (Pa.s) y_1	ρ (g/mL) y_2	Overrun (%) y_3	Fresh foam G' (Pa) y_4	Fresh foam G" (Pa) y_5	Fresh foam δ (°) y_6	Foam aged for 24 h G' (Pa) y_7	Foam aged for 24 h G" (Pa) y_8	Foam aged for 24 h δ (°) y_9
1	-1 (2.00)	-1 (4:1)	7.35	0.56	102.2	1202.0	1317.0	47.85	523,5	811.0	57.1
2	1 (5.00)	-1 (4:1)	17.29	0.48	140.5	2485.0	1808.0	36.21	1024.0	833.8	38.8
3	-1 (2.00)	1 (16:1)	3.15	0.42	180.0	2411.0	1216.0	27.57	928.5	773.0	39.8
4	1 (5,00)	1 (16:1)	11.25	0.39	212.8	4700.0	1909.0	22.35	1434.0	877.8	31.5
5	-1.41 (1.40)	0 (10:1)	3.05	0.45	169.4	1893.0	1036.0	29.72	561.7	693.4	51.0
6	1.41 (5.60)	0 (10:1)	10.32	0.39	198.0	4697.0	1788.0	21.14	1232.0	724.2	30.5
7	0 (3.50)	-1.41 (2:1)	21.48	0.72	46.9	363.1	664.3	61.4	268.6	511.2	62.3
8	0 (3.50)	1.41 (18:1)	7.98	0.40	198.9	3938.0	1603.0	22.3	1163.0	767.2	33.4
9	0 (3.50)	0 (10:1)	10.54	0.41	187.6	4079.0	1738.0	24.91	1145.0	850.8	36.7
10	0 (3.50)	0 (10:1)	7.88	0.42	181.4	2769.0	1214.0	24.73	908.4	596.2	33.3
11	0 (3.50)	0 (10:1)	9.98	0.41	190.5	3962.0	1644.0	22.72	989.4	647.6	33.2

Coded values and ( ) true values of the independent variables; Total biopol. conc.: total biopolymer concentration; η: apparent viscosity; ρ: density; G': elastic modulus; G": viscous modulus; δ: phase angle.

For the responses apparent viscosity, density, overrun, G', and δ, the R^2 and calculated F values (Table 2) are adequate to obtaining the second-order model (Equations y_1, y_2, y_3, y_4, y_6, y_7 e y_9), within the range studied.

Table 2. Percentage of explained variance (R^2), $F_{calculated}$ value and $F_{tabulated}$ of the responses apparent viscosity (η), foaming capacity (density and overrun), rheological properties (G', G" and δ) of fresh foam and foam aged for 24 h.

Response		R^2 (%)	$F_{calculated}$	$F^*_{tabulated}$	Equation
Apparent Viscosity (η) Pa.s		92.0	17.18	4.53	$y_1 = 9.47 + 3.55x_1 - 1.64x_1^2 - 3.67x_2 + 2.40x_2^2$
Density (ρ) (g/mL)		91.0	22,45	4,35	$y_2 = 0.41 - 0.02x_1 - 0.08x_2 + 0.07x_2^2$
Overrun (%)		97.0	72.77	4.35	$y_3 = 186.5 + 14,0x_1 + 45.7x_2 - 30.6x_2^2$
Fresh foam	G' (Pa)	91.0	24.55	4.35	$y_4 = 3453.7 + 943.6x_1 + 1061.3x_2 - 688.6x_2^2$
	G" (Pa)	42.0	6.42	5.12	It was not possible to establish a model
	δ (°)	95.0	48.81	4.35	$y_6 = 24.45 - 3.63x_1 - 11.19x_2 + 8.85x_2^2$
Foam aged for 24h	G' (Pa)	90.0	21.84	4.35	$y_7 = 999.1 + 244.6x_1 + 260.2x_2 - 101.9x_2^2$
	G" (Pa)	25.0	-	---	No regression coefficient was statistically significant ($p > 0.10$)
	δ (°)	90.0	21.98	4.35	$y_9 = 36.79 - 6.96x_1 - 8.18x_2 + 5.38x_2^2$

x_1, x_2 : coded independent variables for total biopolymer concentration and EW:HPMC ratio, respectively. ---:there is no regression coefficient.

The equations in Table 2 were used to generate the contour curves for the dependent variables apparent viscosity of sugar/EW/HPMC mixture before whipping (y_1), foaming capacity (density (y_2) and overrun (y_3)) of fresh foam, rheological properties of fresh foam G' (y_4), and δ (y_6) and foam aged for 24 h G' (y_7) and δ (y_9) (Fig. 1). According to Fig. 1, G' values of foam aged for 24 h are lower than G' values of fresh foam indicating that fresh foams are not stable. After 24 h, the microstructure changes, leading to a less elastic behavior.

The apparent viscosity of sugar/ EW/HPMC mixture before whipping increases with increasing total biopolymer concentration and decreasing EW:HPMC ratio (Fig. 1). Foam presented low density, high overrun, high G' and low δ for fresh sample and sample aged for 24 h at total biopolymer concentration above 5% (w/w) and EW**:**HPMC ratio above 10:1. In the regions of low density and high overrun, G' values are higher and δ values are lower, either for fresh foams and foams aged for 24 h foams. Therefore the elastic behavior, increases air incorporation (increasing overrun) of foams, which is in accordance with previous works (Goff et al., 1995; Thakur, Vial & Djelveh et al., 2008).

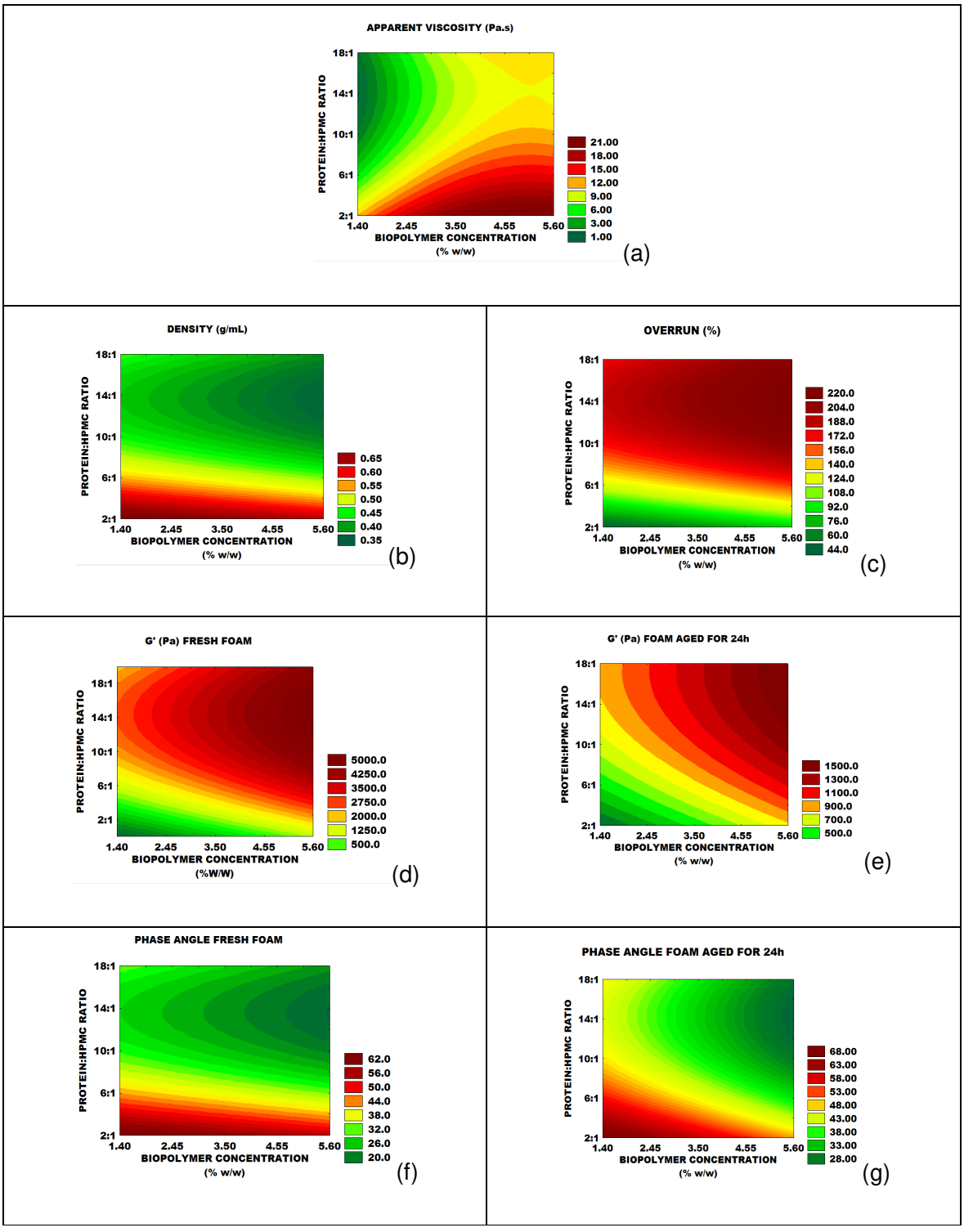
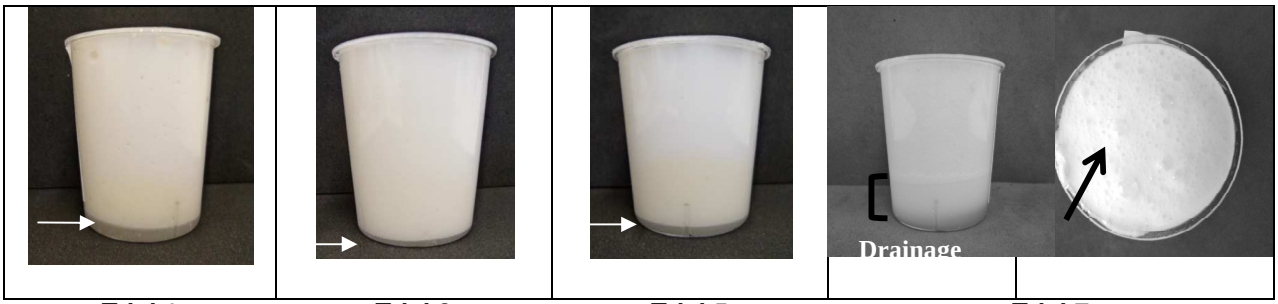


