UNIVERSIDADE FEDERAL DE VIÇOSA

RAQUEL NUNES FERNANDES

ÉSTERES DE SACAROSE: SÍNTESE ENZIMÁTICA E AVALIAÇÃO DE SUAS PROPRIEDADES TENSOATIVAS

VIÇOSA – MINAS GERAIS
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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Doctor Scientiae*.

Orientador: Luis Antonio Minim

Coorientadores: Andréa Alves Simiqueli
Valéria Monteze Guimarães
Valéria Paula Rodrigues Minim

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Assentimento:

Raquel Nunes Fernandes

Raquel Nunes Fernandes
Autora

Luis Antonio Minim
Orientador

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"... Eu sou Deus; também de hoje em diante, eu o sou; e ninguém há que possa fazer escapar das minhas mãos; operando eu, quem impedirá?..."

(Isaías 43:13)

RESUMO

FERNANDES, Raquel Nunes, D.Sc., Universidade Federal de Viçosa, abril de 2022. **Ésteres de sacarose: Síntese enzimática e avaliação de suas propriedades tensoativas.** Orientador: Luis Antonio Minim. Coorientadores: Andréa Alves Simiqueli, Valéria Monteze Guimarães e Valéria Paula Rodrigues Minim.

O presente trabalho objetivou estudar a produção enzimática de ésteres de sacarose e suas propriedades tensoativas. Lipase comercial de *Candida antarctica* B (CALB) imobilizada em resina acrílica foi utilizada em produções em batelada. A maior conversão do ácido esteárico (53,4%) em ésteres de sacarose foi obtida usando 2-metil-2-butanol e proporção molar de 1:3 (sacarose: ácido esteárico), após 72 h a 65 °C. A síntese foi confirmada por cromatografia em camada delgada (CCD) e espectroscopia de infravermelho (FTIR). A tensão superficial de equilíbrio (γ_{eq}) e a concentração micelar crítica (CMC) foram obtidas, e seus valores (45 ± 1 mN/m e 10 mg/L, respectivamente) eram característicos de um surfactante não iônico. As propriedades interfaciais de misturas de ésteres de sacarose e goma guar ou goma xantana, na interface óleo-água foram determinadas. Observou-se que a adição dos polissacarídeos no meio aumentou a tensão na interface. Estudos de viscoelasticidade interfacial mostraram que todos os filmes produzidos eram predominantemente elásticos. Verificou-se ainda, que emulsões produzidas com ésteres de sacarose apresentaram excelente estabilidade ao longo de 28 dias. Posteriormente, foi desenvolvido um biorreator enzimático para a síntese de ésteres de sacarose em modo contínuo. Lipase de *Candida rugosa* (CRL) foi imobilizada em criogel de poliacrilamida. A metodologia de superfície de resposta, com base em um delineamento composto central rotacional, foi utilizada para otimizar a produção enzimática de ésteres de sacarose. A porcentagem máxima de esterificação prevista foi de 93,01% utilizando uma razão molar de 1:3,13 (sacarose: ácido esteárico), temperatura de 55,8 °C e proporção de 7,44:92,56 (DMSO: *tert*-butanol). O modelo ajustado foi validado experimentalmente nas condições ótimas. Novamente, a síntese de ésteres de sacarose foi confirmada por CCD e FTIR e a tensão superficial de equilíbrio e a CMC foram determinadas (44 ± 1 mN/m e 14,5 mg/L, respectivamente). Após 10 ciclos sucessivos, o biorreator manteve uma alta taxa de conversão. Assim, este trabalho apresenta contribuições significativas para o avanço da tecnologia enzimática para síntese de ésteres de sacarose, que devido aos baixos valores de tensão interfacial têm potencial aplicação como emulsificantes para a indústria.

Palavras-chave: Surfactante. Lipases. Otimização. Emulsões. Dinâmica interfacial.

