**UNIVERSIDADE ESTADUAL DE MARINGÁ**
**CENTRO DE CIÊNCIAS AGRÁRIAS**
**Programa de Pós-Graduação em Ciência de Alimentos**

# GOMA DE *ACACIA MEARNSII* DE WILD: UMA ALTERNATIVA À GOMA ARÁBICA COMO ESTABILIZANTE DE ALIMENTOS

**FERNANDO ANTÔNIO ANJO**

Maringá
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Tese apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos.

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# BIOGRAFIA

Fernando Antônio Anjo, nascido em 22 de junho 1991 na cidade de São Pedro da União, Minas Gerais, Brasil. Filho de José Francisco Anjo e Maria Aparecida Maia. Concluiu o ensino médio no ano de 2009 na Escola Estadual Coronel Antônio Domingos Ribeiro, Bom Jesus da Penha, Minas Gerais, Brasil. Iniciou o curso de Engenharia de Alimentos pela Universidade Tecnológica Federal do Paraná – Campus Campo Mourão em 2010 obtendo o título de Engenheiro de Alimentos em 2016. Ingressou no Programa de Pós-graduação em Ciência de Alimentos da Universidade Estadual de Maringá em março de 2016, obtendo o título de Mestre em Ciência de Alimentos em fevereiro de 2018. Ainda em 2018 ingressou no Programa de Pós-graduação em Ciência de Alimentos da Universidade Estadual de Maringá em nível de doutorado, com defesa da tese em março de 2021. Desde 2016 é membro do Grupo de Pesquisa em Alimentos Funcionais da Universidade Estadual de Maringá - UEM. Atua nas áreas de tecnologia de produtos agropecuários, tecnologia de fermentações, alimentos funcionais, compostos bioativos, controle e gestão da qualidade e no estudo de novas gomas brasileiras e sua aplicação na indústria de alimentos.

***Dedico***

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# APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos.

1 Fernando Antônio Anjo; Bianka Rocha Saraiva; Jessica Bassi da Silva; Yasmin Carla Ribeiro; Marcos Luciano Bruschi; Izabel Cristina Riegel-Vidotti; Fernanda Fogagnoli Simas; Paula Toshimi Matumoto-Pintro. *Acacia mearnsii* gum: A residue as an alternative gum Arabic for food stabilizer. Food Chemistry.

2 Fernando Antônio Anjo; Bianka Rocha Saraiva; Jessica Bassi da Silva; Camilla Yara Langer Ogawa; Francielle Sato; Marcos Luciano Bruschi; Izabel Cristina Riegel-Vidotti; Fernanda Fogagnoli Simas; Paula Toshimi Matumoto-Pintro. A new food stabilizer in technological properties of low-fat processed cheese. Food hydrocolloids.

# GENERAL ABSTRACT

**INTRODUCTION.** Used for the commercial production of tannins, charcoal and in the paper industry, *Acacia mearnsii* De Wild trees naturally produce an exudate (gum), which is not applied industrially, and is considered a crop residue. Recent studies show that the gum Arabic obtained from *A. mearnsii* has a chemical composition similar to the commercial gum Arabic, which are obtained from the species *Acacia senegal* and *Acacia seyal*. *A. mearnsii* gum is a complex, hyper-branched amphiphilic heteropolysaccharide, composed of galactose, arabinose, rhamnose, uronic acids and proteins with physicochemical characteristics that allow it to act as a food stabilizer/emulsifier. Gums obtained from trunk exudate and tree branches are among the commercial hydrocolloids commonly used by the food industry. When added to food preparations, gums give the product a thickening effect, stabilize foams, emulsions and dispersions, inhibit syneresis and crystal formation (ice and sugar) and control the release of flavors. Considering the technological applications of gums, their use in the production of dairy products, such as ice cream and processed cheese (products formed by emulsions), can contribute to the development of new products with different technofunctional characteristics than traditional products.

**AIMS.** The objective of this study was to characterize the polysaccharides from *A. mearnsii* gum, to evaluate the safety of their use by cytotoxicological tests and their stabilizing potential by physicochemical, rheological and texture analyzes, in dairy products that formed by emulsions, such as ice cream and processed cheese.

**MATERIAL AND METHODS.** The polysaccharides from *A. mearnsii* were obtained after aqueous extraction of the raw gum, collected from trees grown in the state of Rio Grande do Sul, Brazil (kindly provided by the company Seta SA). Polysaccharides from *A. mearnsii* were characterized by nuclear magnetic resonance (NMR) analysis and Fourier transform infrared spectroscopy (FTIR). The cytotoxicity of *A. mearnsii* gum and cell proliferation analyzes were performed on Balb/3T3 cells (non-tumor murine fibroblasts) and HepG2 (human hepatocarcinoma). *A. mearnsii* gum and the commercial gums of *A. senegal* and *A. seyal* were used as stabilizers in ice cream formulations, AMS - ice cream with *A. mearnsii* gum, ASG - ice cream with *A. senegal* gum and ASY - ice cream with *A. seyal* gum. Ice creams were evaluated for their physicochemical, texture and rheological properties. *A. mearnsii* gum was used as a stabilizer in low-fat processed cheese formulations. Processed cheeses had their fat percentage reduced by 50% and were added with different concentrations of gum. Six formulations were prepared: STAN - standard processed cheese; CONT - low-fat processed cheese without *A. mearnsii* gum; PC125 - low-fat processed cheese and 0.125% *A. mearnsii* gum; PC250 - low-fat processed cheese and 0.250% *A. mearnsii* gum; PC375 - low-fat processed cheese and 0.375% *A. mearnsii* gum and PC500 - low-fat processed cheese and 0.500% *A. mearnsii* gum. The chemical composition, color, physicochemical, textural and viscoelastic properties of processed cheeses were analyzed.

**RESULTS AND DISCUSSION.** *A. mearnsii* gum presented a carbohydrate composition similar to commercial gum Arabic. Galactose, arabinose, rhamnose, aliphatic galactoproteins, uronic acid, arabinogalactan proteins and pyranose rings were identified by NMR and FTIR. The results obtained in cytotoxicity tests show that the use of *A. mearnsii* gum is safe in its various applications, since the treatment of the gum did not affect cell proliferation nor was there a loss of cell viability of both tested cell lines. Ice cream prepared with *A. mearnsii* gum (AMS) showed higher viscosity than with commercial gum Arabic. Protein present in the *A. mearnsii* gum provided an increase in colloidal particles of the ice cream (denatured proteins, caseins, etc.) and increased viscosity. The molecular interactions between mixture components increased the hardness, reducing the overrun of AMS. AMS and ASY show resistance when subjected to a shear stress (higher shear rates to initiate the flow/deformation), indicating the

presence of a more structured and robust three-dimensional network due to molecular interactions, reducing the tendency to melt. The observation of a smaller hysteresis area of AMS allowed to conclude that its structure is recovered more quickly than other samples prepared with commercial gum Arabic when subjected to a deformation; and it is a more stable system - which is an important property of ice cream to be resistant to adverse conditions during the production chain (from processing conditions to consumption). Low-fat processed cheeses were made with and without the addition of *A. mearnsii* gum, and showed a 40% reduction in calories. The gum inclusion in processed cheeses increased the carbohydrates concentration, and PC500 sample had protein content similar to STAN sample. The yellow color of processed low-calorie cheeses was affected, and the gum inclusion made the yellowing index of processed cheeses intermediate in relation to STAN and CONT. The texture profile analysis showed that the low-fat processed cheeses were softer and more spreadable than STAN. In the frequency sweep, low-fat cheeses processed presented smaller elastic (*G'*) and viscous (*G''*) modulus than STAN; but the increase in gum concentration raised the values of *G'* and *G''* according to frequency increase, possibly due to interactions between protein aggregates and the gum in the continuous phase, improving the elastic and viscous properties of PC125, PC250, PC375. PC500 showed higher *G'* and *G''* among low-fat processed cheeses and viscoelastic behavior (same behavior of STAN), probably due to the higher gum concentration, which increases the number of molecular interactions between the constituents, helping in their emulsification, making the processed cheese stronger and with a harder and less spreadable consistency. The oscillation thermometry showed that milk fat has an influence on the viscoelastic characteristics of processed cheeses. STAN showed elastic behavior throughout the temperature scan and the samples with low fat content showed viscoelastic behavior due to possible interactions between protein-protein and protein-carbohydrate, mainly occurring at temperatures above 45 °C. STAN showed changes in viscoelastic properties due to the melting and solidification of milk fat during the temperature variation of the cycles. Processed cheeses with low fat content showed viscous behavior throughout the temperature range tested in the cycles. In all processed cheeses, the data were reproducible, the behavior observed in the first temperature cycle, was repeated in the second. The maintenance of the viscous behavior of processed cheeses with low fat content shows that even after variations in temperature, the processed cheeses maintain a soft texture and preserve their spreadability.

**CONCLUSIONS.** *A. mearnsii* gum is safe to use in food process; however, more toxicity tests need to be carried out. The ice cream made with *A. mearnsii* gum had technological characteristics (physicochemical and rheological) relevant to industrial processes and the commercialization of the product. Fat reduction and the inclusion of *A. mearnsii* gum influence the mechanical and textural properties of processed cheeses. The low-fat processed cheeses and with *A. mearnsii* gum addition became softer and more spreadable than the standard sample, and proved to be stable when subjected to temperature variation cycles. The results show that *A. mearnsii* gum can be used by the food industry as a stabilizer and can become a source of income and not a residue in the production chain of *A. mearnsii*.

**Keywords:** hydrocolloids, gums, ice cream, processed cheese, textural properties, rheological properties.

# RESUMO GERAL

**INTRODUÇÃO.** Utilizada para a produção comercial de taninos, carvão vegetal e na indústria de papel, as árvores da espécie *Acacia mearnsii* De Wild produzem naturalmente um exsudato (goma), que não é aplicado industrialmente, sendo considerado um resíduo do cultivo. Estudos recentes mostram que a goma arábica obtida de *A. mearnsii* possui composição química semelhante às gomas Arábicas comerciais, as quais são obtidas a partir das espécies *Acacia senegal* e *Acacia seyal*. A goma de *A. mearnsii* é um heteropolissacarídeo anfifílico complexo, hiper-ramificado, composto por galactose, arabinose, ramnose, ácidos urônicos e proteínas com características físico-químicas que a permitem atuar como um estabilizador/emulsificante de alimentos. Gomas obtidas do exsudato de tronco e galhos de árvores estão entre os hidrocoloides comerciais comumente usados pela indústria de alimentos. Quando adicionadas a preparações alimentícias as gomas conferem ao produto efeito espessante, estabilizam espumas, emulsões e dispersões, inibem sinérese e formação de cristais (gelo e açúcar) e controlam a liberação de sabores. Considerando as aplicações tecnológicas das gomas, seu uso na elaboração de produtos lácteos, como sorvete e queijo processado (produtos formados por emulsões), pode contribuir no desenvolvimento de novos produtos com características tecnofuncionais diferenciadas dos produtos tradicionais.

**OBJETIVOS.** O objetivo deste estudo foi caracterizar os polissacarídeos da goma de *A. mearnsii*, avaliar a segurança de seu uso a partir de testes citotoxicológicos e por meio de análises físico-químicas, reológicas e de textura, avaliar seu potencial estabilizador em produtos lácteos tipicamente formados por emulsões como, sorvete e queijo processado.

**MATERIAL E MÉTODOS.** Os polissacarídeos de *A. mearnsii* foram obtidos após extração aquosa da goma bruta, coletada de árvores cultivadas no estado do Rio Grande do Sul, Brasil (gentilmente cedida pela empresa Seta SA). Os polissacarídeos da goma de *A. mearnsii* foram caracterizados por análises de ressonância magnética nuclear (RMN) e por espectroscopia no infravermelho com transformada de Fourier (FTIR). A citotoxicidade da goma *A. mearnsii* e análises de proliferação celular foram realizadas em células Balb/3T3 (fibroblastos murinos não tumorais) e HepG2 (hepatocarcinoma humano). Goma de *A. mearnsii* e as gomas comerciais de *A. senegal* e *A. seyal* foram utilizadas como estabilizantes em formulações de sorvetes, AMS - sorvete com goma *A. mearnsii*, ASG - sorvete com goma *A. senegal* e ASY - sorvete com goma *A. seyal*. Os sorvetes foram avaliados quanto as suas propriedades físico-químicas, de textura e reológicas. A goma de *A. mearnsii* foi utilizada como estabilizante em formulações de queijo processado com baixo teor de gordura. Os queijos processados tiveram seu percentual de gordura reduzido em 50% e foram adicionados de diferentes concentrações de goma. Foram preparadas seis formulações: STAN - queijo processado padrão; CONT - queijo processado com baixo teor de gordura sem goma *A. mearnsii*; PC125 - queijo processado com baixo teor de gordura e 0,125% de goma *A. mearnsii*; PC250 - queijo processado com baixo teor de gordura e 0,250% de goma *A. mearnsii*; PC375 - queijo processado com baixo teor de gordura e 0,375% de goma *A. mearnsii* e PC500 - queijo processado com baixo teor de gordura e 0,500% de goma *A. mearnsii*. Foram analisadas a composição química, cor, propriedades físico-químicas, texturais e viscoelásticas dos queijos processados.