Fig. 1. Contour curves of the dependent variables apparent viscosity (η) of sugar/ EW/HPMC mixtures: (y_1) before whipping (a), foaming capacity of fresh foam density (y_2) (b) and *overrun* (y_3) (c), rheological properties of fresh foam G' (y_4) (d) and δ (y_6) (f) and foam aged for 24 h G' (y_7) (e) and δ (y_9) (g).

The apparent viscosity of sugar/EW/HPMC mixture before whipping was measured in order to evaluate its infuence on foaming capacity and foam rheological properties. Above 15 Pa.s, increasing density and decreasing overrun values were observed possibly due to the difficulty to incorporate air bubbles. Since the lower G' values leads to lower foaming capacity, a less elastic and solid foams were obtained. Low apparent viscosity of sugar/EW/HPMC mixture led to greater liquid drainage. Foams from trials 1, 3 and 5 (Table 1), which were prepared with mixtures with apparent viscosity bellow 8 Pa.s showed liquid drainage after one week of storage at 25 ℃ (Figure 2). Foams prepared from sugar/EW/HPMC mixtures with viscosity between 8 to 17 Pa.s did not present drained liquid after 20 days at 25 ℃ (data not shown). On the other hand, mixture with high apparent viscosity such as the one from trial 7 (21.48 Pa.s) resulted in foam with high density value (0.72 g/mL) and low overrun value (46.9%). The high apparent viscosity possibly hampered the incorporation of air bubbles during whipping. High viscosity can also influence molecular diffusion and decrease the adsorption rate of proteins (Yang & Foegeding, 2010). Due to the lower foaming capacity, the foam presented low G' (363.1 Pa) and high δ (61.4º). These values indicate that this foam does not behave as a solid, leading to creaming and liquid drainage after 20 days of storage at 25 ℃ (Figure 2).



Trial 1	Trial 3	Trial 5	Trial 7
Bio.conc.: 2.0% w/w	Bio. conc.: 2.0% w/w	Bio.conc.: 1.4% w/w	Biop. conc.:3.50 % w/w
EW:HPMC ratio 4:1	EW:HPMC ratio16:1	EW:HPMC ratio 10:1	EW:HPMC ratio 2:1
η = 7.35 Pa.s;	η = 3.15 Pa.s;	η = 3.05 Pa.s;	η = 21.48 Pa.s;
ρ = 0.56 g/mL	ρ = 0.42 g/mL	ρ = 0.45 g/mL	ρ = 0.72 g/mL

Fig. 2. Liquid drainage of foams obtained under the conditions of Trial 1, 3 and 5 (pH 3.0; 70 ℃) after 1 week of storage at 25 ℃; drainage and crea ming of Trial 7 after 20 days of storage at 25º C. η: apparent viscosity of sugar/EW/HPMC mixture before whipping; ρ: foam density.

The contour curves (Fig. 1) were jointly analyzed to determine the conditions to obtain high foaming capacity, elastic and solid behavior, which characterize good foam properties. Thus 5% (w/w) total biopolymer concentration and EW:HPMC ratio 14:1 were the conditions to obtain low density, high overrun and G', small **δ** and intermediate apparent viscosity values (9 a 12 Pa.s). Foam obtained at these conditions showed density and δ about 0.35 g/ml and 20º, respectively, which are found in products such as marshmallows, chocolate mousse, whipped cream and dairy toppings (Jackson, 1995; Thakur et al., 2008).

Model validation was carried out under the previous established conditions (5% w/w total biopolymer concentration, 14:1 EW:HPMC ratio). The relative error between the experimental tests and predicted values by the coded model for apparent viscosity, density, overrun, G' (fresh foam), δ (fresh foam), G' (foam aged for 24 h) and δ (foam aged for 24 h) were -2.5, 3.1, 5.5, 9.8, 14.6, -1.2 and 16.5%, respectively. In general, the experimental results were close to the predicted values. The exceptions were the experimental δ (fresh foam and foam aged for 24 h). In spite of this deviation, the results from validation experiments were satisfactory.

3.2. Effect of pH on foaming and rheological properties

Thermodynamic incompatibility of proteins and polysaccharides in solution (Grinberg & Tolstoguzov, 1997) and the effect of sucrose on the thermodynamic properties (protein hydrophilicity and surface activity) of proteins depend on the pH (Antipova et al., 1999). Thus, in order to study the influence of pH on foaming properties in a high sugar content system with EW and HPMC, experiments were carried out under the model validation conditions (5% w/w total biopolymer concentration, 14:1 EW:HPMC ratio, 80 wt% total solid and 70 ℃) at pH 3.0, 4.5 and 6.0. The results are presented in Table 3.

Table 3. Apparent viscosity of sugar/EW/HPMC mixture (5% w/w total biopolymer concentration, 14:1 EW:HPMC ratio, 80 wt% total solid) before whipping at 10 s^{-1}, foam density (ρ), overrun, rheological properties of fresh foam and foam aged for 24 h (elastic modulus G', viscous modulus G" and phase angle δ at 1 Hz) obtained at pH 3.0, 4.5 and 6.0.

pH	η	Density	Overrun	Fresh foam			Foam aged for 24h		
	(Pa.s)	(g/ml)	(%)	G' (Pa)	G" (Pa)	δ (°)	G' (Pa)	G" (Pa)	δ (°)
3.0	9.74 ± 0.91[a]	0.38 ± 0.00[a]	206.1 ± 10.7[a]	5325.8 ± 227.3[a]	1924.5 ± 57.3[a]	20.28 ± 0.54[a]	1359.9 ± 115.4[a]	850.4 ± 35.7[a]	32.03 ± 2.09[a]
4.5	1.34 ± .63[a]	0.42 ± 0.01[b]	168.0 ± 4.2[b]	3538.0 ± 721.2[b]	1837.3 ± 266.9[a]	27.48 ± 0.90[b]	1234.56 ± 75.6[a]	1230.4 ± 32.5[b]	44.89 ± 2.00[b]
6.0	8.72 ± 2.10[a]	0.51 ± 0.02[c]	139.5 ± 15.2[c]	903.0 ± 226.0[c]	1380.3 ± 114.2[b]	60.63 ± 1.60[c]	465.9 ± 104.0[b]	524.6 ± 72.2[c]	53.73 ± 2.42[c]

Values are mean ± SD of triplicates, except G' and δ fresh sample that are mean ± SD of duplicates. For the same response, mean with different small letters in the same column differ significantly ($p < 0.05$) by Tukey's test; density (ρ), *overrun,* rheological properties of fresh sample and sample aged for 24 hours (elastic modulus G', viscous modulus G" and δ).

According to Table 3, pH did not affect the apparent viscosity of sugar/EW/HPMC mixture before whipping. However, the foams obtained at pH 3.0, 4.5 and 6.0 showed differences ($p<0.05$) in density, overrun and δ. The highest foaming capacity (density and overrun) was obtained at pH 3.0. At this pH, the foam showed G' and δ values which characterized elastic and solid behavior for fresh foam and foam aged for 24 h. At pH 3.0 and 4.5, G' of the foams aged for 24 h did not differ ($p>0.05$) while at pH 4.5, G" values were higher than at pH 3.0 ($p<0.05$). At pH 6.0 it was obtained the lowest foaming capacity and G" value higher than G' for fresh foam and foam aged for 24 h (Table 3), indicating viscous behavior.