ABSTRACT

FERNANDES, Raquel Nunes, D.Sc., Universidade Federal de Viçosa, April 2022. **Sucrose esters: Enzymatic synthesis and evaluation of their surfactant properties**. Advisor: Luis Antonio Minim. Co-advisors: Andréa Alves Simiqueli, Valéria Monteze Guimarães and Valéria Paula Rodrigues Minim.

The present work aimed to study the enzymatic production of sucrose esters and their surfactant properties. Commercial lipase from *Candida antarctica* B (CALB) immobilized on acrylic resin was used in batch productions. The highest conversion of stearic acid (53.4%) to sucrose esters was obtained using 2-methyl-2-butanol and a molar ratio of 1:3 (sucrose: stearic acid), after 72 h at 65 °C. The synthesis was confirmed by thin layer chromatography (TLC) and infrared spectroscopy (FTIR). Equilibrium surface tension (γ_{eq}) and critical micelle concentration (CMC) were obtained, and their values (45 ± 1 mN/m and 10 mg/L, respectively) were characteristic of a non-ionic surfactant. The interfacial properties of mixtures of sucrose esters and guar gum or xanthan gum at the oil-water interface were determined. It was observed that the addition of polysaccharides in the medium increased the tension at the interface. Interfacial viscoelasticity studies showed that all films produced were predominantly elastic. It was also verified that emulsions produced with sucrose esters showed excellent stability over 28 days. Subsequently, an enzymatic bioreactor was developed for the synthesis of sucrose esters in continuous mode. *Candida rugosa* lipase (CRL) was immobilized on polyacrylamide cryogel. The response surface methodology, based on a central composite rotational design, was used to optimize the enzymatic production of sucrose esters. The maximum percentage of esterification predicted was 93.01% using a molar ratio of 1:3.13 (sucrose: stearic acid), temperature of 55.8 °C and proportion of 7.44:92.56 (DMSO: *tert*-butanol). The fitted model was experimentally validated under optimal conditions. Again, the synthesis of sucrose esters was confirmed by TLC and FTIR and the equilibrium surface tension and CMC were determined (44 ± 1 mN/m and 14.5 mg/L, respectively). After 10 successive cycles, the bioreactor maintained a high conversion rate. Thus, this work presents significant contributions to the advancement of enzymatic technology for the synthesis of sucrose esters, which, due to their low interfacial tension values, have potential application as emulsifiers for industry.

Keywords: Surfactant. Lipases. Optimization. Emulsions. Interfacial dynamics.

SUMÁRIO

INTRODUÇÃO GERAL

O uso de surfactantes sintéticos derivados do petróleo tem sido relacionado a vários riscos à saúde humana, incluindo reações alérgicas, irritações nos olhos e na pele, distúrbios intestinais e câncer (FLOYD; ZAROGIANNIS; FOX, 2006). Por outro lado, ésteres de ácidos graxos de açúcar, ou simplesmente ésteres de açúcar, são surfactantes seguros tanto para humanos quanto para o meio ambiente, uma vez que são compostos não tóxicos, não irritantes, insípidos, inodoros, biodegradáveis e biocompatíveis, além de possuirem alta seletividade e eficiência em condições extremas de temperatura e concentração de sais, o que os tornam adequados para uma variedade de aplicações industriais (ABDULMALEK; HAMIDON; ABDUL RAHMAN, 2016; PAPPALARDO et al., 2017; ZAGO et al., 2021).

Os ésteres de açúcar são moléculas anfifílicas baseadas em uma fração de açúcar hidrofílica e uma cauda de ácido graxo hidrofóbica. No geral, ésteres de açúcar são considerados surfactantes não iônicos e suas propriedades incluem formação de espuma, emulsificação, estabilização e alto poder de dispersão (COELHO; ORLANDELLI, 2021; ZAGO et al., 2021). Devido, principalmente, às suas propriedades tensoativas, ésteres de açúcar podem ser utilizados na indústria alimentícia, farmacêutica, cosmética e química (ZAGO et al., 2021). Tais propriedades estão associadas, especialmente, à estrutura molecular dos ésteres, como comprimento da cadeia carbônica e tamanho da fração hidrofílica do açúcar (AN et al., 2019; ZAGO et al., 2021).