**RESULTADOS E DISCUSSÃO.** A goma de *A. mearnsii* apresentou composição de carboidratos semelhante as gomas arábicas comerciais. Galactose, arabinose, ramnose, galactoproteinas alifáticas, ácido urônico, proteínas arabinogalactanas e anéis de piranose foram identificados por RMN e FTIR. Os resultados obtidos em

testes de citotoxicidade mostram que o uso da goma de *A. mearnsii* é seguro em suas várias aplicações, pois, o tratamento da goma não afetou a proliferação celular nem houve perda de viabilidade celular de ambas as linhas celulares testadas. Sorvete preparado com goma de *A. mearnsii* (AMS) apresentou maior viscosidade do que o preparado com as gomas Arábicas comerciais. As proteínas presentes na goma de *A. mearnsii* proporcionaram o aumento de partículas coloidais do sorvete (proteínas desnaturadas, caseínas, etc.) e o aumento da viscosidade. As interações moleculares entre os componentes da mistura aumentaram a dureza, diminuindo o *overrun* de AMS. AMS e ASY apresentam resistência quando submetidos a uma tensão de cisalhamento (maiores taxas de cisalhamento para iniciar o escoamento/deformação), indicando a presença de uma rede tridimensional mais estruturada e robusta devido às interações moleculares, reduzindo a tendência ao derretimento. A observação de uma menor área de histerese de AMS permitiu concluir que sua estrutura é recuperada mais rapidamente do que outras amostras preparadas com gomas comerciais quando submetida a uma deformação; e é um sistema mais estável - que é uma propriedade importante do sorvete para ser resistente a condições adversas durante a cadeia produtiva (das condições de processamento até o consumo). Queijos processados com baixo teor de gordura foram elaborados com e sem adição de goma de *A. mearnsii*, e apresentaram redução de 40% de calorias. A inclusão de goma em queijos processados elevou a concentração de carboidratos, e a amostra PC500 apresentou conteúdo proteico similar a amostra STAN. A cor amarela dos queijos processados de baixa caloria foi afetada, e a inclusão da goma tornou o índice de amarelecimento dos queijos processados intermediário em relação ao STAN e CONT. A análise do perfil de textura mostrou que os queijos processados com baixo teor de gordura apresentaram-se mais macios e espalháveis que a amostra STAN. Na varredura de frequência, queijos processados com baixo teor de gordura apresentaram menores módulos de elástico (*G'*) e viscoso (*G"*) que STAN; mas o aumento na concentração de goma elevou os valores de *G'* e *G"* de acordo com o aumento da frequência, possivelmente, devido a interações entre agregados de proteínas e a goma na fase contínua, melhorando as propriedades elásticas e viscosas de PC125, PC250, PC375. O PC500 apresentou maior *G'* e *G"* entre os queijos processados com baixo teor de gordura e comportamento viscoelástico (mesmo comportamento de STAN), provavelmente devido à maior concentração de goma, que aumenta o número de interações moleculares entre os constituintes, auxiliando na sua emulsificação, tornando o queijo processado mais forte e com consistência mais dura e menos espalhável. A termorreometria de oscilação mostrou que a gordura láctea tem influência sobre as características viscoelásticas dos queijos processados. STAN apresentou comportamento elástico ao longo de toda varredura de temperatura e as amostras com baixo teor de gordura apresentaram comportamento viscoelástico devido a possíveis interações entre proteína-proteína e proteína-carboidrato principalmente ocorridas em temperaturas acima de 45°C. STAN apresentou alterações nas propriedades viscoelásticas devido ao derretimento e solidificação da gordura láctea durante a de variação de temperatura dos ciclos. Queijos processados com baixo teor de gordura apresentaram comportamento viscoso em toda a faixa de temperatura testada nos ciclos. Em todos os queijos processados, os dados foram reproduzíveis, o comportamento observado no primeiro ciclo de temperatura, repetiu-se no segundo. A manutenção do comportamento viscoso dos queijos processados com baixo teor de gordura mostra que mesmo após variações de temperatura os queijos processados mantêm uma textura macia e preservam sua espalhabilidade.

**CONCLUSÕES.** Goma de *A. mearnsii* é segura para uso em processos alimentícios,

entretanto, mais testes de toxicidade precisam ser realizados. O sorvete elaborado com goma *A. mearnsii* apresentou características tecnológicas (físico-químicas e reológicas) relevantes para os processos industriais e a comercialização do produto. A redução de gordura e a inclusão da goma *A. mearnsii* influenciam as propriedades mecânicas e texturais de queijos processados. Os queijos com baixo teor de gordura e com adição de goma *A. mearnsii* tornaram-se mais macios e espalháveis que a amostra padrão, mostraram-se estáveis quando submetidos a ciclos de variação de temperatura. Os resultados mostram que a goma *A. mearnsii* pode ser utilizada pela indústria de alimentos como estabilizante e pode se tornar uma fonte de renda e não um resíduo na cadeia produtiva de *A. mearnsii*.

**Palavras chaves:** hidrocoloides, gomas, sorvete, queijo processado, propriedades texturais, propriedades reológicas.

# ARTICLE 1

## *Acacia mearnsii* gum: a residue as an alternative gum Arabic for food stabilizer

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# *Acacia mearnsii* gum: A residue as an alternative gum Arabic for food stabilizer

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ABSTRACT

*Acacia mearnsii* gum is not commercially exploited, being characterized as residue from *A. mearnsii* cultivation. This work investigated the *A. mearnsii* gum polysaccharide composition, its cytotoxicity and the technological effect as a stabilizer in ice cream. *A. mearnsii* gum showed a similar chemical structure to commercial gum Arabic and did not decrease the viability and proliferation of fibroblast cells (Balb/3T3) and hepatocarcinoma (HepG2). Rheological tests showed that the ice cream stabilized by the *A. mearnsii* gum had a more structured system (more interactions between the mixture components) and the same melting characteristics as the ice cream samples made with commercial gum Arabic. The results showed that *A. mearnsii* gum, which is actually an agro-industrial residue from tannin production for industry, is a potential stabilizing gum for the food industry, contributing to the economic development of the exploitation chain of *A. mearnsii* products and by-products.

## 1. Introduction

The species *Acacia mearnsii* De Wild is cultivated in several countries (Australia, South Africa, Zimbabwe, Kenya, Tanzania, Brazil and China) and used for commercial tannins production from bark and the trunk, while its wood is used in energy production from charcoal and in the paper industry (Brown & Ko, 1997). *A. mearnsii* naturally produce an exudate (gum), which has no industrial applicability, being considered a residue from the *A. mearnsii* cultivation that is left in the environment (Brown & Ko, 1997).

Gums obtained from the exudate of trunk and tree branches are among the commercial hydrocolloids commonly used by the food industry (Li & Nie, 2016). Hydrocolloids are polymers (polysaccharides or proteins) that disperse in water, providing a thickening effect, gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavors, etc (Glicksman, 2019). They confer viscosity to food systems when added in low concentrations (<1%) (FDA, 2019), stabilize emulsions and suspensions, control crystallization, inhibit syneresiss and film formation and have a great influence on food's sensory properties (texture, color and lightness) (Milani & Maleki, 2012; Rodríguez et al., 2003).

Gum exudates from *Acacia senegal* and *Acacia seyal* species are used commercially as a stabilizer (gum Arabic – International Numbering System – INS – E414) in food products (FAO, 2006; FDA, 2019). It is known that *A. senegal* gum has better interfacial properties than *A. seyal* gum, therefore, it exhibits better emulsification/stabilization properties, this is one of the characteristics that make *A. senegal* gum the most used commercially (Elmanan et al., 2008; Fauconnier et al., 2000).

The exudate obtained from *A. mearnsii* has a similar chemical composition to *A. senegal* and *A. seyal* exudate and their polysaccharides have a surfactant behavior, being able to act as stabilizers in oil/water emulsions (Grein et al., 2013). *A. mearnsii* gum is composed of carbohydrates with different molar masses and different protein contents (arabinogalactan, arabinogalactan-protein and glycoproteins),

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providing its interfacial activity. *A. mearnsii* gum is amphiphilic, being able to act in interfaces, with the polysaccharide (hydrophilic) portion facing the aqueous solution and the protein (hydrophobic) portion facing the nonpolar phase (Grein et al., 2013). Studies by Grein-Iankovski et al., 2018 and Grein et al., 2013 showed that *A. mearnsii* has a higher protein content, better distinctive behavior in tensiometry tests and greater emulsion stabilizing capacity than commercial gum Arabic, characteristics that can contribute to the improvement of the technological properties of food products.

The Food and Drug Administration (FDA) and Food and Agriculture Organization of the United Nations (FAO) only allow the commercialization of gums obtained from exudates of the species *A. senegal* and *A. seyal*, which are classified as Generally Recognized as Safe – GRAS (FAO, 2006; FDA, 2019). According to the FDA's own legislation (21 CFR 170.3), for an additive to be classified as "safe" all relevant information is considered as consensus among scientists, data on the exposure of the human diet projected to the additive, the toxicological data of the additive, and the published literature (FDA, 2019). The generation of information on the technological effect of *A. mearnsii* gum on food production may contribute to the future classification of the gum as a GRAS additive by the FDA, favoring its production chain and preventing the disposal of an exudate with potential for technological application.

The present study aimed to increase the necessary knowledge about cytotoxicity and to compare technologically the stabilizing effect of *A. mearnsii* gum and commercial gum Arabic in ice cream preparation; a product characterized as an emulsion composed of cryo-concentrated phase, ice crystals, fat globules and air, and which contain stabilizers in their formulation to maintain the dispersion stability of their immiscible phases (fat-in-water emulsion), without food additive use the phase's separation is inevitable (Goff & Hartel, 2013).

## 2. Materials and methods

### 2.1. Materials and reagents

*Acacia senegal* (Instantgum™ AA) and *Acacia seyal* (Instantgum™ BA) gums for food were provided by Nexira Brasil Comercial Ltda. (Sao Paulo, Brazil) and *A. mearnsii* gum crude was provided by SETA company (Rio Grande do Sul, Brazil). Ice cream ingredients were acquired in the local market. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), thyazolyl blue tetrazolium bromide (MTT), neutral red (NR) and crystal violet (CV) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

### 2.2. Extraction of Acacia mearnsii gum polysaccharide

The *A. mearnsii* polysaccharide was obtained after aqueous extraction of crude gum according to Grein et al. (2013). Gum, which was collected from trees planted in the state of Rio Grande do Sul, Brazil (kindly furnished by Seta S.A. company) was stirred with water for 16 h and filtered using a fine cloth. The aqueous extract was recovered, ethanol (3:1 v/v) was added, the mixture was centrifuged (12,000 g, 30 min, 10 °C), and the polysaccharide fraction was obtained. It was dialyzed against distilled water (48 h) a membrane with a 12–14 kDa $M_w$ cutoff (Spectra/Por® Cellulose Ester) and freeze-dried to yield the *A. mearnsii* polysaccharide (37g).

### 2.3. Nuclear magnetic resonance analysis of polysaccharides

Gum polysaccharides were analyzed by 2D-NMR heteronuclear ($^1H/^{13}C$) HSQCed (Edited Heteronuclear Single Quantum Coherence), which were obtained using Bruker AVANCE III 400 MHz spectrometer, at 70 °C, with a 5 mm inverse probe. The chemical shifts of $^1H$ and $^{13}C$ were expressed in δ (ppm).

### 2.4. Acacia mearnsii gum cytotoxicity and its effect on cell proliferation

*A. mearnsii* gum cytotoxicity and cell proliferation analyses were performed on Balb/3T3 (non-tumoral murine fibroblasts) (ATCC) and HepG2 (human hepatocarcinoma) (BCRJ code 0291), which are both good cell models to test the cytotoxicity of new food additives (Boyle, 2008; Knasmüller et al., 2002). The cell lines were cultured in DMEM supplemented with 10% FBS and cell cultures were maintained in a humidified incubator at 37 °C and an atmosphere with 5% $CO_2$. *A. mearnsii* gum was sterilized by exposure to ultraviolet light for 16 h and solubilized in DMEM without FBS. Cells were seeded in 96-well plates (2000 and 3000 cells/well for Balb/3T3 and HepG2 respectively) and after 24 h they were exposed to *A. mearnsii* gum at 10, 100, 1000, and 2500 μg/mL (final concentration), the gum concentrations used for the treatment of cells were determined from the highest concentration (12.5 mg/mL) of gum soluble in cell culture medium (ICCVAM, 2006). Plates were incubated at 37 °C in an atmosphere with 5% $CO_2$ for 72 h.