The highest foaming capacity obtained at pH 3.0 is possibly due to the interaction between EW and HPMC. At pH 3.0 EW is partially unfolded, which increases its surface hidrofobicity and flexibility and improves its foaming properties (Liang & Kristinsson, 2005). At pH 4.5 the foaming capacity is lower than at pH 3.0 possibly because pH 4.5 is close to protein pI, which favor aggregation of ovalbumin. In addition, in presence of sucrose due to strengthening of the protein-protein net attractive interactions, significant aggregation of protein occurs leading to decrease of ovalbumin surface activity (Antipova et al., 1999).

At pH 6.0, the lowest foaming capacity and the highest foam instability (Fig. 3i) were possibly due to the interaction between ovalbumin and sucrose which lead to increase protein hydrophilicity in the bulk medium and decrease the protein surface activity (Antipova et al., 1999). Moreover, thermodynamic incompatibility between biopolymers takes place at pH higher than protein isoelectric point (Grinberg & Tolstoguzov, 1997; Rodríguez Patino & Pilosof, 2011). The thermodynamic incompatibility at the interfacial film affects the foam stability (Damodaran & Razumovsky, 2003).

The bubble size distribution of foams aged for 24 h obtained at pH 3.0, 4.5 and 6.0 are presented in Fig. 3. At pH 3.0, foam presented the smallest average bubble diameter (d_{32}) and bimodal bubble size distribution (Fig. 3b). The splitting suggests that the smaller bubbles may be evolving into the larger ones due to gas diffusion from smaller bubble to larger bubble (disproportionation). After 30 days of storage, foam at pH 3.0 did not present drainage (Fig 3c). At pH 4.5 the d_{32} was larger than at pH 3.0 and presented monodisperse bubble size distribution. At this pH, the foam did not show drainage which led to greater stability concerning the disproportionation and coalescence (Fig. 3f). The foam stability increases at pH near the pI due to lower repulsion of proteins that increase the interactions at interface air-water and a more stable and firm protein film is created (Kuropatwa et al., 2009). The foam prepared at pH 6.0 showed the largest bubble d_{32} (56.5 μm) and the widest bubble size distribution (Fig. 3h). These factors led to larger foam instability such as creaming and liquid drainage after 30 days of storage at 25 ℃ (Fig. 3i).

In a previous study (thesis chapter 3), foam prepared with EW/HPMC aqueous solution showed the same behavior at pH 3.0 and 4.5 concerning the foaming capacity and stability. The exception was that aerated high sugar system at pH 4.5 showed no liquid drainage, possibly because the sugar increased the liquid continuous phase viscosity. At pH 6.0, the foaming capacity was higher comparing to foam in high sugar content but aqueous solution and high sugar content foam presented high instability.

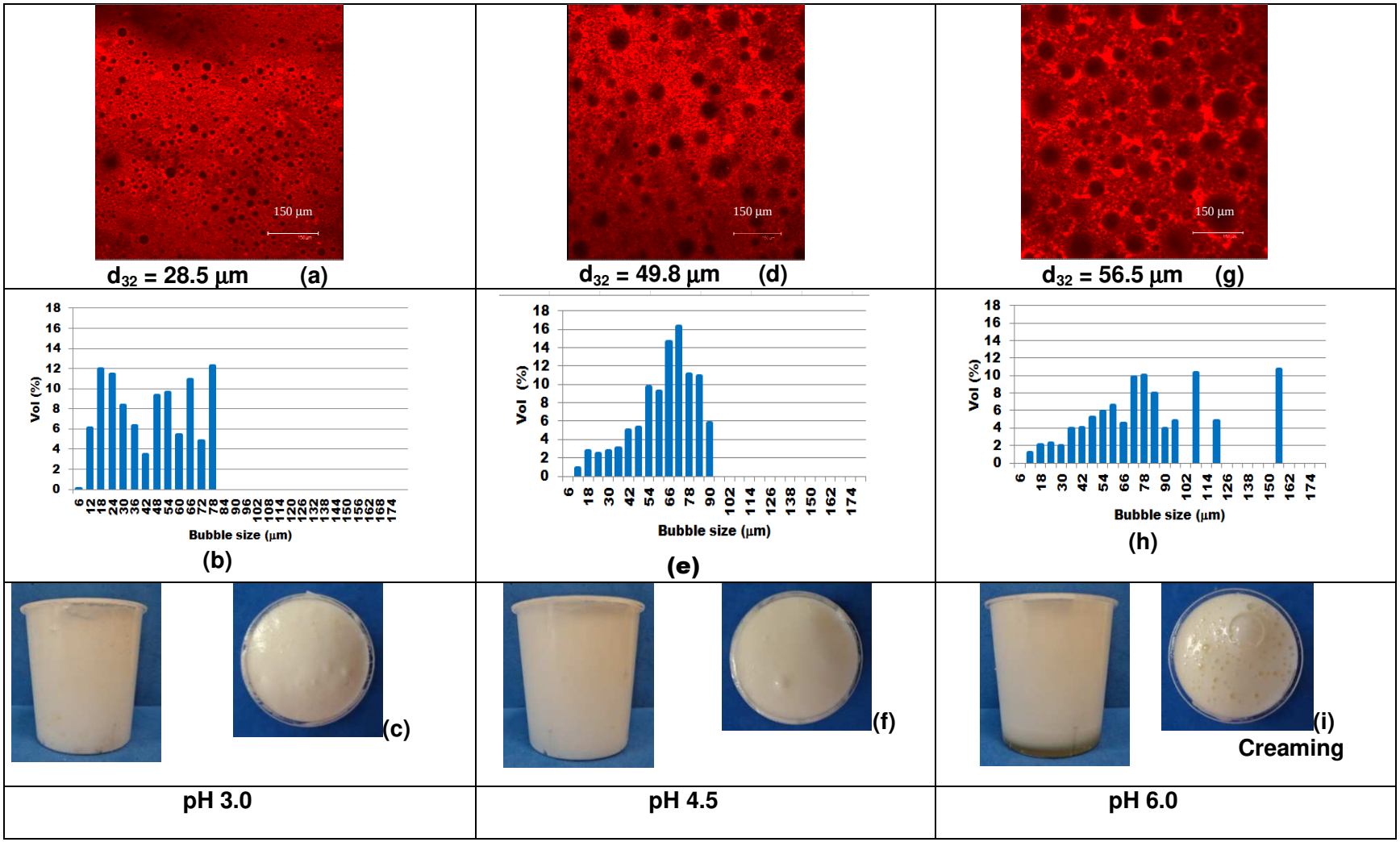


Fig. 3. Confocal microscopy (after 24h of storage at 25 ℃) (a, d, g), bubble size distribution (b, e, h) and photographs (after 30 days of storage at 25 ℃) (c, f, i) of aerated samples cont aining 5 %w/w biopolymer and EW:HPMC ratio 14:1 at pH 3.0, pH 4.5 and pH 6.0. Average bubble diameter: d_{32}

4. Conclusion

At pH 3.0, the apparent viscosity of sugar/ EW/HPMC mixture before whipping increases with increasing total biopolymer concentration and decreasing EW:HPMC ratio. Foam presented low density, high overrun, high G' and low δ at total biopolymer concentration above 5% (w/w) and EW:HPMC ratio above 10:1, within the studied range. In the regions of high foaming capacity, foams showed high G' and low δ values indicating that air incorporation enhanced the elastic and solid behaviors.At pH 3.0, the apparent viscosity of sugar/EW/HPMC mixture before whipping seems to influence foaming and rheological properties. Intermediate apparent viscosity (from 8 to 17 Pa.s) led to good foaming properties, elastic and solid behavior. Low apparent viscosity led to liquid drainage whereas those with high apparent viscosity resulted in a lower foaming capacity, lower G' and higher δ values (less elastic and solid behavior) leading to creaming and drainage. Under the best conditions to obtain foam with desirable characteristics, the properties of high sugar/ EW/HPMC mixture foams, were influenced by pH, but not by apparent viscosity before whipping. Thus the interactions between EW and HPMC are important to define foaming properties. At pH 3.0, foam presented the highest foaming capacity, elastic and solid behavior, without drainage whereas foam prepared at pH 4.5 presented lower foaming capacity, although with better stability (concerning disproportionation and coalescence) than foam at pH 3.0. At pH 6.0 foam showed the lowest foaming capacity, the highest instability and viscous behavior. Thus, HPMC could be considered to increase the stability of aerated confectionery at pH close to pI of EW. Further work will aim at evaluating surface properties (surface tension and rheology) of sugar/EW/HPMC mixtures and correlate to foaming capacity and foam stability.

Acknowledgements

The authors are grateful for the financial support (FAPESP 2011/50067-9) and EMBRAPA for the PhD scholarship granted to the author Sadahira MS.