Dentre esta classe de surfactantes à base de carboidratos, estão os ésteres de sacarose que têm se destacado comercialmente (INPRAKHON et al., 2017; MENG et al., 2014). Ésteres de sacarose são obtidos de sacarose e ácidos graxos derivados de gorduras e óleos comestíveis. Como a molécula de sacarose possui oito grupos hidroxila livres, ela pode ser esterificada com até oito ácidos graxos para formar ésteres que variam de monoésteres a octaésteres. Como resultado, estes surfactantes podem fornecer várias propriedades hidrofílico-lipofílicas com valores de balanço hidrofílico-lipofílico (BHL) variando de 1 a 16 (CHANSANROJ; BETZ, 2010) que podem atender a necessidades específicas de uma variedade de produtos industriais.

Ésteres de açúcar podem ser extraídos naturalmente de plantas ou sintetizados por rota química ou enzimática (MORA VARGAS et al., 2020). O método químico apresenta baixa seletividade, requer mais energia, o uso de catalisadores químicos e altas temperaturas (PAPPALARDO et al., 2017). A combinação de alta temperatura e catalisador alcalino, muito utilizada em processos químicos, leva à formação de subprodutos que são difíceis de remover,

acarretando em custos adicionais que são indesejáveis para a indústria. Alguns destes subprodutos são tóxicos, alergênicos e possivelmente cancerígenos (GUMEL et al., 2011). Em contrapartida, o método enzimático é uma alternativa ecológica que requer condições operacionais mais brandas, além de oferecer alta especificidade e regiosseletividade da reação (AN et al., 2019). Reações catalisadas por enzimas para a síntese de ésteres são conduzidas em temperaturas mais amenas e baixa pressão, proporcionam bons rendimentos e produtos com maior pureza (AN et al., 2019; PAPPALARDO et al., 2017; ZAGO et al., 2021).

Dessa forma, a síntese catalisada por enzimas tem sido a opção preferida para a produção de ésteres de ácidos graxos de açúcar. Porém, diferentes fatores podem afetar as reações enzimáticas e impactar o rendimento do processo de esterificação (GUMEL et al., 2011; NGUYEN et al., 2021). Dentre esses fatores, a solubilidade dos substratos desempenha um papel fundamental, por isso a escolha do solvente na síntese de ésteres de açúcar é um fator determinante (ZAGO et al., 2021). O uso de solvente orgânico com alta polaridade aumenta a solubilidade do açúcar, mas por outro lado pode resultar em baixa solubilidade do ácido graxo, diminuir a atividade da enzima e favorecer reações secundárias indesejadas, como a hidrólise (AN et al., 2019). Por sua vez, a utilização de solventes orgânicos com baixa polaridade aumenta a solubilidade dos ácidos graxos, mas acarreta em baixa solubilidade do açúcar e pode induzir a secagem da camada de hidratação da enzima e, consequentemente, reduzir seu poder catalítico e sua estabilidade (GUMEL et al., 2011; SALEM et al., 2010).

Assim, para garantir uma síntese bem sucedida de ésteres de açúcar, o solvente deve proporcionar uma boa solubilização dos dois substratos (açúcar e ácido graxo), mantendo inalterada a atividade enzimática e evitando a hidrólise dos produtos (AN et al., 2019). Contudo, a maioria dos solventes orgânicos apolares, como *n*-hexano e *n*-heptano, têm uma boa solubilidade de ácidos graxos, mas muito baixa solubilidade de açúcares, e não são apropriados para reações nas quais os dois substratos diferem muito em termos de polaridade. Solventes com maior polaridade, que podem dissolver açúcares e lipídios incluem dimetilsulfóxido, piridina e dimetilformamida, mas esses solventes geralmente inativam a enzima e são incompatíveis com aplicações em alimentos (GANSKE; BORNSCHEUER, 2005; JIA et al., 2010).