The following assays were performed: MTT - Thyazolyl Blue Tetrazolium Bromide, to visualize mitochondrial metabolism (Fotakis & Timbrell, 2006); NR – Neutral Red, that visualizes the neutral red dye retention capacity in acid intracellular vesicles (Repetto et al., 2008); and CV – Crystal Violet, a nucleic acid dye, to indicate the cell density adhering to the culture plate (Bonnekoh et al., 1989).

### 2.5. Ice cream processing

The ice cream formulation and manufacturing process was elaborated according to Goff and Hartel (2013) with modifications. Ice cream formulation contained 62.5% pasteurized whole milk, 5% whole milk powder, 10% commercial sucrose, 10% pasteurized milk cream, 1% starch, 5% glucose syrup, 5% invert sugar, 1% commercial emulsifier (water, emulsifiers: distilled fatty acid monoglycerides, potassium stearate, sorbitan monostearate, and sorbitan monostearate polyoxyethylene) and 0.5% stabilizer (w/w). For the purposes of technological comparison, *A. senegal* and *A. seyal* gums were used as standard stabilizers. Three ice cream formulations were prepared with different stabilizers, AMS – ice cream with *Acacia mearnsii* gum, ASG - ice cream with *Acacia senegal* gum and ASY - ice cream with *Acacia seyal* gum.

Ice cream mix was prepared using pasteurized whole milk and milk cream under heating (50 °C/10 min). Solids (milk powder, sucrose, starch and stabilizer) were added and heated at 90 °C/10 min. Ice cream mix was refrigerated at 8 °C for 15 h (aging of mix) and the glucose syrup, invert sugar and emulsifier were added and stirred in a food blender at 3500 rpm for 10 min and frozen in an ice cream maker (Cuisinart, East Windsor, USA) for 30 min (initial vessel temperature was −18 °C). Ice cream (30 g) was placed in polypropylene cups and stored at −18 °C for 24 h before analyses.

### 2.6. Ice cream rheological properties

#### 2.6.1. Continuous shear analysis

Each ice cream formulation was analyzed at −2 °C using a Mars II rheometer (Haake®, Thermo Fisher Scientific Inc., Newington, Germany) fitted with a 35 mm diameter cone-plate separated by 0.104 mm fixed gap. Flow curves for each sample were determined over shear rates ranging from 0 to 2000 $s^{-1}$, increasing the shearing rate over a period of 150 s, held at the upper limit for 10 s, and then decreased over a period of 150 s. The data of flow curves were evaluated and fitted according to the rheological model of Herschel-Bulkley (Eq. (1)) using RheoWin 4.10.0000 (Haake®) software.

$$\tau = \tau_0 + k.\dot{\gamma}^{n} \quad (1)$$

where $\tau$ is the shear stress (Pa), $\tau_0$ is the yield stress value (Pa), $k$ represents the consistency index ([Pa.s]$^n$), $\dot{\gamma}$ is the shear rate ($s^{-1}$) and $n$ is

the flow behavior index (dimensionless).

*2.6.2. Oscillatory rheology*

Firstly, the linear viscoelastic region (LVR) was determined by increasing the torque sweep at a fixed frequency (1 Hz) for each sample. A deformation rate within the LVR was selected for subsequent frequency sweep analyses from 0.1 to 10.0 Hz. G′ and G′′ were calculated using RheoWin 4.20.0003 (Haake®) software.

*2.7. Ice cream physicochemical and texture profile analysis*

Ice cream pH was analyzed with a pH-meter (Tecnopon, mPA-210). Ice cream color was analyzed using a colorimeter (Chroma Meter CR-400, Minolta, USA) and the CIELAB color scale, reading L* (100 = white; 0 = black), a* (+, red; −, green) and b* (+, yellow; −, blue). The volume of air incorporated (overrun) during the freezing process was determined by volume difference from before and after freezing (Marshall et al., 2003). Melting properties were evaluated on 30 g samples which remained at 25 ± 2 °C until complete melting; melting mass was noted every 5 min, and presented as melting curves. Melting rate was obtained from linearization curves and first drop time (Tekin et al., 2017).

Texture profile analysis (TPA) was performed using a texture analyzer CT-III (Brookfield, Middleborough, USA). Each sample (30 g, 44 mm diameter, 18 mm height) was removed from storage (-18 °C) and immediately analyzed (room temperature 25±2 °C) using an acrylic circular probe TA/4-1000 (38.1 mm diameter and 20 mm height) with 50 g trigger, and 2 mm/s speed.

*2.8. Statistical analysis*

All experiments were performed three times using technical triplicates in each one and expressed as arithmetic mean and standard deviation. The physical analysis results and the rheological parameters obtained from the mathematical models were subjected to analysis of variance (ANOVA) analysis and, when significantly different ($p \leq 0.05$), means were compared by post-hoc test, using Statistical Analysis System (SAS) 9.1 software package (SAS Institute Inc., Cary, NC, USA). Cytotoxicity and cell proliferation results represent at least three independent experiments performed in triplicate and are presented as floating bars (min to max) with lines at media. All treated groups were compared to the untreated control (dashed line, normalized as 1) of each respective experiment. One-way ANOVA with Tukey's multiple comparison test were used for comparison. Gray filled space shows the interval of 0–30% reduction of each parameter.

## 3. Results and discussion

*3.1. NMR characterization of polysaccharides*

The anomeric regions of 2D-NMR HSQCed spectra of polysaccharides (Fig. 1) showed that even though there are similarities, there were fine structural differences between then. *A. mearnsii* polysaccharide showed typical signals compared to those previously published (Grein et al., 2013). There were eight anomeric signals indicating the high structural complexity of the sample. $^{1}H/^{13}C$ signals at $\delta$ 5.011/107.3, $\delta$ 5.108/106.8, $\delta$ 5.187/109.2, and $\delta$ 5.326/108.0 were attributed to H-1/C-1 correlations of 5-*O*-, 3,5-di-*O*-, 3-*O*-substituted, and non-reducing end units of α-$_{L}$-Ara*f* units, respectively (Grein et al., 2013; Petkowicz et al., 1998; Tischer et al., 2002). Signals at $\delta$ 4.414/102.9 and $\delta$ 4.662-/103.03 were assigned to H-1/C-1 of β-$_{D}$-Gal*p* main-chain units and β-$_{D}$-Glc*p*A side-chain units, respectively (Tischer et al., 2002). H-1/C-1 signals at $\delta$ 4.734/100.3 and $\delta$ 5.026/101.2 could be from α-$_{L}$-Rha*p* units in different chemical environments (Delgobo et al., 1999; Gorin & Mazurek, 1975).

Comparing anomeric regions of HSQC spectra of both commercial gums (Fig. 1) it could be noticeable some important differences. *A. seyal* polysaccharide spectrum had fourteen anomeric signals while that from *A. senegal* had only seven, indicating the more complex structure of *A. seyal* polysaccharide. Both commercial gums showed signals from H-1/C-1 correlations of 3-*O*-substituted ($\delta$ 5.203/109.5 and $\delta$ 5.170/109.2) and non-reducing end units ($\delta$ 5.322/108.2 and $\delta$ 5.283/108.3) of α-$_{L}$-Ara*f* units. However, only *A. seyal* polysaccharide HSQC spectrum had signals at $\delta$ 5.018/107.5, which were from α-$_{L}$-Ara*f* 5-*O*-substituted (Petkowicz et al., 1998). Signals from β-$_{D}$-Gal*p*, β-$_{D}$-Glc*p*A, and α-$_{L}$-Rha*p* units were detected in both spectra at $\delta$ 4.415(4.409)/103.0, $\delta$ 4.630(4.609)/103.5(104.3), and $\delta$ 4.692(4.683)/100.7, respectively. Beyond β-$_{D}$-Gal*p* units, *A. seyal* polysaccharide have also α-$_{D}$-Gal*p* units. Anomeric signals at $\delta$ 5.256/97.2, $\delta$ 5.209/98.1, $\delta$ 5.134/97.3, $\delta$ 5.125/ 99.9, and $\delta$ 5.059/976 could be attributed to 3,4-di-*O*-, 3,4,6-tri-*O*-, 3,6- di-*O*-, 3-*O*-, and non-reducing end units of α-$_{D}$-Gal*p* units (Li et al., 2020). Taken together HSQC results showed that all three polysaccharides have intrinsic structural differences, which in turn can influence their properties and some particularities in their applications.

*3.2. Cytotoxicity and cell proliferation*

*A. mearnsii* gum cytotoxicity and its effect on cell proliferation are shown in Fig. 2. Tests were performed on Balb/3T3 cells (non-tumoral murine fibroblasts), which are cells used for the assessment and general toxicity comparison, and on HepG2 cells (human hepatocarcinoma), which were cells that have many characteristics of normal hepatic cells and are used as an alternative animal model, and on isolated hepatocytes