5. References

Antipova, A. S., Semenova, M. G., & Belyakova, L. E. (1999). The effect of sucrose on the thermodynamic properties of ovalbumin and sodium caseinate in bulk solution and at air–water interface. *Colloids and Surfaces B: Biointerfaces*, *12*(3-6), 261–270.

AOAC. (2010). Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, USA: Association of Official Methods Analytical Chemists.

Bryant, C. M., & Mcclements, D. J. (2000). Influence of sucrose on NaCl-induced gelation of heat denatured whey protein solutions, *33*, 649–653.

Campbell, G. M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science & Technology*, *10*, 283–296.

Damodaran, S. (2005). Protein Stabilization of Emulsions and Foams. *Journal of Food Science*, *70*(3), R54–R66.

Damodaran, S. (2008). Amino Acids, Peptides, and Proteins. In O. R. Damodaran, Srinivasan; Parkin, Kirk L.; Fennema (Ed.), *Food Chemistry* (4th ed., pp. 217–329). Boca Raton: CRC Press.

Damodaran, S., & Razumovsky, L. (2003). Competitive adsorption and thermodynamic incompatibility of mixing of b -casein and gum arabic at the air – water interface, *17*, 355–363.

Dickinson, E. (2003). Hydrocolloids at interfaces and the in ¯ uence on the properties of dispersed systems q. *Food Hydrocolloids*, *17*, 25–39.

Dickinson, E. (2011). Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids*, *25*(8), 1966–1983.

Goff, H. D., Freslon, B., Sahagian, M. E., Hauber, T. D., Stone, A. P., & Stanley, D. W. (1995). Structural development in ice cream - dynamic rheological measurements. *Journal of Texture Studies*, *26*, 517–536.

Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*(2), 145–158.

Guzey, D., Mcclements, D. J., & Weiss, J. (2003). Adsorption kinetics of BSA at air – sugar solution interfaces as affected by sugar type and concentration. *Food Research International*, *36*, 649–660.

Jackson, E. B. (1995). Liquorice paste, cream paste and aeratd confectionery. In E. B. Jackson (Ed.), *Sugar Confectionery Manufacture* (pp. 218–235). London: Black Academic and Professional.

Kuropatwa, M., Tolkach, A., & Kulozik, U. (2009). Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. *Food Hydrocolloids*, *23*(8), 2174–2181.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of thehead of bacteriophage T4. *Nature*, *227*, 680–685.

Lau, C. K., & Dickinson, E. (2005). Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids*, *19*, 111–121.

Lau, K., & Dickinson, E. (2004). Structural and Rheological Properties of Aerated High Sugar Systems Containing Egg Albumen. *Journal of Food Science*, *69*(5), E232–E239.

Lees, R., & Jackson, E. B. (1992). *Sugar confectionery and chocolate manufacture*. (R. Lees & E. B. Jackson, Eds.) (pp. 299–323). Glasglow: Chapman & Hall.

Liang, Y., & Kristinsson, H. (2005). Influence of pH-Induced Unfolding and Refolding of Egg Albumen on Its Foaming Properties. *Journal of Food Science*, *70*(3), C222–C230.

Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. *Trends in Food Science & Technology*, *6*, 225–232.

Murray, B. S., & Ettelaie, R. (2004). Foam stability : proteins and nanoparticles. *Current Opinion in Colloid & Interface Science*, *9*, 314–320.

Perez, O. E., Carrera Sanchez, C., Rodriguez Patino, J. M., & Pilosof, A. M. . (2007). Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl–methyl–cellulose mixtures at the air-water interface. *Food Hydrocolloids*, *21*(5-6), 794–803. doi:10.1016/j.foodhyd.2006.11.013

Raikos, V., Campbell, L., & Euston, S. R. (2007). Effects of sucrose and sodium chloride on foaming properties of egg white proteins, *40*, 347–355.

Rao, M. A. (1999). Rheological behavior of processed fluid and semisolid foods. In M. A. Rao (Ed.), Rheology of fluid and semi solid foods: principles and applications. (Chapter 3 and 5, pp. 105–108, 244–254).

Rodrigues, M. I., & Iemma, A. F. (2012). *Experimental Design and Process Optimization* (2 nd.). Campinas: Carita Editora.

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*(8), 1925–1937.

Schellart, W.P. (2001). Rheology and density of glucose syrup and honey: Determining their suitability for usage in analogue and fluid dynamic models of geological processes. *Journal of Structural Geology,* 33, 1079-1088.

Stansell, D. I. (1995). The composition and structure of confectionery. In E. B. Jackson (Ed.), *Sugar Confectionery Manufacture* (pp. 298–311). London: Black Academic and Professional.

Steffe, J. F. (1996). Rheological methods in food process engineering (2nd ed.). East

Lansing, MI: Freeman Press.

Thakur, R. K., Vial, C., & Djelveh, G. (2008). Effect of composition and process parameters on elasticity and solidity of foamed food. *Chemical Engineering and Processing*, *47*, 474–483.

Walsh, D. J., Russell, K., & Fitzgerald, R. J. (2008). Stabilisation of sodium caseinate hydrolysate foams. *Food Research International*, *41*, 43–52.

Walstra, P., & Vliet, T. van. (2008). Dispersed systems: basic considerations. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Food Chemistry* (4th ed., pp. 783–847). Boca Raton.

Wills, D. (1998). Water activity and its importance in making candy. *The Manufacturing Confectioner*, *78*(8), 71–74.

Yang, X., & Foegeding, E. A. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids*, *24*(2-3), 227–238.

Zmudzinsk, D., Ptaszek, P., Kruk, J., Kaczmarczyk, K., Roznowski, W., Berski, W., … Grzesik, M. (2014). The role of hydrocolloids in mechanical properties of fresh foams based on egg white proteins. *Journal of Food Engineering*, *121*, 128–134.

CAPÍTULO 7. CONCLUSÕES GERAIS

Capítulo 7. Conclusões gerais

Na região entre pH 2,0 a 4,9, há interação eletrostática atrativa entre as proteínas da clara de ovo e a pectina em sistemas aquosos. A proporção proteína:pectina afeta a carga total do sistema (potencial zeta), o tipo e o tamanho do complexo eletrostático formado, solúvel ou insolúvel. Estes fatores influenciaram tanto a capacidade de aeração (*overrun* e densidade) como a estabilidade da espuma. Os complexos que apresentaram neutralidade elétrica (complexos insolúveis) apresentaram maior tamanho e construíram uma rede interfacial viscoelástica na interface ar-água de baixa permeabilidade gasosa conduzindo a uma maior estabilidade com relação à desproporção. A presença dos complexos solúveis na *lamella* aumentou a viscosidade da fase contínua líquida, resultando em uma menor drenagem do líquido. Os complexos solúveis formaram uma camada secundária que contribuiu para a formação de um filme estável na interface ar-água, inibindo a coalescência. Em um sistema com alto teor de açúcares/proteínas da clara de ovo/pectina, pode ser observado comportamento semelhante com relação aos complexos proteínas da clara de ovo/pectina solúveis.

Para sistemas contendo proteínas da clara de ovo e HPMC, o pH foi o fator principal que influenciou a interação desses componentes em solução aquosa e suas propriedades espumantes. No pH 3,0, a compatibilidade termodinâmica entre a proteína e HPMC ocorreu, provavelmente, devido às interações hidrofóbicas e pontes de hidrogênio entre os biopolímeros, resultando em espumas com melhores propriedades espumantes. Enquanto, a incompatibilidade termodinâmica entre os biopolímeros entre os pH 4,5 e 6,0 conduziu a uma menor estabilidade com relação à desproporção comparadas com espumas sem HPMC. A espuma obtida a partir da mistura açúcar/proteínas da clara de ovo/HPMC no pH 3,0, apresentou uma alta capacidade de aeração e ausência de líquido drenado, enquanto que no pH 4,5 apresentou uma baixa capacidade de aeração, porém maior estabilidade com relação à desproporção e coalescência. No pH 6,0, a espuma não apresentou boas propriedades espumantes.

De forma geral, as espumas das misturas com maior capacidade de aeração apresentaram valores de G' alto e δ baixo, indicando que o seu comportamento elástico, melhora a incorporação de bolhas de ar.

As interações proteína/polissacarídeo (aniônico ou neutro) têm um papel importante na capacidade de aeração e nos mecanismos de instabilidade de uma espuma. O controle destas

interações é um fator chave para o desenvolvimento de produtos aerados com maior estabilidade física.