No estudo de Jia et al. (2010), foi avaliada a síntese enzimática de dilauroil maltose em meio orgânico. Os autores observaram que em sistemas únicos de solventes altamente apolares ou solventes altamente polares não ocorreu esterificação. Por outro lado, ao testarem sistemas mistos, verificaram que um solvente com alta solubilidade de maltose, como acetona, e outro que confere boa estabilidade à enzima como, *n*-hexano, resultavam em uma síntese enzimática

seletiva de ésteres de açúcar, com uma melhor taxa de conversão. Portanto, também é possível escolher misturas adequadas de dois ou mais solventes para reações de esterificações catalisadas por enzimas, que não alteram a atividade enzimática e proporcionam boa solubilidade dos substratos.

O uso de substrato em excesso também pode influeciar no rendimento da reação, uma vez que é capaz de deslocar seu equilíbrio para a síntese do produto de esterificação (AN et al., 2019). Abdulmalek et al. (2016) testaram diferentes razões molares de açúcar e ácido graxo na síntese enzimática de um éster de açúcar (caproato de xilose). Os autores verificaram que uma razão equimolar era menos eficiente na conversão de ácido graxo (45%) do que o uso de um excesso de três equivalentes molares de ácido capróico (64%). Em outro estudo, a razão molar do substrato variou de 1:1 a 1:5 (galactose: ácido oleico) e observou-se que a maior conversão (87%) foi alcançada para a razão molar 1:3. Porém, quando a razão molar aumentou até 1:5, a conversão caiu. Os autores concluíram que isso pode ter ocorrido devido às maiores quantidades de ácido oleico limitarem a transferência de massa e reduzirem a concentração de açúcar dissolvido (ABDULMALEK et al., 2012). Portanto, geralmente, a taxa de conversão de esterificação pode ser melhorada fornecendo um excesso de ácido graxo. Porém, deve-se considerar que uma concentração muito alta de ácido graxo pode resultar em diminuição da solubilidade do açúcar, ao afetar a polaridade do meio de reação (GUMEL et al., 2011).

A temperatura é também um parâmetro importante que interfere no equilíbrio da reação, bem como na solubilidade do substrato e na atividade da enzima. Apesar de altas temperaturas melhorarem a transferência de massa no meio, enzimas podem ser termossensíveis e geralmente apresentam atividade em uma faixa de temperatura específica (ZAGO et al., 2021). Arcens et al. (2018) mostraram o efeito de diferentes valores de temperatura (20 °C, 30 °C, 45 °C, 60 °C e 70 °C) na síntese de palmitato de 6-*O*-glicose. Estes autores observaram que as taxas de conversão foram maiores em temperaturas mais altas e que o aquecimento a 60 °C reduziu significativamente o tempo de reação, já que 94% de conversão foram alcançados em apenas 20 h, enquanto a 45 °C precisou-se de um tempo de reação de 40 h para atingir a mesma taxa de conversão. Já Zaidan et al. (2012) estudaram o efeito da temperatura na síntese de caprato de lactose catalisada por lipases e verificaram que as conversões de ácidos graxos foram cerca de 20% maiores com o aumento da temperatura de 45 °C para 55 °C, devido, provavelmente, ao aumento da frequência de contato das moléculas no meio reacional ocasionado pela aplicação de maiores níveis de energia ao sistema. Contudo, na temperatura de 60 °C foi observada uma diminuição da taxa de conversão, em consequência da perda de atividade da enzima livre, devido a desnaturação térmica.