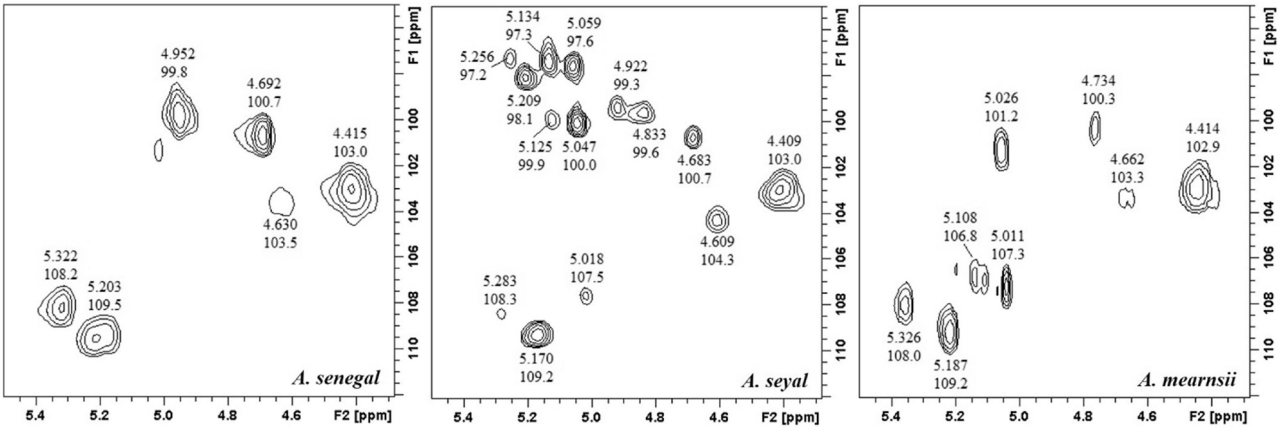


**Fig. 1.** Anomeric regions of 2D-NMR heteronuclear ($^{1}H/^{13}C$) HSQCed spectra of gums polysaccharides.

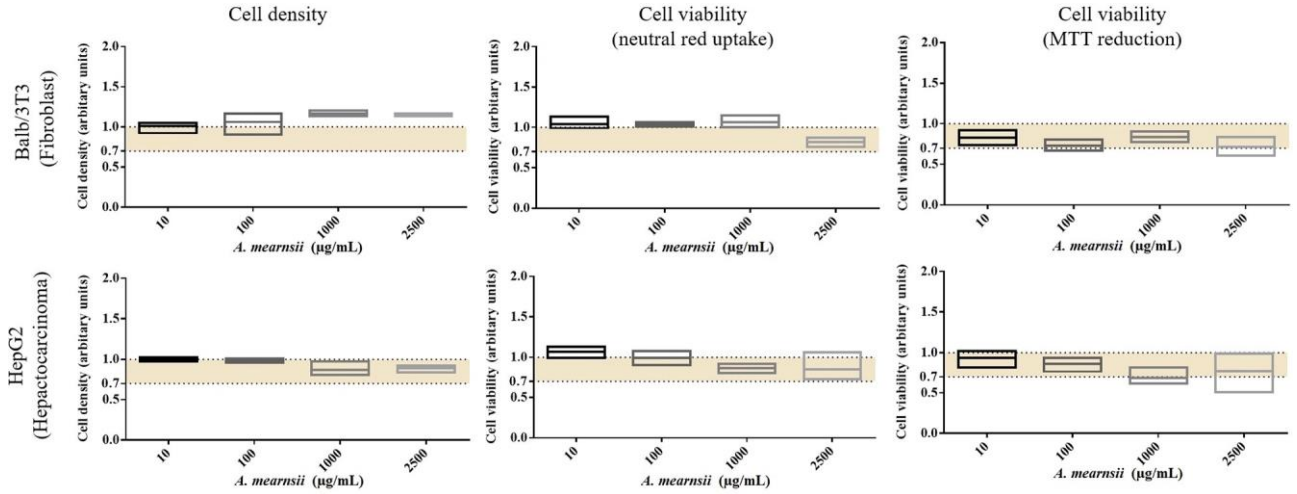


**Fig. 2.** *Acacia mearnsii* gum cytotoxicity screening. Mouse fibroblasts (Balb/3T3) or human hepatocarcinoma cells (HepG2) were cultivated in the presence of four concentrations of *A. mearnsii* gum for 72 h. Cells were colorimetric assayed for determination of cell density (by crystal violet dye) and cell viability (by neutral red dye uptake or MTT reduction). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Radko et al., 2013). Results showed that Balb/3T3 and HepG2 cells are able to capture neutral red and maintain mitochondrial metabolism after 72 h of being exposed to *A. mearnsii* gum from 10 to 2500 µg/mL (Fig. 2) indicating that gum did not induce loss in cell viability. Moreover, *A. mearnsii* gum treatment did not affect cell proliferation of both tested cell lines (Fig. 2). In addition to statistical analysis, a threshold of more than 30% reduction in any of the studied parameters (represented by the gray filled space in Fig. 2) was considered as biologically significant cytotoxicity (Standardization, 2009). Neither statistical nor biologically significant changes were detected in all range of tested concentrations. Cytotoxicity tests are necessary for the new food ingredients approval and based on the similar *A. mearnsii* gum chemical characteristics with commercial gum Arabic (Grein et al., 2013), on the results obtained in cytotoxicity tests and in studies showing that gum Arabic use is safe and its various applications (Al-Yahya et al., 2009; Gado & Aldahmash, 2013; Gamal-Eldeen et al., 2014; Gamal el-din, 2003; Renuga Devi et al., 2015), these results contribute to understanding of the *A. mearnsii* gum food safety.

### 3.3. Ice cream rheological properties

Flow behavior analyses of ice cream samples were performed to evaluate apparent viscosity and shear stress in response to shear rate. All samples presented characteristic shear-thinning non-Newtonian behavior (Fig. 3A and 3B), in which the viscosity decreases and shear stress increases with increased shear rates. This is considered a typical behavior for polymeric macromolecular systems, in which the three-dimension network of the molecules exhibit a tendency to align on the flow direction, dissociate or assume another conformation and thus reduce viscosity (Schramm, 2006). Ice cream is an emulsion formed by four phases (cryo-concentrate, ice crystals, fat globules and air), with complex composition, and when submitted to a shear stress the new rearrangement of particles decreases resistance to flow and apparent viscosity. Ice cream prepared with *A. mearnsii* gum has higher viscosity than those prepared with commercial gums. This is maybe due the relative higher viscosity of *A. mearnsii* gum as described previously (Grein et al., 2013). A time-dependent flow behavior was observed for all samples, since the downward ramp was not superimposed on upward ramp. However, samples prepared with *A. mearnsii* gum showed lower hysteresis area (Table 1) being its structure recovered more rapidly than other samples that were prepared with commercial gums.

The gums when used in ice cream production contribute to mixture viscosity, this increase in viscosity helps in emulsion proteins stabilization avoiding the loss of serum, help in the suspension of flavoring particles, create a stable foam with easy cut. During storage, they slow the migration of moisture or air from the product to packaging and prevent the overrun reduction. Gums give body to the ice cream, reduce the growth of ice crystals and lactose during storage, especially when there are temperature variations (Bahramparvar & Tehrani, 2011).

*A. mearnsii* gum has a higher degree of branching, greater dispersion and lower average molar mass than commercial gum Arabic (Grein-Iankovski et al., 2016; 2018). Among commercial gums, *A. senegal* gum has a less branched and compact structure than *A. seyal* gum (Elmanan et al., 2008). This differentiated structure of each gum allows for greater or lesser interaction and structuring between the components, and influences the overrun, melting and hardness. As seen in Table 1, AMS and ASG have similar hardness and both have greater hardness than ASY. ASY showed also statistical difference at overrun, which was higher than AMS but similar with ASG. No statistical differences were observed between the melting profile (Fig. 4 and Table 1). The structure of *A. mearnsii* gum and *A. senegal* gum, generates a greater increase in viscosity to the system, highly viscous systems do not favor foaming capacity, but do favor foam stability, forming a dense and less creamy ice cream (Wildmoser et al., 2004).

In addition to gums degree of branching, the protein content may also have influenced aeration (overrun). *A. mearnsii* gum has 7% protein (Grein et al., 2013), *A. senegal* gum has 2% and *A. seyal* gum has 0.7% (Elmanan et al., 2008). The gums protein is important for its performance as a stabilizer, a role played due to the polar (serine and histidine) and non-polar (alanine and valine) amino acids present in the gum. The amphiphilic proteins in the gums (complex polysaccharides) help the ice cream stabilization process; protein chains will form interactions with hydrophilic (milk solid-not-fat) and hydrophobic (milkfat) phases, and the polysaccharide chains interact with the water phase (milk) (da Silva et al., 2015). Proteins are soluble polymers of high molecular weight, this increases viscosity, even at low concentrations due to the increased interactions between proteins and the mixture components. These interactions provide an increase in colloidal particles (denatured proteins, caseins, etc.) in suspension from the other ingredients, and increase the ice cream viscosity (Fig. 3B) (Daw & Hartel, 2015).

The rheological parameters obtained from fitting the flow curves to Herschel-Bulkley model are shown in Table 1. AMS and ASY shows greater resistance when subjected to a shear stress (higher shear rates to start the flow/deformation); these samples showed higher yield stress values ($\tau_0$). The necessary tension to start the shear is related to sample's

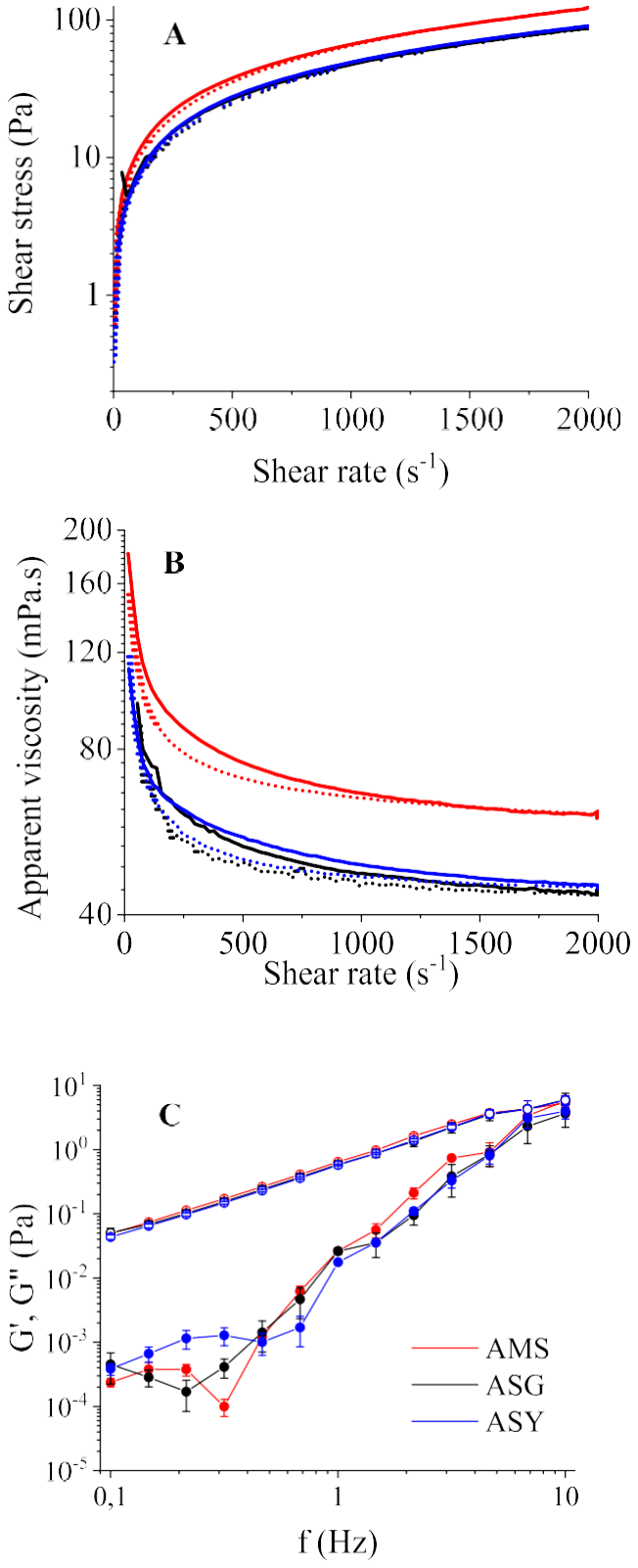


**Fig. 3.** Ice cream continuous flow rheology curves: (A) shear stress as a function of shear rate; (B) apparent viscosity as a function of shear rate. Upward curve - solid line; downward curve – dotted line; (C) ice cream oscillatory rheology curves. G′ – storage module (solid symbols); G″ – loss module (open symbols); f – frequency. AMS – ice cream with *Acacia mearnsii* gum; ASG – ice cream with *Acacia senegal* gum; ASY – ice cream with *Acacia seyal* gum.

**Table 1**
Physicochemical properties, texture profile analysis (TPA) and Herschel-Bulkley fit of ice cream.

| | AMS | ASG | ASY |
|---|---|---|---|
| *Physicochemical properties* | | | |
| pH | 7.57 ± 0.08$^{a}$ | 7.29 ± 0.15$^{b}$ | 7.49 ± 0.03$^{a}$ |
| L* | 85.53 ± 0.77$^{b}$ | 88.08 ± 0.70$^{a}$ | 88.53 ± 0.29$^{a}$ |
| a* | −1.74 ± 0.06$^{a}$ | −4.06 ± 0.24$^{b}$ | −3.89 ± 0.13$^{b}$ |
| b* | 12.01 ± 0.65$^{b}$ | 13.02 ± 1.13$^{a}$ | 12.76 ± 0.65$^{ab}$ |
| Overrun (%) | 19.27 ± 2.90$^{b}$ | 24.33 ± 2.09$^{ab}$ | 27.96 ± 2.16$^{a}$ |
| Melting rate (g/min) | 0.64 ± 0.06$^{a}$ | 0.55 ± 0.03$^{a}$ | 0.59 ± 0.04$^{a}$ |
| First drop times (min) | 0.28 ± 0.07$^{a}$ | 0.37 ± 0.02$^{a}$ | 0.33 ± 0.10$^{a}$ |
| *TPA* | | | |
| Hardness (N) | 125.40 ± 15.77$^{a}$ | 117.37 ± 12.03$^{a}$ | 88.94 ± 6.78$^{b}$ |
| Gomosity (N) | 21.81 ± 4.79$^{a}$ | 22.14 ± 4.78$^{a}$ | 17.82 ± 1.68$^{a}$ |
| Adhesiveness (mJ) | 3.60 ± 0.00$^{a}$ | 5.20 ± 0.00$^{a}$ | 2.10 ± 0.00$^{a}$ |
| Cohesiveness | 0.21 ± 0.04$^{a}$ | 0.19 ± 0.02$^{a}$ | 0.20 ± 0.02$^{a}$ |
| Elasticity (mm) | 1.45 ± 0.19$^{ab}$ | 1.66 ± 0.36$^{a}$ | 1.24 ± 0.24$^{b}$ |
| *Herschel-Bulkley model* | | | |
| $R^2$ | 0.999 | 1.000 | 0.998 |
| $\tau_0$(Pa) | 2.235 ± 0.087$^{a}$ | 1.056 ± 0.369$^{b}$ | 2.595 ± 0.677$^{a}$ |
| $k$ ([Pa.s]$^{n}$) | 0.144 ± 0.037$^{a}$ | 0.099 ± 0.037$^{a}$ | 0.085 ± 0.037$^{a}$ |
| $n$ (dimensionless) | 0.875 ± 0.026$^{a}$ | 0.884 ± 0.030$^{a}$ | 0.920 ± 0.068$^{a}$ |
| Hysteresis area (Pa.s) | 2.840 ± 0.008$^{b}$ | 4.193 ± 1.276$^{a}$ | 4.588 ± 0.313$^{a}$ |

Results are expressed as mean ± standard deviation. Different letters in the same line are significantly different ($p < 0.05$). AMS – ice cream with *Acacia mearnsii* gum; ASG – ice cream with *Acacia senegal* gum; ASY – ice cream with *Acacia seyal* gum. $\tau_0$: yield value; $k$: consistency index; $n$: flow behavior index.