A formação de complexo eletrostático entre a pectina (polissacarídeo aniônico) e proteínas da clara de ovo em pH abaixo do ponto isoelétrico da proteína tem a propriedade funcional de estabilizar a espuma em sistema aquoso e de alto teor de açúcar. Em sistema aquoso, a diminuição da proporção proteína:pectina (aumento da concentração de pectina) favoreceu a formação de complexos insolúveis (neutros), aumentando a estabilidade quanto a desproporção, na faixa estudada. A diminuição da proporção proteína:HPMC (aumento da concentração de HPMC), devido às propriedades emulsificantes do HPMC (polissacarídeo neutro), levou, possivelmente, à adsorção competitiva pela interface ar-água entre HPMC e proteínas, resultando em uma maior instabilidade das espumas. Para sistemas com alto teor de açúcares, os resultados alcançados neste estudo abrem possibilidades de novas pesquisas, entre as quais destacamos:

- Para o sistema açúcar/proteína da clara de ovo/pectina: estudo das interações eletrostáticas dos biopolímeros em solução de açúcares para melhor entendimento dos mecanismos de estabilidade das espumas;
- Para o sistema açúcar/proteínas da clara de ovo/HPMC: estudo das propriedades interfaciais (tensão superficial e reologia) dos biopolímeros na presença de açúcares.

ANEXO A

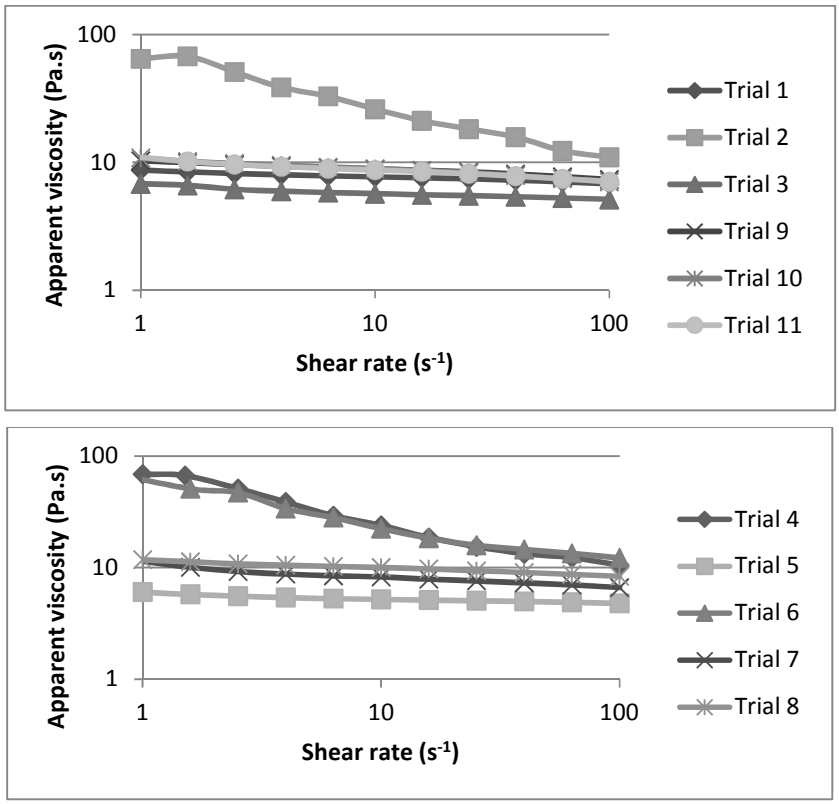


Fig. A1. Flow curves for sugar/EW/pectin mixtures before whipping of trials from Table 1 (thesis chapter 5)

Table A1. Power law index *n*, K and R^2 of trials for sugar/EW/pectin mixtures before whipping of trials from Table 1 (thesis chapter 5)

Trial	K	n	R^2
1	8.61	0.95	1.00
2	62.18	0.62	0.97
3	6.59	0.94	1.00
4	63.8	0.60	0.98
5	5.84	0.95	1.00
6	59.09	0.62	0.99
7	10.52	0.90	1.00
8	11.68	0.93	1.00
9	10.58	0.93	1.00
10	10.74	0.91	1.00
11	10.62	0.91	1.00

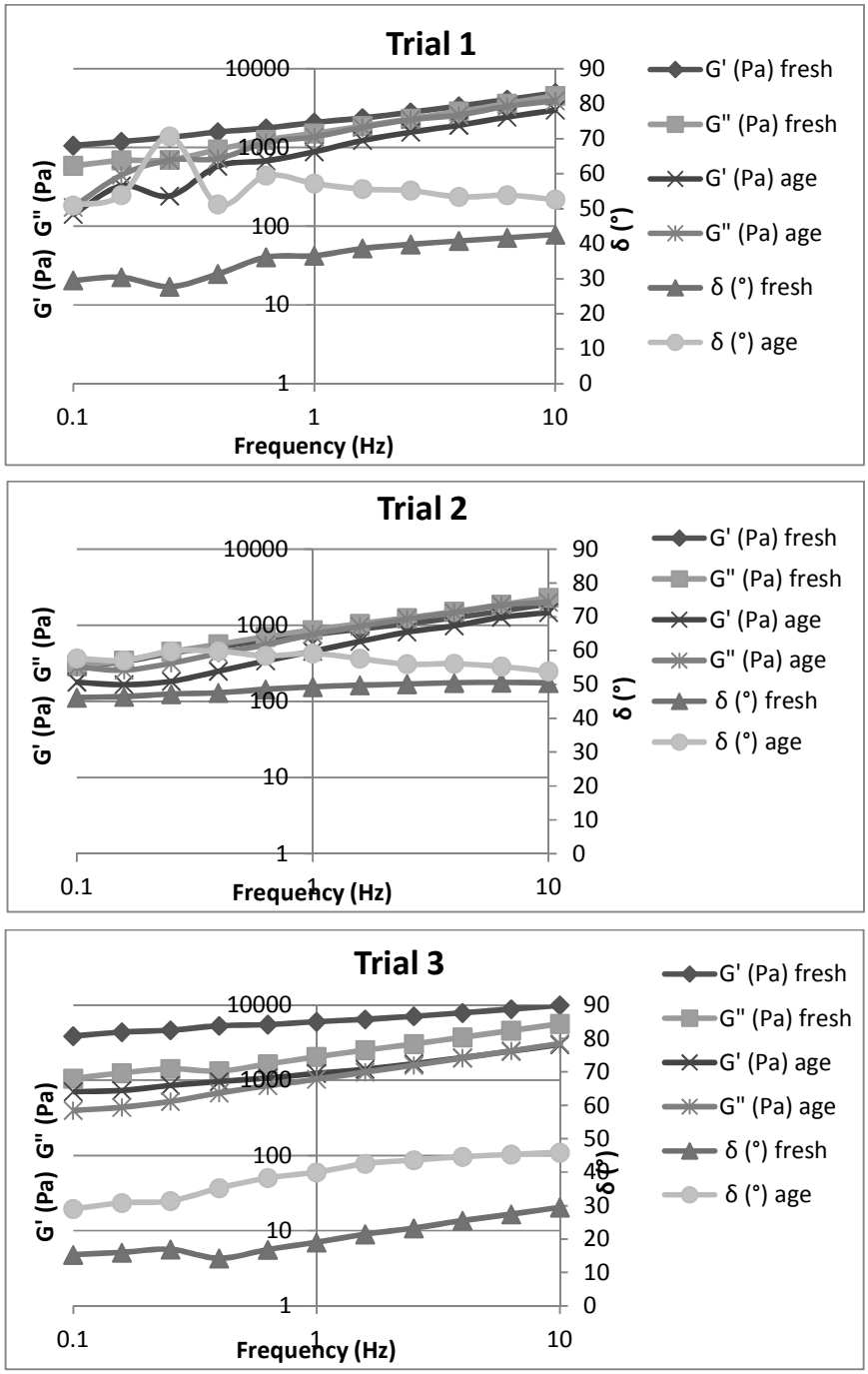


Fig. A2. Frequency sweep of fresh and aged for 24 h foams of Trial 1, 2 and 3 from Table 1 (thesis chapter 5). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