Lipases, proteases e esterases são os tipos de enzimas que catalisam eficientemente as reações de esterificação em meio orgânico (VAN DEN BROEK; BOERIU, 2013; ZAGO et al., 2021). Todas essas enzimas apresentam em comum a tríade catalítica serina-histidina-ácido aspártico no sítio ativo, onde ocorrem as reações enzimáticas. No entanto, para a seleção de biocatalisadores que catalisam a formação de ligações éster entre o receptor (carboidrato) e o doador (ácido graxo) de acil, as lipases (triacilglicerol acil hidrolases, EC 3.1.1.3) são as mais amplamente utilizadas em razão de sua alta especificidade de substrato (NGUYEN et al., 2021; NIETO et al., 2021; PAPPALARDO et al., 2017; VAN DEN BROEK; BOERIU, 2013). Atributos como eficiência catalítica, versatilidade e alta estabilidade explicam o papel central das lipases nas indústrias alimentícia, química e farmacêutica. Atualmente, a maioria das lipases que dominam o mercado e são utilizadas para pesquisas e processos industriais é de origem microbiana (CASTIGLIONI et al., 2020).

Entretanto, apesar do método enzimático possuir muitas vantagens em relação ao método químico, como visto, são vários os fatores que afetam a atividade catalítica das enzimas. Assim, as aplicações industriais de enzimas dependem, em sua maioria, da introdução de técnicas de imobilização eficientes. Os principais objetivos da imobilização são tornar as enzimas insolúveis no meio de reação, aumentar a estabilidade operacional, aumentar o controle do processo (separação produto/enzima e formação do produto), automatizar processos (projetar biorreatores para processos contínuos), minimizar a inibição alostérica de enzimas e permitir a reutilização do biocatalisador, que é uma característica altamente desejável dado o alto custo das enzimas (LINDEQUE; WOODLEY, 2019; REMONATTO et al., 2022). A estabilização induzida pelo processo de imobilização pode conferir um maior rendimento de reação, principalmente, ao melhorar o desempenho do biocatalisador em maior faixa de temperatura e aumentar sua tolerância a solventes orgânicos (DAL MAGRO et al., 2020; PEREIRA GONÇALVES et al., 2019; REMONATTO et al., 2022).

Vários protocolos de imobilização de enzimas já foram propostos, contudo, há uma busca constante por rotas mais simples e eficientes para a obtenção de derivados imobilizados para aplicações industriais. No geral, a imobilização enzimática pode ser obtida por meio dos métodos de aprisionamento ou encapsulação, adsorção e ligação covalente (MEENA et al., 2021; RAFIEE; REZAEE, 2021). No caso das lipases, os métodos de imobilização mais comuns são a adsorção e a ligação covalente. A adsorção é uma técnica simples e rápida que geralmente causa poucas alterações estruturais nas lipases, pois as interações formadas entre enzima e suporte são fracas (FERNANDEZ-LAFUENTE et al., 1998; RODRIGUES et al., 2019). Já na ligação covalente as enzimas são ligadas covalentemente ao suporte por meio de grupos

funcionais em sua superfície, assim, esta técnica reduz a possibilidade de dessorção enzimática ao induzir a formação de fortes interações entre enzima e suporte (GUISAN et al., 2020; RAFIEE; REZAEE, 2021; REMONATTO et al., 2021).

O processo de imobilização de enzimas consiste basicamente no confinamento ou ligação da enzima em um suporte físico, produzindo um biocatalisador/biorreator heterogêneo. Com isto, as propriedades das enzimas também passam a depender do material do suporte (MONTEIRO et al., 2021). O uso de um suporte inadequado pode impactar negativamente nas características do biocatalisador, levando, possivelmente, à distorção da estrutura terciária da enzima induzida por interações com o suporte, impedimento estérico causado pela orientação da enzima após a ligação ao suporte, lixiviação enzimática, baixas cargas enzimáticas, e limitações na taxa de transferência de massa pela difusão de substratos e produtos (BOUDRANT; WOODLEY; FERNANDEZ-LAFUENTE, 2020; TOMKE; RATHOD, 2020). Desse modo, a seleção da matriz de imobilização é de suma relevância tanto para o desempenho catalítico quanto para o custo de produção em escala industrial. A porosidade, o tamanho dos poros, a resistência mecânica e a estabilidade química são parâmetros cruciais para a seleção do suporte, afetando diretamente sua eficiência para as aplicações desejadas (LIESE; HILTERHAUS, 2013).