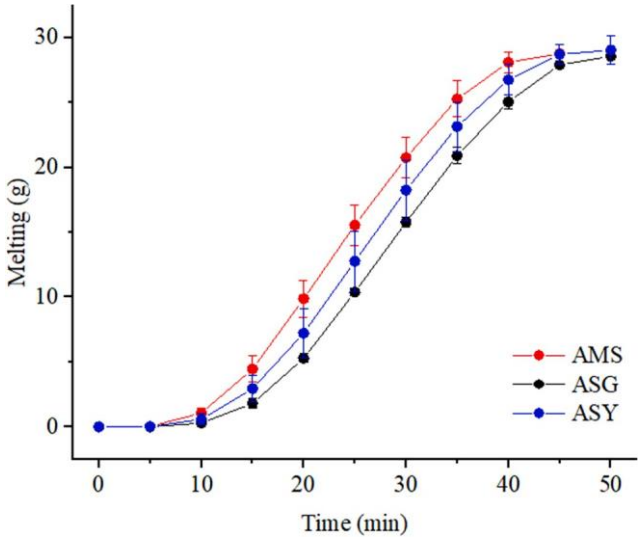


**Fig. 4.** Ice cream melting. AMS – ice cream with *Acacia mearnsii* gum; ASG – ice cream with *Acacia senegal* gum; ASY – ice cream with *Acacia seyal* gum.

viscosity and consistency, showing a more structured and stronger three-dimensional network due to molecular interactions, reducing the tendency to melt (Goff & Hartel, 2013). The AMS $\tau_0$ value indicates that the sample has greater resistance to shear, and therefore, is a more stable system — this is an important property because the ice cream must be resistant to adverse conditions that may occur in the course of its production chain (from the conditions in processing plants until consumption).

Different acacia gum species used in ice cream do not influence the consistency index ($k$). Both samples show shear-thinning behavior (Fig. 3A and 3B), with $n < 1$ (Table 1). All ice cream samples were characterized as thixotropic (with hysteresis area), a common behavior in several foods, as they are heterogeneous systems composed of a dispersed phase. These foods, when subjected to stress, undergo a reversible deformation, as they do not present resistance to stress and

shear rate application (Barnes, 1997). The hysteresis cycle area represents the energy consumed in the structural breakdown; AMS had a smaller hysteresis area (Table 1) that results in a greater degree of stability of the suspension and less time dependence. Ice cream produced with *A. mearnsii* gum (AMS), has a greater resistance to shear, as shown by the $\tau_0$ value (Table 1) and when subjected to stress its structure is altered but it returns to its initial state in a shorter time, and has a faster system restructuring speed (Barnes, 1997).

The deformation oscillation test allows study of the ice cream microstructure, due to its sensitivity to mechanical treatment, and results are shown in Fig. 3C. The storage modulus or elastic modulus ($G'$) represents the energy stored in the material, and the loss modulus or viscous modulus ($G''$), represents the energy dissipation by the system during deformation (Wildmoser et al., 2004). An increase in $G'$ and $G''$ values was observed, due to the increased frequency, decreasing the molecules' mobility and thus increasing the material elastic properties. Both samples showed $G'' > G'$, characterizing the liquid-like (viscous) behavior; this behavior is due to the increase in viscosity provided by acacia gum, indicating that the samples behave as structured fluids (Cavender & Kerr, 2020). The samples' viscous (liquid-like) behavior can be correlated with the ice cream flowability and scoopability. These characteristics are relevant during industrial processes, where the product will be transported and pumped via pipes, and during the handling of the final product during its commercialization and consumption (Wildmoser et al., 2004).

### *3.4. Ice cream physicochemical and texture profile characteristics*

AMS and ASY had the highest pH (Table 1). *Acacia* gums are heterogeneous, have highly branched polysaccharides, acids and contain several ionizable groups that can cause changes in chain conformation due to electrostatic interactions and ion distribution (Grein-Iankovski et al., 2018). *A. mearnsii* gum is an acid arabinogalactan, and has a lower proportion of units loaded with uronic acids (4%) than commercial gum Arabic (17%); this may have influenced the AMS pH and contributed to better system stabilization, since the *A. mearnsii* gum interfacial characteristics are not affected by pH due to the small proportion of charged uronic acid units (Grein et al., 2013).

*A. mearnsii* gum decreases the ice cream's lightness (Table 1 and Fig. 5). As shown in Fig. 5, *A. mearnsii* gum is darker than *A. senegal* and *A. seyal* gums, possibly due to the different process. In industrial processing, raw gums are diluted with water, clarified by centrifugation or filtration, heated or pasteurized and then atomized to obtain a fine, clear powder (Glicksman, 2019).

Ice cream melting is shown in Table 1 and Fig. 4. Different acacia gum species used as stabilizers did not affect the melting profile, melting rate, and first drop time. The stabilizer is responsible for binding the water molecules; without them the ice cream would not present a soft texture, and there would be migration of free water and the appearance of ice crystals (Goff & Hartel, 2013). This behavior is associated with the consistency index ($k$) and cohesiveness (Table 1), parameters that indicate the degree of fluid resistance, and molecules tended to stick together and showed no difference between the samples.

The samples' texture profile is shown in Table 1. AMS is one of the samples that presented greater hardness, indicating a system with a greater number of interactions between the mixture components, decreasing the overrun (Table 1) and increasing the viscosity (Fig. 3B). Ice cream gomosity, adhesiveness, and cohesiveness were not influenced by gums. These TPA results are associated with the behavior observed in Fig. 4 and Table 1, which show that the use of different stabilizers did not affect the samples' melting profile and the *A. mearnsii* gum stabilizing potential in relation to commercial gum Arabic.

## 4. Conclusions

*A. mearnsii* gum polysaccharide showed a similar chemical structure to commercial gum Arabic. Based on *A. mearnsii* gum cytotoxicity tests, it can be inferred that *A. mearnsii* gum use in food is safe; however, more

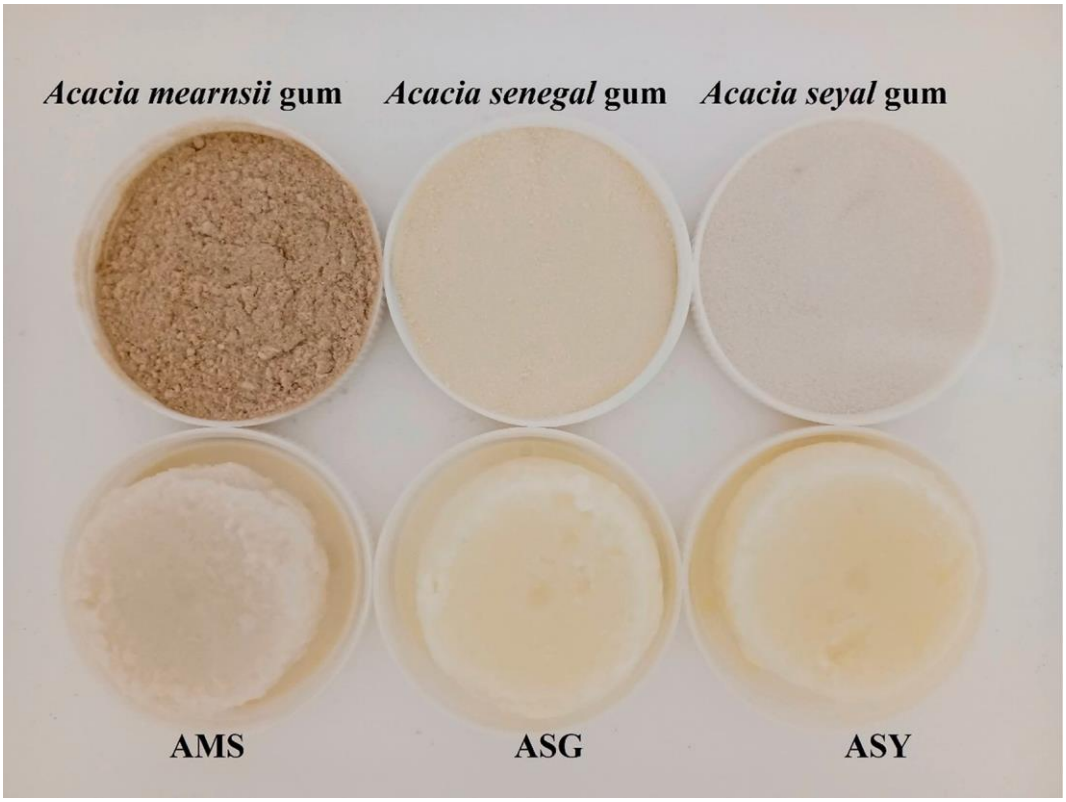


**Fig. 5.** Gum Arabic and ice cream. AMS – ice cream with *Acacia mearnsii* gum; ASG – ice cream with *Acacia senegal* gum; ASY – ice cream with *Acacia seyal* gum.

toxicity tests need to be performed. The ice cream made with *A. mearnsii* gum showed technological characteristics (physicochemical and rheological) relevant to industrial processes and to product commercialization. The results show that *A. mearnsii* gum can be used by the food industry as a stabilizer and that *A. mearnsii* gum can become a source of income, as it is currently characterized as a residue of the *A. mearnsii* productive chain.

## CRediT authorship contribution statement

**Fernando Antônio Anjo:** Investigation, Writing - original draft, Writing - review & editing, Project administration, Formal analysis, Conceptualization. **Bianka Rocha Saraiva:** Investigation. **Jessica Bassi Da Silva:** Investigation. **Yasmin Carla Ribeiro:** Investigation. **Marcos Luciano Bruschi:** Supervision, Writing - review & editing, Resources. **Izabel Cristina Riegel-Vidotti:** Supervision, Conceptualization, Resources. **Fernanda Fogagnoli Simas:** Supervision, Writing - original draft, Writing - review & editing, Formal analysis, Conceptualization, Resources. **Paula Toshimi Matumoto-Pintro:** Supervision, Writing - review & editing, Project administration, Conceptualization, Resources.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# ARTICLE 2

## A new food stabilizer and its effect on the technological properties of low-fat processed cheese

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FOOD HYDROCOLLOIDS

**A new food stabilizer in technological properties of low-fat processed cheese**

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**Abstract**

Mechanical and textural properties of processed cheeses are affected by low-fat, and hydrocolloids are commonly used as fat mimetics due their ability to make connections with water molecules. The aim of this study was to evaluate the stabilizing effect of *Acacia mearnsii* gum in low-fat processed cheeses, through physicochemical, composition, rheological and texture analyses. Processed cheeses were made with a 50% fat reduction and 0.125, 0.250, 0.375 and 0.500% of *A. mearnsii* gum (AMS). Also, were prepared samples full-fat (standard sample) and low-fat without gum (control sample). The chemical composition, physicochemical, textural and viscoelastic properties of processed cheeses were analyzed. Samples with *A. mearnsii* gum showed a higher amount of carbohydrates and protein content similar to the standard sample. The low-fat content influenced the rheological and textural properties of the processed cheeses, they had liquid-like behavior and were softer and more spreadable. Processed low-fat cheese with a higher AMS concentration showed viscoelastic behavior similar to the full-fat sample in the frequency sweep. Only the full-fat sample showed changes in viscoelastic behavior in the thermoreversibility test, and all samples were considered stable when subjected to temperature variation cycles, as they showed identical behavior in both cycles. The addition of *A. mearnsii* gum promotes greater molecular interactions, capable of stabilizing products with low fat content, evidencing its potential for industrial application.

**Keywords:** *Acacia mearnsii* De Wild, gum, fat-reduced, viscoelastic properties.

## 1. Introduction

Processed cheese may be defined as any cheese that has completely destroyed the original clot structure (Van Dender, 2014). Its processing depends on the fusion of two elements: fat and protein. The addition of emulsifying salts prevents the fat and water separation, making processed cheese as a homogeneous oil-in-water emulsion (Silva et al., 2016; Van Dender, 2014).

Consistency of processed cheeses is an important parameter to be evaluated, since it has influence on purchase decision by consumer (Ferrão et al., 2016; Kapoor & Metzger, 2008). Consistency of processed cheese can be influenced by four factors: composition of ingredients (type, composition, coagulation, and maturation of the cheese mass, emulsifying salts, water and fat), processing parameters (stirring speed, melting temperature and cooling rate of the cheese), final product composition (protein, fat, moisture and pH) and storage conditions (packaging, temperature and humidity) (Černíková et al., 2017; Ferrão et al., 2016; Johnson et al., 2009; Kapoor & Metzger, 2008).

Rheological and textural properties of low-fat processed cheeses are affected and require a study about consistency phenomenon (Ferrão et al., 2016). Instrumental analyzes of small or large deformations (dynamic oscillation rheometry and texture profile analysis - TPA) are commonly applied to evaluate the microstructure of processed cheeses (Černíková et al., 2017; Hosseini-Parvar et al., 2015; Lee et al., 2004; Silva et al., 2016).