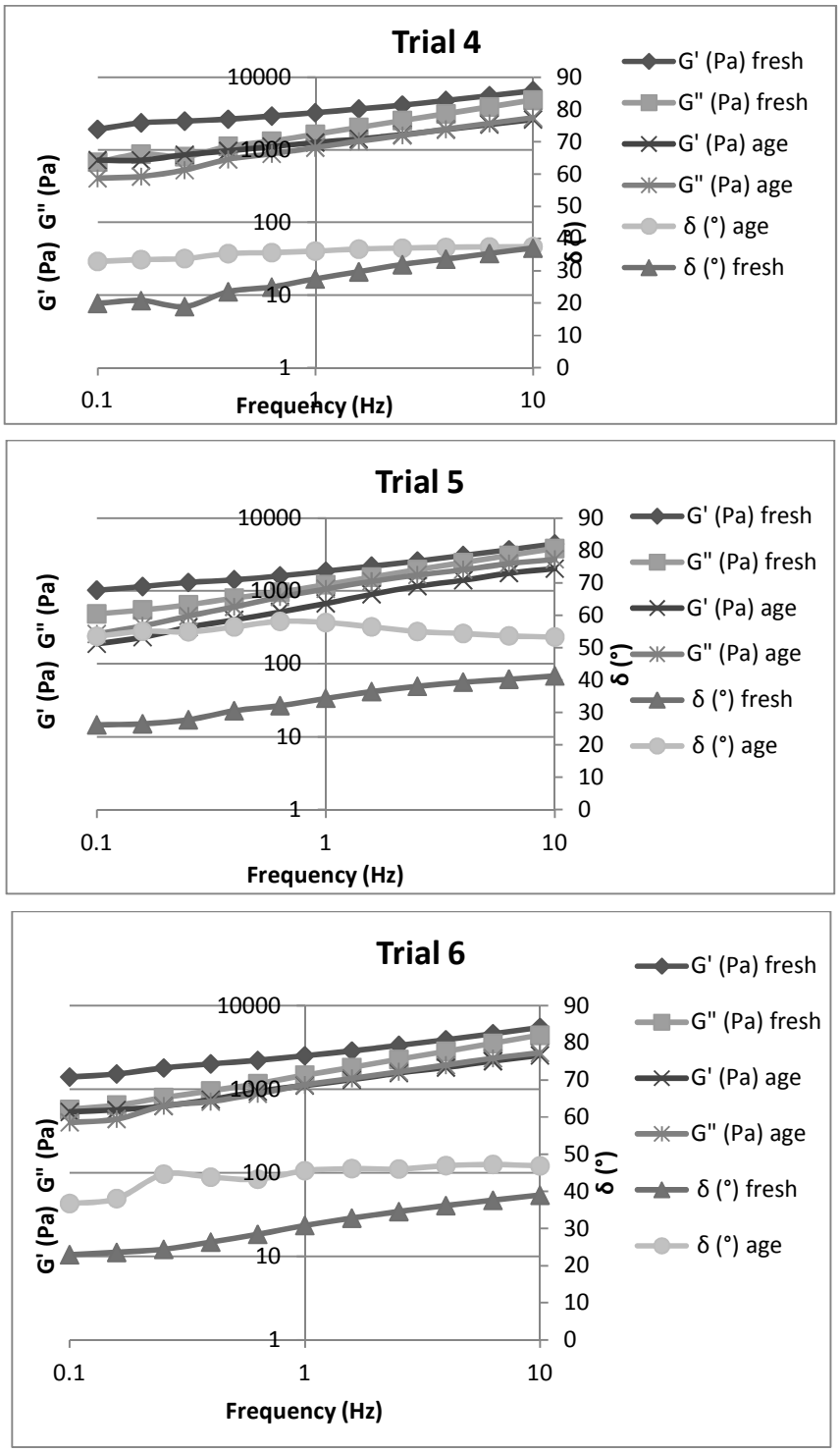


Fig. A3. Frequency sweep of fresh and aged for 24 h foams of Trial 4, 5 and 6 from Table 1 (thesis chapter 5). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

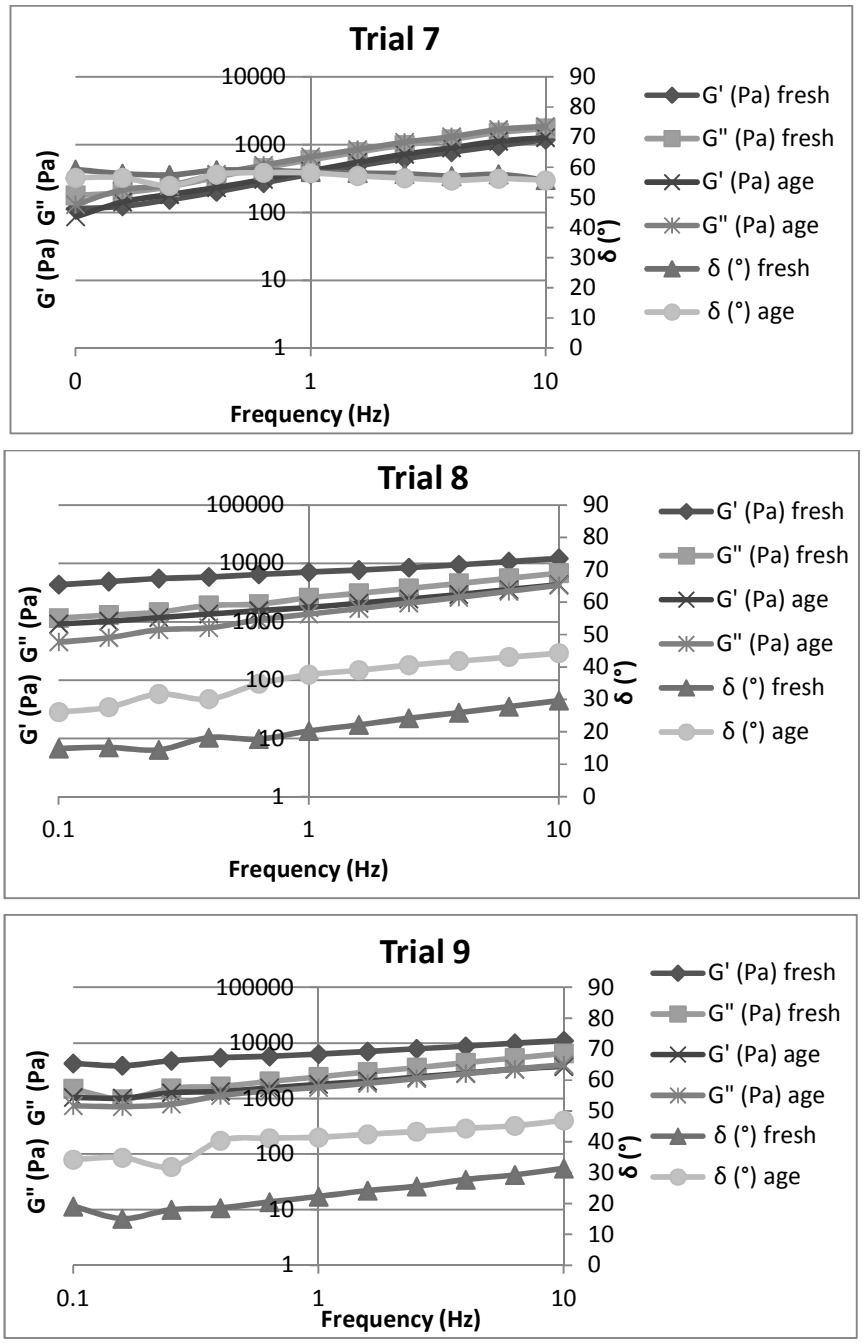


Fig. A4. Frequency sweep of fresh and aged for 24 h foams of Trial 7, 8 and 9 from Table 1 (thesis chapter 5). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

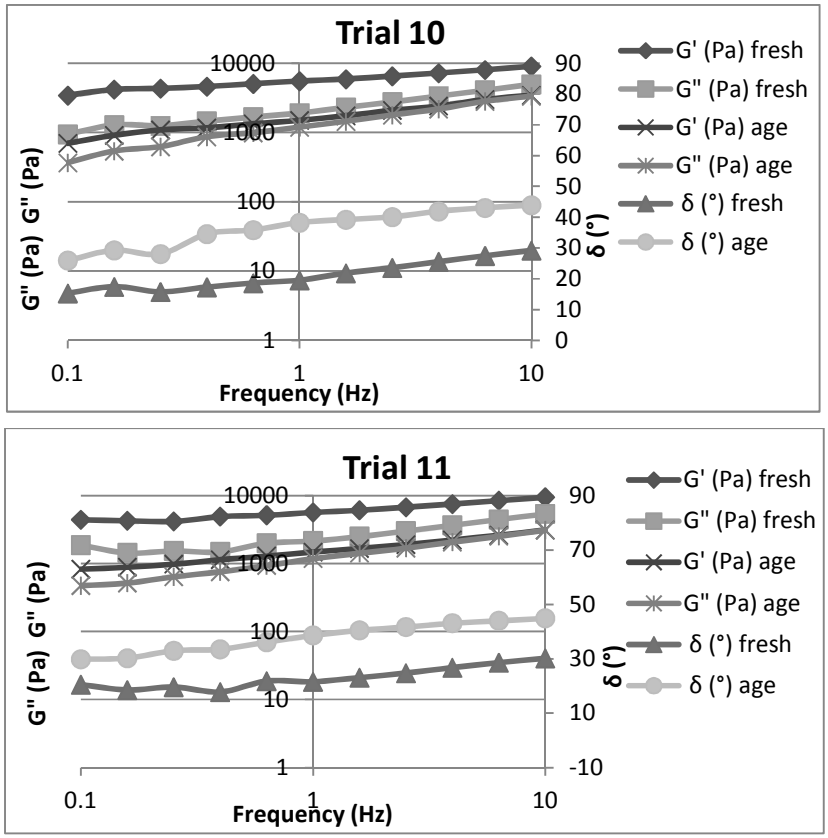


Fig. A5. Frequency sweep of fresh and aged for 24 h foams of Trial 10, 11 from Table 1 (thesis chapter 5). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