Currently, research related to low-fat processed cheese production is categorized in two areas: (1) processed cheese production with low fat cheese , and (2) fat substitutes or mimetics compounds in processed cheese formulation (Johnson et al., 2009). Fat substitutes are macromolecules with high fats similarity, as sucrose fatty acid esters and polyesters, carbohydrate fatty acid esters, mono- and diglycerides, lecithin and structured lipids. Fat mimetics are hydrocolloids able to perform similar physical and sensory properties with water

connections (Johnson et al., 2009).

Several studies have described the effects of carbohydrates as fat mimetics on the consistency of processed cheese. For example, when using locust bean gum, carrageenan and guar gum it was possible to observe an improvement in texture in processed cheeses with more than 60% moisture (Brummel & Lee, 1990). Swenson et al., (2000) found that processed cheeses that hydrocolloids were used as fat mimetics had more uniform and smoother consistencies than those without. The use of xylitol resulted in less hardness and different viscoelastic behavior than the full-fat sample (Kommineni et al., 2012). Cheeses processed with 50% fat reduction with the addition of konjac glucomannan showed the greatest hardness and strong elastic behavior and cheeses with konjac glucomannan or konjac flour presented a more stable structure, as they were less susceptible to melting (Silva et al., 2016). The inulin addition reduced the hardness and increased the adhesiveness of the processed cheese and had a positive effect on the spreadability (El-Assar et al., 2018). Saraiva et al., (2020) when producing processed cheese with 50% fat reduction with addition of inulin obtained a product with softer and more stable, and with stable elastic behavior during heating.

*Acacia mearnsii* De Wild is cultivated for tannins production and not for use in food applications. Studies show that gum obtained from *A. mearnsii* has chemical composition similar to commercial gums, it is a complex, hyper-branched, amphiphilic heteropolysaccharide composed of galactose, arabinose, rhamnose, uronic acids and proteins with stabilizer/emulsifier physicochemical characteristics (Aspinall et al., 1968; Grein-Iankovski et al., 2016, 2018; Grein et al., 2013; Silva et al., 2015). Some of these studies evaluate the stabilizing/emulsifying characteristics of *A. mearnsii* gum (AMS) in classic laboratory tests, which do not consider the complexity of a food matrix, with proteins, carbohydrates, lipids, minerals, water and vitamins. Considering the influence that hydrocolloids have on the consistency of low-fat processed cheeses and the AMS stabilizing potential, this study aimed

to evaluate the stabilizing effect of AMS in low-fat processed cheeses, through physicochemical, composition, rheological and texture analyses.

## 2. Materials and methods

### *2.1. Extraction and characterization of Acacia mearnsii gum (AMS) polysaccharides*

The AMS polysaccharide was obtained after aqueous extraction of crude gum according to Grein et al. (2013). Gum (kindly furnished by Seta S.A. company, Rio Grande do Sul, Brazil) was stirred with water under mechanical stirrer during 16 h. After that the mixture was filtered using a fine cloth where insoluble residues were retained and aqueous extract was pass through. Ethanol (3:1 v/v) was added in aqueous extract yielding precipitated compound, isolated by centrifugation (12,000 × g, 30 min, 10 ºC), which corresponded to polysaccharide fraction. It was dialyzed against distilled water (48 h) through a membrane with a 12–14 kDa $M_w$ cutoff (Spectra/Por® Cellulose Ester) and freeze-dried.

Fourier Transform Infrared Spectrometer (FTIR) (Vertex 70v model, Bruker, Germany) coupled to an attenuated total reflectance (ATR) accessory (Platinum model, Bruker, Germany) was used to verify that the new batch of AMS polysaccharides were chemically similar to those reported in the literature (Aspinall et al., 1968; Grein-Iankovski et al., 2018; Grein et al., 2013). The gum was positioned and primed on ATR diamond crystal, so that all the diamond was in contact with them during the measurements. Each spectrum obtained is an average at 128 scans, with a spectral resolution at 4 $cm^{-1}$. The range used for analysis was 4000 to 400 $cm^{-1}$.

### *2.2. Processed cheese production*

The processed cheeses were made according to Silva et al., (2016) with modifications. Fresh cheese curd (26.16% fat, 14.51% protein, 59.7% moisture), butter (85% fat), water, emulsifying salt (sodium polyphosphates) and AMS were used to make 6 low-fat processed

cheeses according Table 1. The ingredients were homogenized into a stainless-steel trough and heated to 85 ± 2 °C at 3000 rpm. Cheese curd and emulsifying salt were homogenized for 2 min, butter was added and homogenized for 2 min, water containing AMS previously solubilized (500 rpm/10min) was added and, mixed for 2 min. Processed cheese (15 g) was placed in polypropylene cups and stored at 4 °C for 24 h before analyses.

*2.3. Physicochemical composition and texture profile analysis of processed cheese*

Processed cheeses samples were analyzed for protein, fat, ash, and moisture content (AOAC, 1990). Carbohydrate content was determined by subtracting the sum of moisture, protein, fat and ash percentages from 100%. The caloric values were estimated according to Equation (1) (Silva et al., 2016).

$$Caloric\ values\ (kcal/100\ g)\ =\ 4\ x\ protein +\ 4\ x\ carbohydrate +\ 9\ x\ fat \quad (1)$$

The color was evaluated using a colorimeter (CR-410, Minolta Sensing Konica, Inc., Tokyo, Japan) and the whiteness (WI, Eq. (2), Balthazar et al., 2017) and yellow index (YI, Eq. (3), Pathare et al., 2013) were calculated.

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (2)$$

$$YI = 142.86\frac{b^*}{L^*} \quad (3)$$

Where, $L^*$ is the lightness, $a^*$ is the color variation between green and red, and $b^*$ is the color variation between blue and yellow.

Texture profile analysis (TPA) was performed using a texture analyzer CT-III (Brookfield,

Middleborough, USA). Each sample (15g, 25.07 mm diameter, 31.65 mm height) was removed from storage (4 °C) and immediately analyzed (room temperature 25 ± 2 °C) using an acrylic circular probe TA/4-100 (12.77 mm diameter and 35.25 mm height) with 10 g trigger, compression distance of 5 mm, and 2 mm/s speed.

*2.4. Processed cheese rheological measurements*

Each processed cheese formulation was analyzed using a Mars II rheometer (Haake®, Thermo Fisher Scientific Inc., Newington, Germany) fitted with a 35 mm diameter cone-plate separated by 0.104 mm fixed gap. Dynamic oscillatory measurements were performed at 4 °C, the linear viscoelastic region (LVR) was determined by increasing the torque sweep at 1 Hz frequency for each sample. A deformation rate within the LVR was selected for subsequent frequency sweep analyses from 0.1 to 10.0 Hz and *G'* and *G''* were calculated.

In the oscillatory thermo-rheometry (OTR) the measuring temperature was continuously increased from 4 °C to 60 °C and cooled from 60 °C to 4 °C at a rate of 3 °C/min at 1 Hz frequency and 1% strain. A sample hood was used to prevent water evaporation. All analyses were carried out at least in duplicate.

Temperature cycles stability was evaluated by heating processed cheese from 4 °C to 25 °C and cooled from 25 °C to 4 °C (3 °C/min) at 1Hz frequency and 1% strain. A sample hood was used to prevent water evaporation. The heating and cooling process was repeated twice, for each processed cheese sample.

*2.5. Statistical analysis*

The experiments were performed twice and analyzed using technical triplicates in each one and the results expressed as arithmetic mean and standard deviation. The physicochemical analysis results were subjected to analysis of variance (ANOVA) and, when significantly

different ($p \leq 0.05$), means were compared by post-hoc test (Tukey), using SPSS program (v.20.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows.

## 3. Results and discussions

### *3.1. Characterization of AMS polysaccharides*

In the AMS FTIR spectrum (Fig. 1) an absorption band at 3326 $cm^{-1}$ represents the presence of an OH group (Bashir & Haripriya, 2016; Daoub et al., 2018; Venkatesham et al., 2012). The absorption band at 2923 $cm^{-1}$ is attributed to C-H bond of alkanes and sugar aldehyde, galactose, arabinose and rhamnose, these monosaccharides correspond to 95% of AMS carbohydrates (Grein et al., 2013) and have been identified in several studies (Anjo et al., 2021; Aspinall et al., 1968; Delgobo et al., 1999; Petkowicz et al., 1998; Tischer et al., 2002). The 1608 $cm^{-1}$ band is characteristic of (C=C) (Daoub et al., 2018). Specific vibrations (C=O, -OH) of uronic acid were identified at 1417 and 1361 $cm^{-1}$, respectively. Uronic acid is the acid component present in AMS, its percentage is around to 13% and is responsible for characterizing the gum as an acid arabinogalactan (Grein-Iankovski et al., 2018). At 1373 $cm^{-1}$ were identified δ(CH3) alkane bend, ν(C-C) aromatic, ν(C-C) ketone, ν(C-O) carboxylic acid, ν(C-O) anhydrides and ν(C-N) amine from polysaccharides and galactoproteins (Daoub et al., 2018), suggesting the presence of arabinogalactan proteins (AGPs) which represent the second largest component of gum Arabic and which, by being adsorbed to interface, promote steric repulsion, a factor believed to be responsible for the gum emulsification capacity (Grein et al., 2013). Alkane δ(CH3), alcohol ν(C-O), ether ν(C-O-C), carboxylic acid ν(CO), amines and alkyl ν(C-N) have been identified at 1255 $cm^{-1}$ due to the main sugar chain showing alkane flexion and alcohol stretch. CN and CO stretches are characteristics from galactoproteins and ether stretch due to attachment of two galactose molecules (Daoub et al., 2018). Bands between 700 and 400 $cm^{-1}$ were attributed to vibrations of the pyranose rings (Bashir & Haripriya, 2016).

*3.2. Composition and physicochemical properties of the processed cheese*

The chemical composition and physicochemical properties of processed cheese are shown in Table 2. An inverse relationship was observed in terms of moisture and fat content. The low-fat processed cheeses showed higher moisture than full-fat sample, the opposite occurred with fat. Fat reduction impacted the caloric value of the processed cheeses. As expected, the CONT, PC125, PC250, PC375, and PC500 showed an average 40% reduction in caloric value compared to STAN.

The carbohydrate content was affected by the use of AMS as a stabilizer. STAN had a lower carbohydrates concentration than low-fat processed cheeses, and samples that contained greater AMS inclusions had higher carbohydrates concentrations. The carbohydrates increase in processed chesses stabilized with AMS, which is a hydrocolloid composed of polysaccharide chains (Grein et al., 2013). The fat reduction and moisture increase in CONT, promoted the mixture components dilution, decreasing the proteins concentration. The low-fat formulations with AMS showed similar proteins to STAN. AMS is a gum that in its composition has polysaccharides and proteins, contains approximately 70% more protein than commercial gum Arabic (Elmanan et al., 2008; Grein et al., 2013). The ash content was not altered by caloric reduction or using AMS.

Low-fat processed cheese color is a factor to be considered because, the reduction of fat reduces the light scattering centers determining factor for whiteness index (WI) (Wadhwani & McMahon, 2012). Processed cheeses WI was affected by low-fat and AMS addition (Table 2), STAN presented the highest WI, followed by CONT. The color influence of AMS processed on a laboratory scale was also observed in a previous study (Anjo et al., 2021).

The AMS use above 0.25% for low-fat processed cheese showed intermediate YI values. STAN showed a higher YI due to a greater amount of fat in the formulation; low-fat promotes a decrease in pigments (carotenoids) concentration from milk origin, influencing the processed

cheese yellow color (Slots et al., 2009; Wadhwani & McMahon, 2012).

The TPA of processed cheeses (Table 2) showed that the low-fat makes processed cheeses softer (< hardness). They need less work to overcome the forces of attraction between the food and the surface in contact (adhesiveness), such as tongue, teeth or palate at the time of ingestion or, in the contact of the food with the packaging, allowing a better use of the product, or even easier flow during industrial transport through pipes and equipment. TPA showed that low-fat processed cheeses need less energy for their disintegration (gomosity).

Processed cheeses that contained the higher AMS concentrations showed greater cohesiveness. AMS is an amphiphilic hydrocolloid and can act on interfaces (Grein et al., 2013), and in processed cheese can increased the interactions between formulation components making less disruption of its internal bonds.

The elasticity of processed cheese can be associated with the speed at which it returns to its original shape after a deformation force is removed (Van Dender, 2014). This deformation can be caused by transporting the product within the industry (piping and pumping), transporting it to the consumer, or when the consumer removes part of the product from the packaging with a spoon or knife. Processed cheese with higher AMS concentration (PC500) showed similar elasticity to other samples with low-fat, however, statistically it is also similar to the standard sample (STAN) ($p < 0.05$).

*3.3.Processed cheese viscoelastic properties*

The oscillatory rheology (Fig. 2) showed the increasement of viscoelastic properties (*G'* and *G"*) of all processed cheeses when frequency increases. STAN is characterized as a viscoelastic fluid, due to crossover presence (Schramm, 2006). It has highest *G'* and *G"*, with lower slope than low-fat processed cheeses, indicating its less susceptibility to frequency variation (Belsito et al., 2017), therefore, a processed cheese with a tougher and less spreadable

consistency (Černíková et al., 2017). This behavior can also be associated with greater STAN hardness and fat content (Table 2).

Low-fat processed cheeses (CONT, PC125, PC250, PC375 and PC500) have less elastic and viscous modulus than STAN. Most of these processed cheeses (CONT, PC125, PC250 and PC375) showed viscous behavior – liquid-like properties (*G'' > G'*) (Schramm, 2006). The low-fat content, low AMS concentration, and increased moisture (approximately 21.23%) (Table 2), contributed to the viscous modulus predominance in these samples. Systems with lower fat content usually presents lower values of *G'* (Silva et al., 2016), which was observed mainly in CONT. The AMS inclusion in the formulations may foster interactions between protein aggregates and AMS in the continuous phase, improving the elastic and viscous modulus. PC500 demonstrates a different behavior (Fig. 2) in comparison with the samples containing less gum. Like STAN, PC500 is a viscoelastic fluid (Schramm, 2006). A higher AMS concentration increased the number of molecular interactions between the constituents, modifying the processed cheese viscoelastic properties. These modifications possibly will help in sample emulsification, making the processed cheese (PC500) with a harder and less spreadable consistency than the samples with lower AMS concentration.

The effect of temperature on the processed cheeses viscoelastic properties is shown in Fig 3. STAN presented elastic behavior (*G' > G''*) along the temperature sweep (4 to 60 °C). A trend towards a change in viscoelastic behavior was observed between 15 to 25 °C (*G'* and *G''* values approximation). Fat content is responsible for texture and viscoelastic properties in processed cheeses (Rønholt et al., 2012; Silva et al., 2016; Wright et al., 2001). The elastic properties of milk fat are frequently associated with its large quantity and variety of triacylglycerol species, which present particular melting temperature each. This variation in triacylglycerols content makes a wide melting range of -40 to 40 °C (Wright et al., 2001), explaining the viscoelastic behavior of STAN.

Melting and deformation of fat globules from processed cheese samples during rising temperatures can weaken protein-protein interactions and cause the protein matrix to flow (Hennelly et al., 2006; Solhi, Azadmard-Damirchi, et al., 2020). There is a decrease in *G'* and *G''* modulus, with predominance liquid-like behavior (*G'' > G'*) (Schramm, 2006) up to 45 °C in low-fat processed cheeses (Fig. 3). However, at high temperatures, interactions between proteins and also between proteins and polysaccharides (AMS) decrease the liquid behavior of processed cheeses (Hennelly et al., 2006; Solhi, Azadmard-Damirchi, et al., 2020), with a predominance of elastic behavior (*G' > G''*) in low-fat processed cheeses.

In processed cheeses with or without AMS, *G''* and, especially, *G'* (downward curve), have their values increased at temperatures below 15 °C (Fig. 3). The elastic modulus (*G'*) is related to the solid fat content (saturated fatty acids), cooling accelerates the nucleation rate and, crystals making the fat solid at temperatures below 20 °C growth (Rønholt et al., 2012; Wright et al., 2001), increasing the processed cheese elastic properties.

Elastic (*G'*) and viscous (*G''*) modulus dependence of processed cheeses with or without AMS for two cycles of increase and decrease temperature (4 °C until 25 °C and vice verse) are shown in Fig. 4. The thermoreversibility test simulates the processed cheese consumption by consumer, evaluating the processed cheeses viscoelastic properties during the period of storage (4 °C) and, also, when the product is exposed to room temperature (25 °C).

STAN was the only processed cheese that showed a change in viscoelastic properties due to temperature variation cycles (Fig. 4). Viscous properties (*G'' > G'*) predominance was observed at temperatures above 15 ºC, due to the fat melting. The data was reproducible, since the viscoelastic changes occurred repeatedly at the same point (around 15 °C). Moreover, this behavior demonstrated to be reversible.

Low-fat processed cheeses showed viscous behavior over the entire temperature range (*G'' > G'*) (Fig. 4). The data was reproducible, the behavior observed on the first cycle of

temperature, repeated itself on second one. The low-fat processed cheeses viscous behavior maintenance shows that even after variations in temperature the processed cheeses maintain a soft texture (Table 2) and preserve their spreadability.

## 4. Conclusions

Low-fat had an impact on chemical composition, physicochemical, color, textural and rheological properties of processed cheese. The use of *Acacia mearnsii* gum as a stabilizer in low-fat processed cheese provides the product with the same amount of protein as the full-fat sample, increases the content of carbohydrates and influences the viscoelastic properties of processed cheeses.

The low-fat processed cheese that contained the highest concentration of *Acacia mearnsii* gum exhibited the viscoelastic behavior equal to full-fat sample, but showed greater softness and more spreadability. Only the full-fat sample showed changes in viscoelastic behavior in the thermoreversibility test, and all samples were considered stable when subjected to temperature variation cycles, as they showed identical behavior in both cycles.

The results show that *Acacia mearnsii* gum can be used by the food industry as a stabilizer. The commercialization of gum can contribute economically to the production chain, and further studies must be carried out in order to determine more technofunctional properties of *Acacia mearnsii* gum.

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## Conflict of interest

The authors have declared no conflicts of interest for this article.

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**Figure Caption**

**Fig. 1.** ATR–FTIR spectra of *Acacia mearnsii* gum polysaccharide.

**Fig. 2.** Effect of frequency sweep on the storage modulus (*G'* - solid symbols) and loss modulus (*G"* - open symbols) of the processed cheeses with and without *Acacia mearnsii* gum (AMS). STAN: standard processed cheese; CONT: low-fat processed cheese without AMS; PC125: low-fat processed cheese and 0.125% AMS; PC250: low-fat processed cheese and 0.250% AMS; PC375: low-fat processed cheese and 0.375% AMS; PC500: low-fat processed cheese and 0.500% AMS.

**Fig. 3.** Effect of temperature sweep (4 – 60 °C) on the storage modulus (*G'* - solid symbols) and loss modulus (*G"* - open symbols) of the processed cheeses with and without *Acacia mearnsii* gum (AMS). STAN: standard processed cheese; CONT: low-fat processed cheese without AMS; PC125: low-fat processed cheese and 0.125% AMS; PC250: low-fat processed cheese and 0.250% AMS; PC375: low-fat processed cheese and 0.375% AMS; PC500: low-fat processed cheese and 0.500% AMS.

**Fig. 4.** Dependence of the storage (*G'* - solid symbols) and loss modulus (*G"* - open symbols) and two cycles of temperature (4 - 25 °C and 4 – 25 °C) on time with a rate of 3 °C/min at 1% strain deformation and frequency 1 Hz of processed cheeses with and without *Acacia mearnsii* gum (AMS). STAN: standard processed cheese; CONT: low-fat processed cheese without AMS; PC125: low-fat processed cheese and 0.125% AMS; PC250: low-fat processed cheese and 0.250% AMS; PC375: low-fat processed cheese and 0.375% AMS; PC500: low-fat processed cheese and 0.500% AMS.

**Fig. 1**

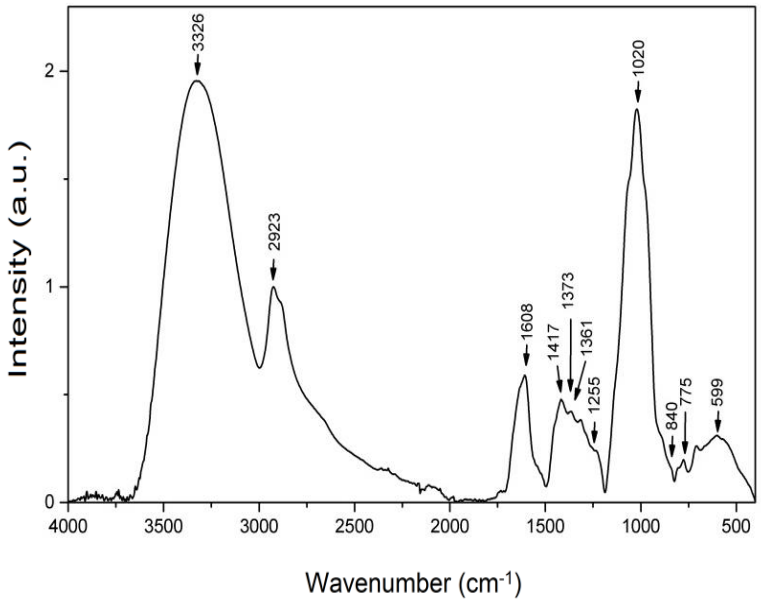
3326
2923
1608
1417
1373
1361
1255
1020
840
775
599
Intensity (a.u.)
Wavenumber (cm-1)
0
1
2
4000
3500
3000
2500
2000
1500
1000
500

**Fig. 2**

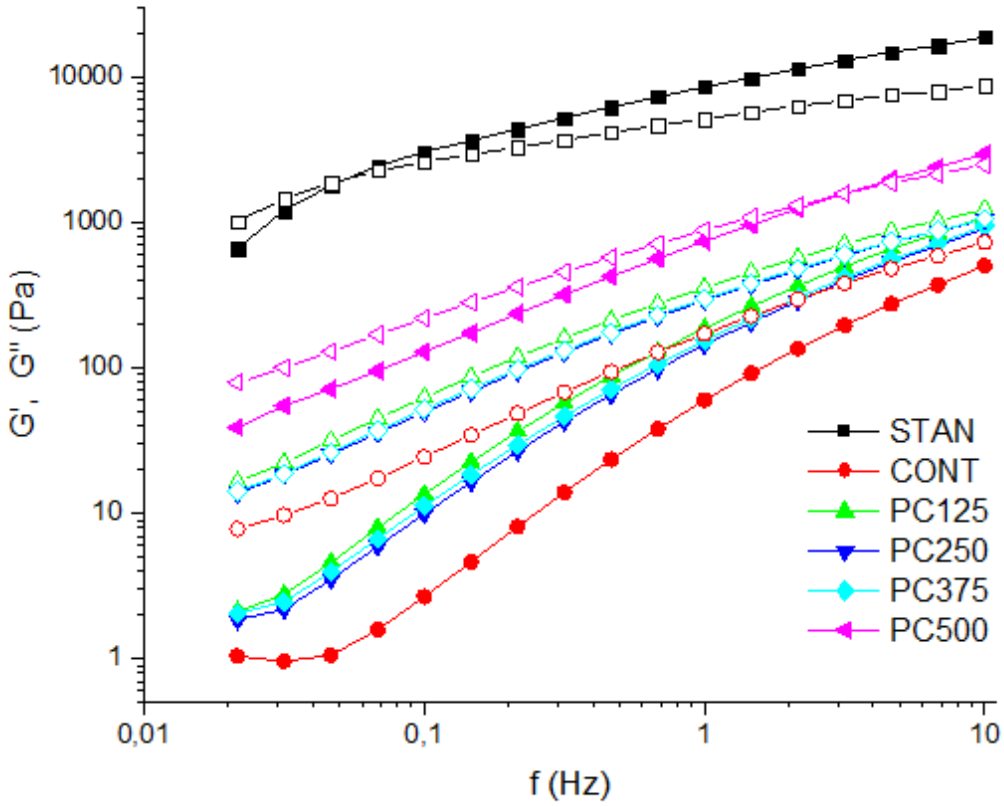
10000
1000
100
10
1
G', G'' (Pa)
0,01
0,1
1
10
f (Hz)
STAN
CONT
PC125
PC250
PC375
PC500