ANEXO B

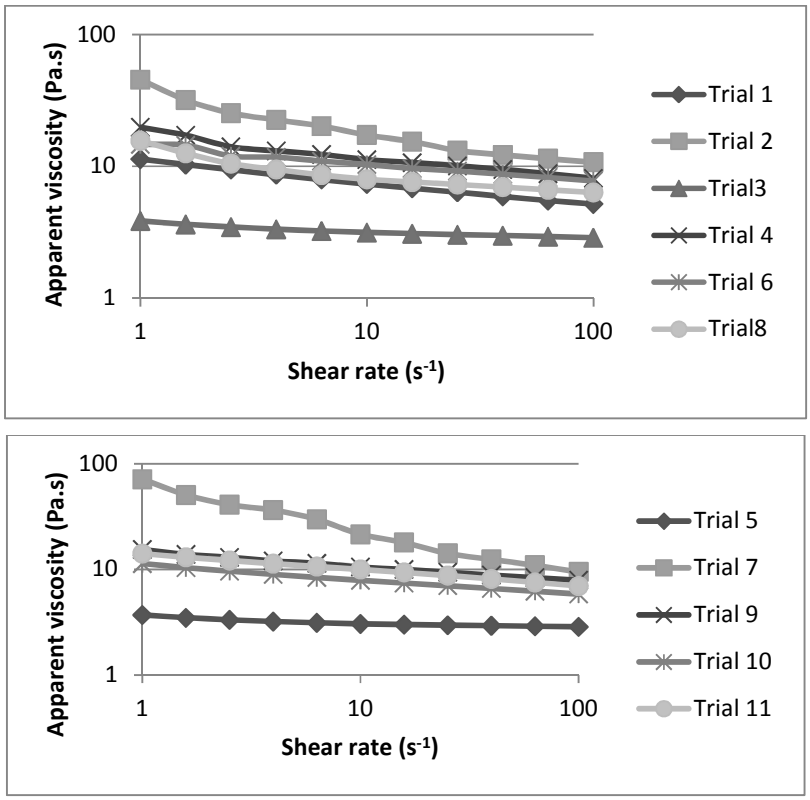


Fig. B1. Flow curves for sugar/EW/HPMC mixtures before whipping of trials from Table 1 (thesis chapter 6).

Table B1. Power law index *n*, K and R^2 of trials for sugar/EW/HPMC mixtures before whipping of trials from Table 1 (thesis chapter 6).

Trial	K	n	R^2
1	12.32	0.79	1.00
2	39.43	0.68	0.99
3	4.17	0.90	0.99
4	18.65	0.81	1.00
5	3.96	0.91	1.00
6	16.11	0.82	0.99
7	66.53	0.55	0.99
8	13.99	0.80	0.99
9	15.21	0.85	1.00
10	11.17	0.85	1.00
11	14.12	0.85	1.00

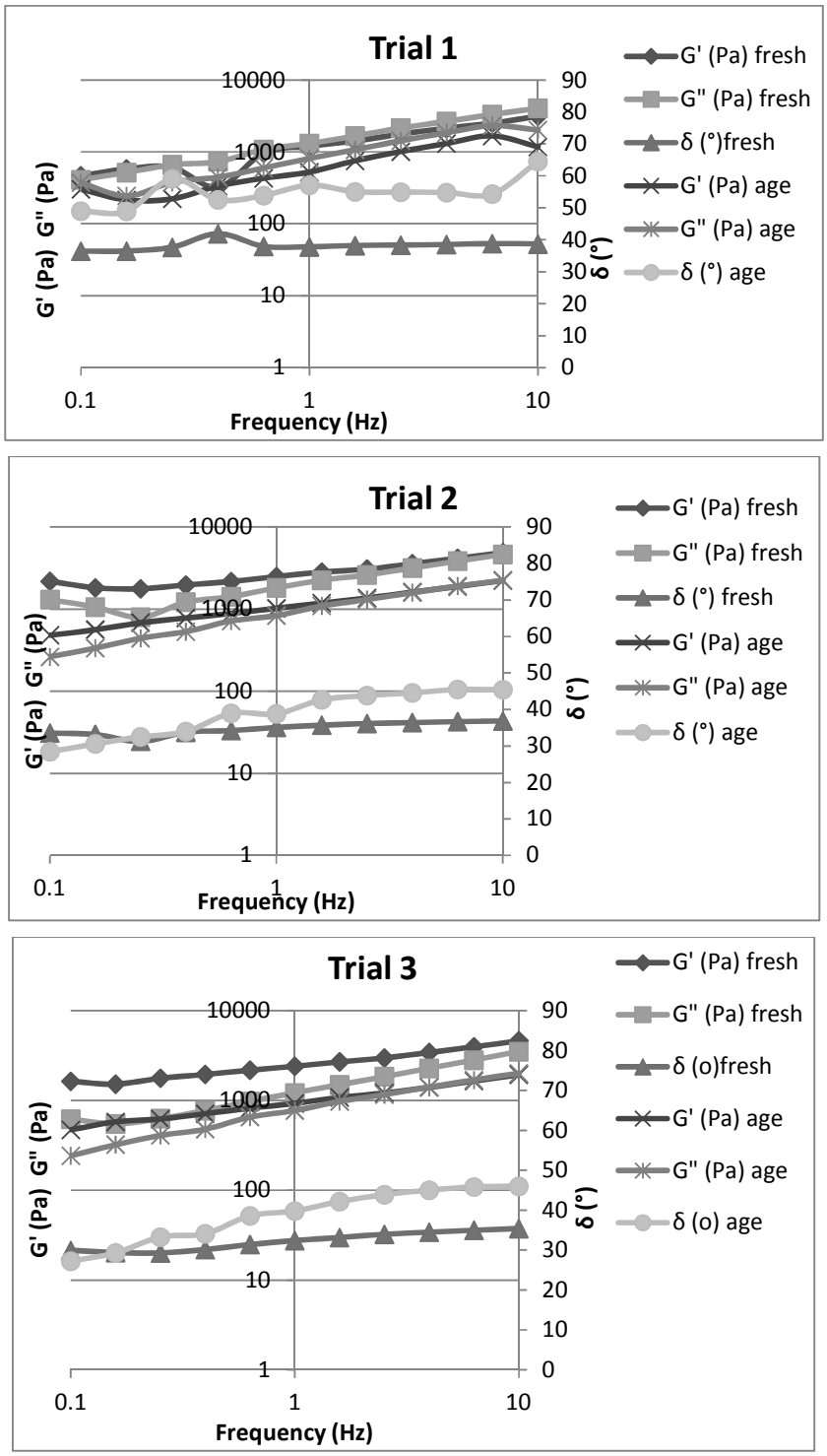


Fig. B2. Frequency sweep of fresh and aged for 24 h foams of Trial 1, 2, 3 from Table 1 (thesis chapter 6). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