**Fig. 3**

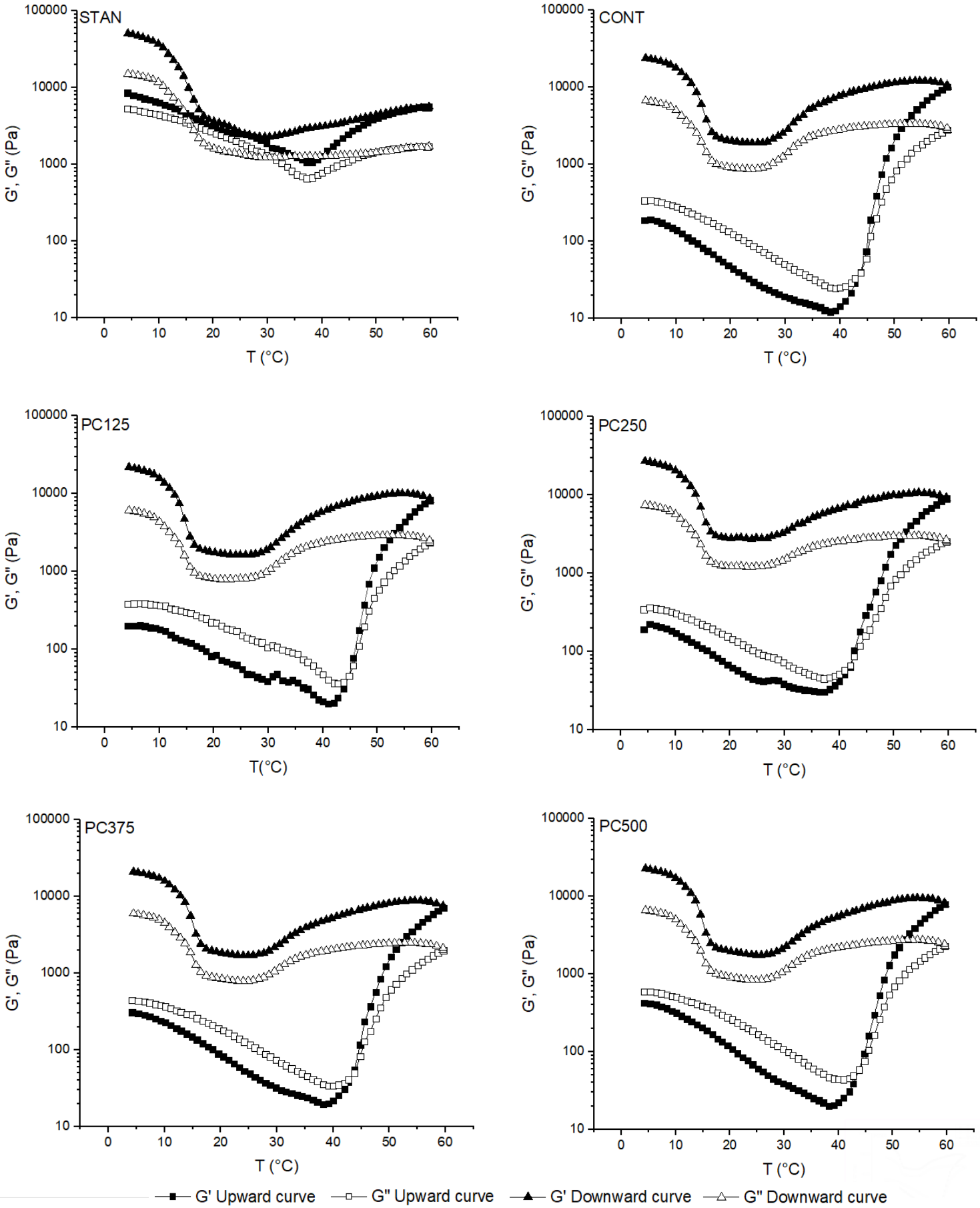
STAN
CONT
PC125
PC250
PC375
PC500
G', G" (Pa)
T (°C)
T(°C)
100000
10000
1000
100
10
0
10
20
30
40
50
60
G' Upward curve
G" Upward curve
G' Downward curve
G" Downward curve

**Fig. 4**

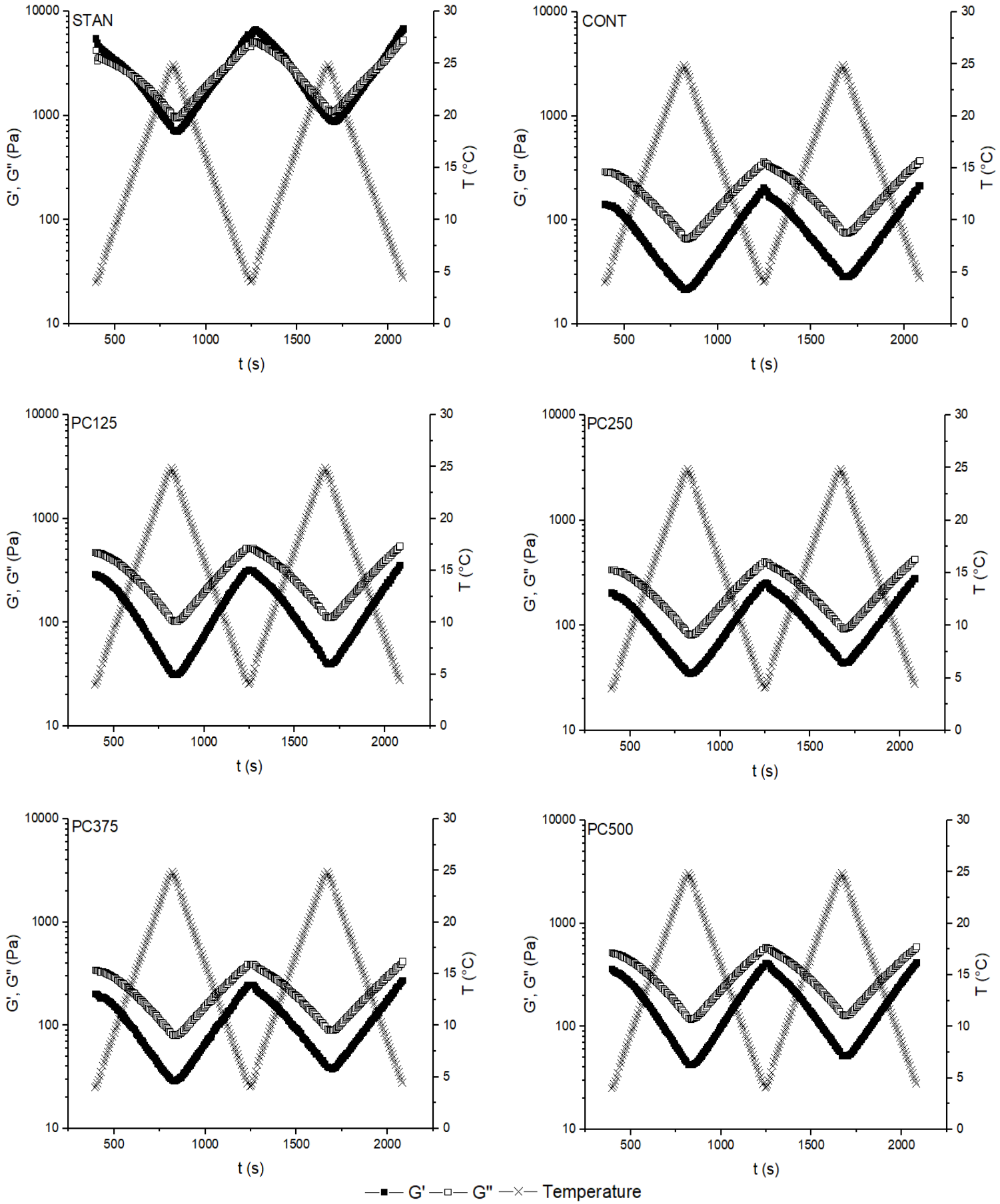

**Table 1.** Ingredients (%) of low-fat processed cheeses without and with *Acacia mearnsii* gum (AMS).

| | Cheese curd | Emulsifying salt | Water | Butter | AMS |
|---|---|---|---|---|---|
| STAN | 51.70 | 1.00 | 28.000 | 19.30 | - |
| CONT | 51.70 | 1.00 | 37.650 | 9.65 | - |
| PC125 | 51.70 | 1.00 | 37.525 | 9.65 | 0.125 |
| PC250 | 51.70 | 1.00 | 37.400 | 9.65 | 0.250 |
| PC375 | 51.70 | 1.00 | 37.375 | 9.65 | 0.375 |
| PC500 | 51.70 | 1.00 | 37.150 | 9.65 | 0.500 |

**Table 2.** Chemical composition (%) and physicochemical proprieties of processed cheeses without and with *Acacia mearnsii* gum (AMS).

| | STAN | CONT | PC125 | PC250 | PC375 | PC500 |
|---|---|---|---|---|---|---|
| *Chemical composition* | | | | | | |
| Moisture | 52.67 ± 0.86$^{b}$ | 64.87 ± 0.71$^{a}$ | 63.92 ± 0.67$^{a}$ | 63.42 ± 0.30$^{a}$ | 63.77 ± 0.63$^{a}$ | 63.25 ± 0.71$^{a}$ |
| Fat | 22.40 ± 0.36$^{a}$ | 8.15 ± 1.10$^{b}$ | 9.15 ± 0.75$^{b}$ | 8.52 ± 0.92$^{b}$ | 7.72 ± 0.30$^{b}$ | 7.35 ± 0.91$^{b}$ |
| Carbohydrate | 13.33 ± 0.51$^{c}$ | 16.40 ± 0.99$^{b}$ | 15.83 ± 0.88$^{b}$ | 16.75 ± 1.10$^{ab}$ | 17.32 ± 0.41$^{ab}$ | 18.30 ± 0.53$^{a}$ |
| Protein | 8.87 ± 0.15$^{a}$ | 8.22 ± 0.17$^{c}$ | 8.57 ± 0.17$^{ab}$ | 8.60 ± 0.08$^{ab}$ | 8.57 ± 0.12$^{ab}$ | 8.60 ± 0.18$^{ab}$ |
| Ash | 2.70 ± 0.00$^{a}$ | 2.35 ± 0.35$^{a}$ | 2.57 ± 0.19$^{a}$ | 2.65 ± 0.06$^{a}$ | 2.67 ± 0.05$^{a}$ | 2.62 ± 0.09$^{a}$ |
| Caloric values (kcal/100g) | 290.53 ± 5.11$^{a}$ | 171.92 ± 6.18$^{b}$ | 179.77 ± 5.14$^{b}$ | 178.32 ± 3.75$^{b}$ | 172.95 ± 3.43$^{b}$ | 173.60 ± 6.81$^{b}$ |
| *Color* | | | | | | |
| WI | 77.64 ± 0.33$^{a}$ | 75.64 ± 1.86$^{b}$ | 74.08 ± 0.77$^{c}$ | 73.84 ± 0.31$^{cd}$ | 73.46 ± 0.70$^{cd}$ | 72.80 ± 0.43$^{d}$ |
| YI | 34.75 ± 0.36$^{a}$ | 28.01 ± 0.74$^{c}$ | 29.95 ± 2.96$^{c}$ | 32.37 ± 0.39$^{b}$ | 32.46 ± 0.64$^{b}$ | 33.21 ± 0.85$^{b}$ |
| *TPA* | | | | | | |
| Hardness (g) | 277.50 ± 15.55$^{a}$ | 35.00 ± 0.00$^{b}$ | 41.25 ± 2.50$^{b}$ | 30.00 ± 0.00$^{b}$ | 31.25 ± 2.50$^{b}$ | 42.50 ± 2.89$^{b}$ |
| Adhesiviness (mJ) | 4.35 ± 0,06$^{a}$ | 1.02 ± 0.05$^{c}$ | 1.32 ± 0.30$^{c}$ | 1.45 ± 0.17$^{bc}$ | 1.60 ± 0.34$^{bc}$ | 2.07 ± 0.62$^{b}$ |
| Cohesiviness* | 0.46 ± 0.02$^{c}$ | 0.60 ± 0.08$^{bc}$ | 0.62 ± 0.04$^{bc}$ | 0.70 ± 0.04$^{ab}$ | 0.75 ± 0.16$^{ab}$ | 0.87 ± 0.03$^{a}$ |
| Elasticity (mm) | 5.33 ± 0.91$^{a}$ | 3.85 ± 0.13$^{b}$ | 4.18 ± 0.34$^{b}$ | 3.73 ± 0.29$^{b}$ | 4.21 ± 0.20$^{b}$ | 4.35 ± 0.45$^{ab}$ |
| Gomosity (g) | 152.50 ± 7.19$^{a}$ | 21.25 ± 1.50$^{b}$ | 25.00 ± 3.16$^{b}$ | 25.75 ± 3.59$^{b}$ | 25.75 ± 0.96$^{b}$ | 25.00 ± 1.63$^{b}$ |

Results are expressed as mean ± standard deviation. Different letters in the same line are significantly different ($p<0.05$). STAN: standard processed cheese; CONT: low-fat processed cheese without AMS; PC125: low-fat processed cheese and 0.125% AMS; PC250: low-fat processed cheese and 0.250% AMS; PC375: low-fat processed cheese and 0.375% AMS; PC500: low-fat processed cheese and 0.500% AMS. WI: whiteness index; YI: yellow index; TPA: texture profile analysis. *Dimensionless.