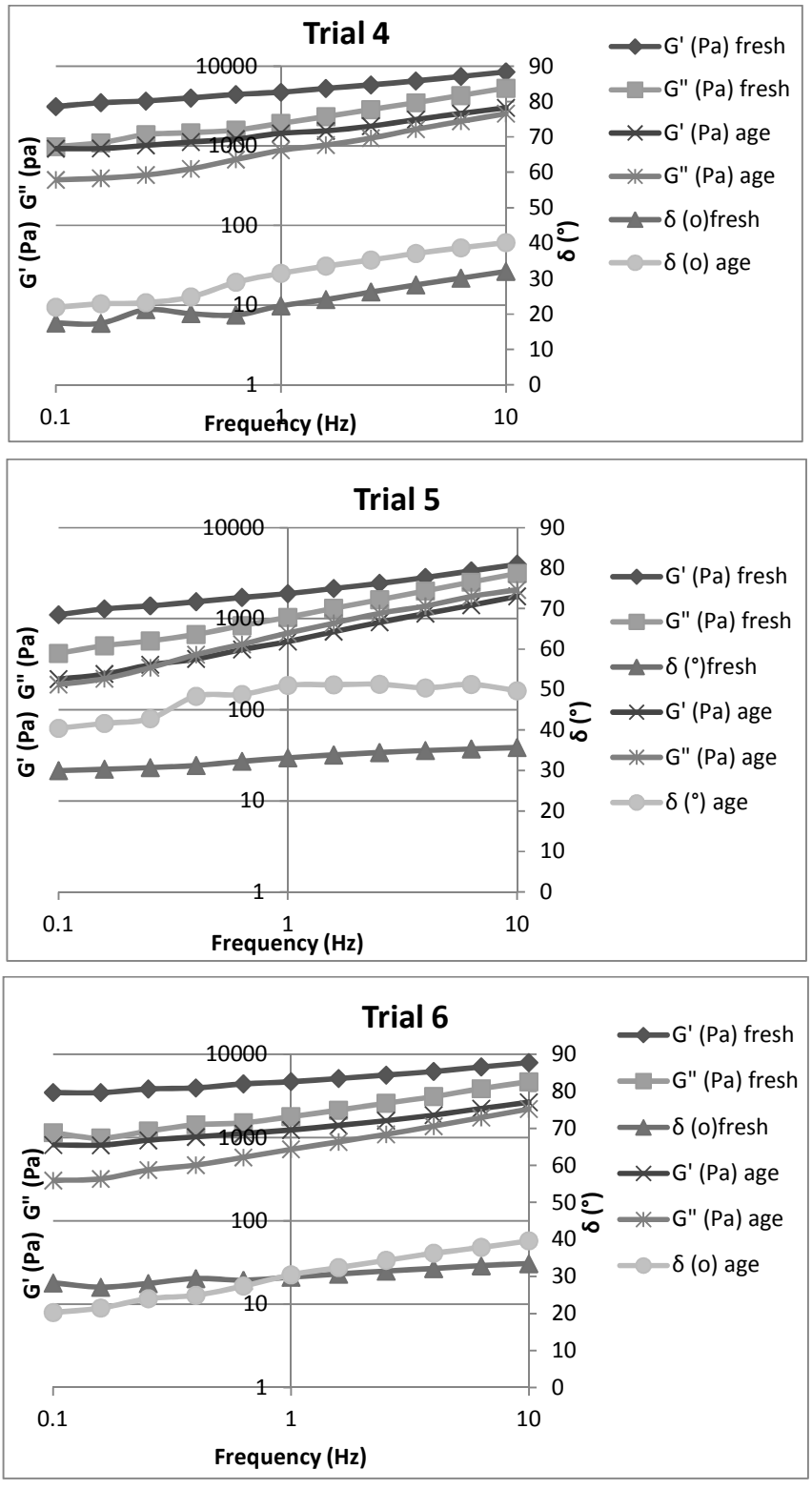


Fig. B3. Frequency sweep of fresh and aged for 24 h foams of Trial 4, 5, 6 from Table 1 (thesis chapter 6). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

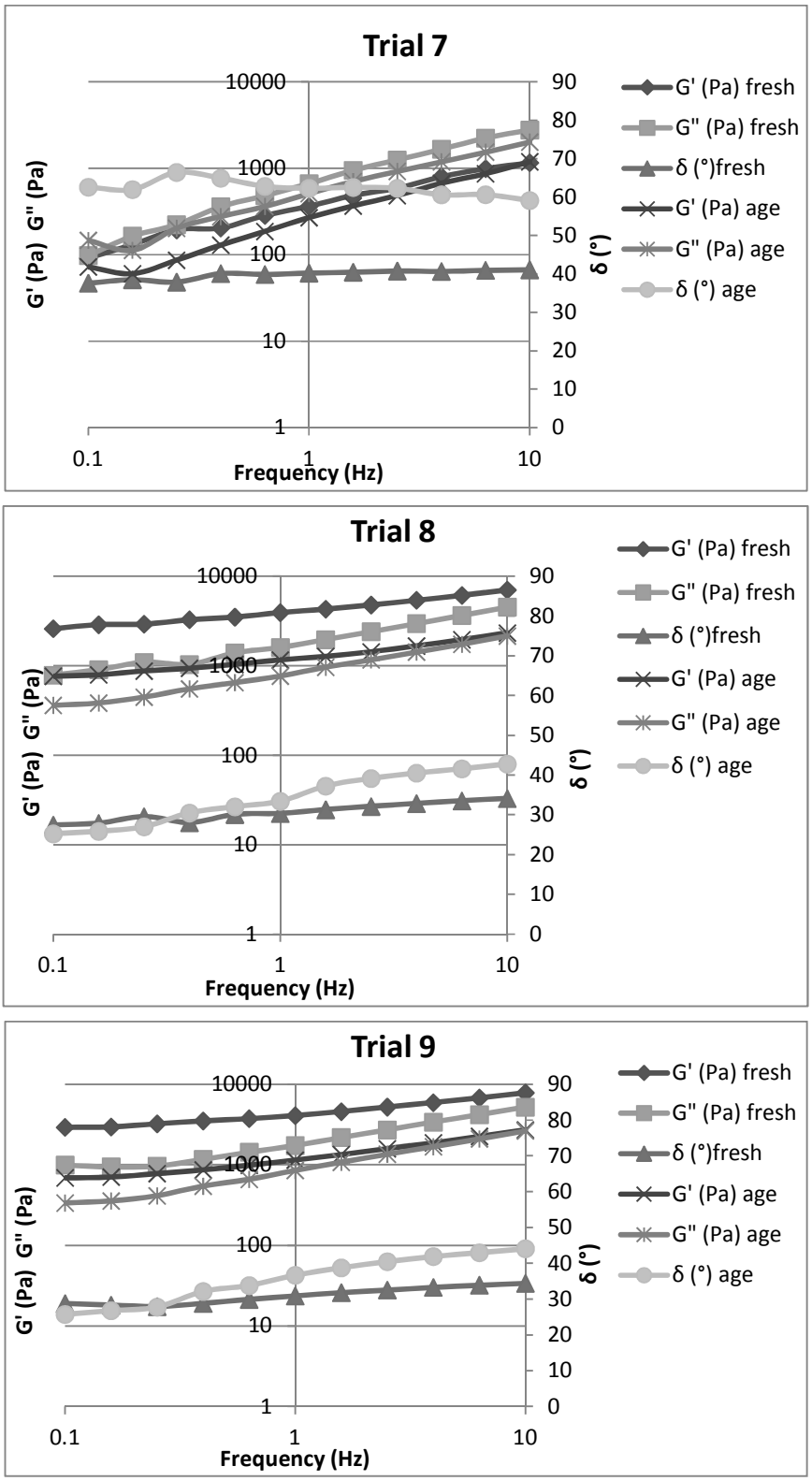


Fig. B4. Frequency sweep of fresh and aged for 24 h foams of Trial 7, 8, 9 from Table 1 (thesis chapter 6). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.

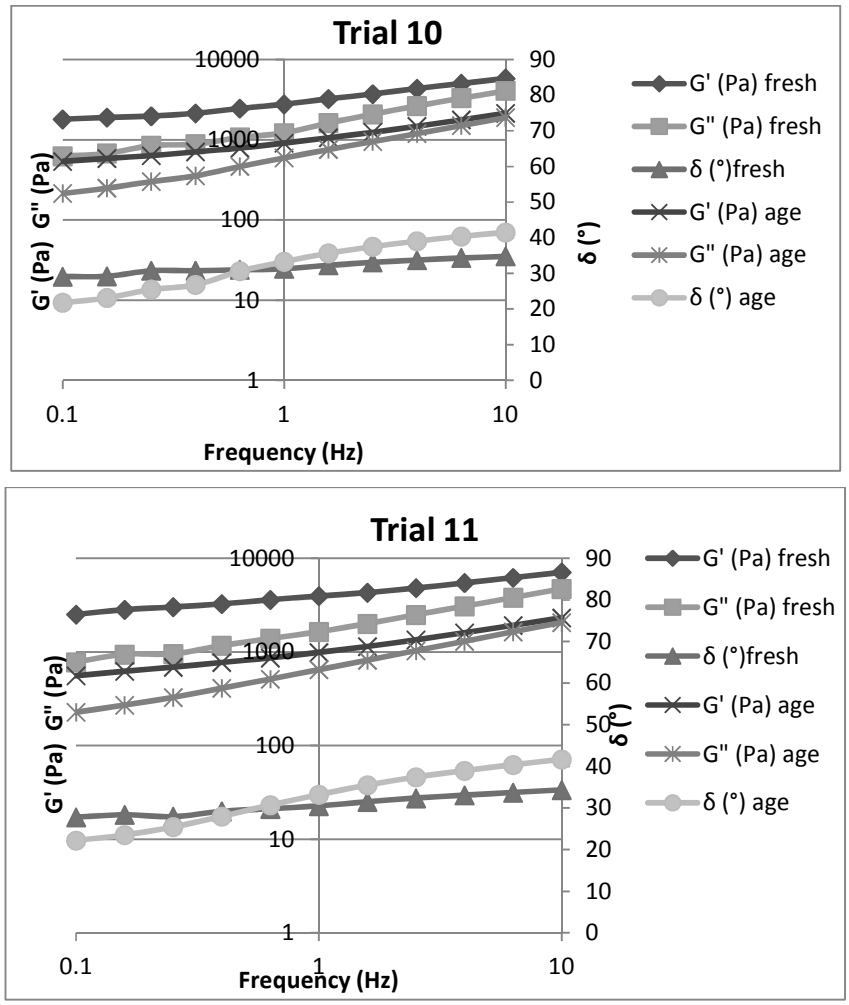


Fig. B5. Frequency sweep of fresh and aged for 24 h foams of Trial 10, 11 from Table 1 (thesis chapter 6). Elastic modulus G', Viscous modulus *G"* and phase angle δ are shown.