Devido a necessidade contínua de otimizar as condições reacionais, bem como desenvolver novos métodos de catálise enzimática para a produção de ésteres de açúcar, com o intuito de aumentar o rendimento do processo de esterificação e torná-lo economicamente viável, a utilização de enzimas imobilizadas é uma alternativa apropriada para alcançar tais finalidades. Dessa forma, o objetivo desta tese foi explorar a síntese enzimática de ésteres de sacarose a partir de enzimas imobilizadas. Como a síntese enzimática de ésteres de açúcar em modo contínuo foi pouco investigada até o momento, neste estudo além da utilização em modo batelada de lipase comercial de *Candida antartica* B imobilizada, foi produzido um biorreator enzimático, a partir da imobilização de lipase de *Candida rugosa* em criogel de poliacrilamida, para a otimização da síntese contínua de ésteres de sacarose.

Além disso, foram avaliadas as propriedades dilatacionais de ésteres de sacarose na interface óleo-água. Investigou-se também o efeito da interação entre ésteres de sacarose e goma guar ou goma xantana na interface óleo-água, juntamente com a estabilidade de emulsões produzidas a partir de soluções mistas desses ésteres com as gomas, uma vez que polissacarídeos e surfactantes são comumente encontrados juntos em sistemas reais e o conhecimento sobre as interações potenciais de ésteres de sacarose e polissacarídeos, até o momento, é escasso e limitado.

REFERÊNCIAS

ABDULMALEK, E. et al. Improved enzymatic galactose oleate ester synthesis in ionic liquids. **Journal of Molecular Catalysis B: Enzymatic**, v. 76, p. 37–43, 2012.

ABDULMALEK, E.; HAMIDON, N. F.; ABDUL RAHMAN, M. B. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents. **Journal of Molecular Catalysis B: Enzymatic**, v. 132, p. 1–4, 2016.

AN, D. et al. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 577, p. 257–264, 2019.

ARCENS, D. et al. 6-O-glucose palmitate synthesis with lipase: Investigation of some key parameters. **Molecular Catalysis**, v. 460, p. 63–68, 2018.

BOUDRANT, J.; WOODLEY, J. M.; FERNANDEZ-LAFUENTE, ROBERTO. Parameters necessary to define an immobilized enzyme preparation. **Process Biochemistry**, v. 90, p. 66–80, 2020.

CASTIGLIONI, G. Z. et al. Production of volatile compounds by yeasts using hydrolysed grape seed oil obtained by immobilized lipases in continuous packed-bed reactors. **Bioprocess and Biosystems Engineering**, v. 43, n. 8, p. 1391–1402, 2020.

CHANSANROJ, K.; BETZ, G. Sucrose esters with various hydrophilic-lipophilic properties: Novel controlled release agents for oral drug delivery matrix tablets prepared by direct compaction. **Acta Biomaterialia**, v. 6, n. 8, p. 3101–3109, 2010.

COELHO, A. L. S.; ORLANDELLI, R. C. Immobilized microbial lipases in the food industry: a systematic literature review. **Critical Reviews in Food Science and Nutrition**, v. 61, n. 10, p. 1689–1703, 2021.

DAL MAGRO, L. et al. Pectin lyase immobilization using the glutaraldehyde chemistry increases the enzyme operation range. **Enzyme and Microbial Technology**, v. 132, 2020.

FERNANDEZ-LAFUENTE, R. et al. Immobilization of lipases by selective adsorption on hydrophobic supports. **Chemistry and Physics of Lipids**, v. 93, p. 185–197, 1998.

FLOYD, P.; ZAROGIANNIS, P.; FOX, K. **SCHER Non surfactant Organic Ingredients and Zeolite-based Detergents**. Scientific Committees, European Commission, 2006. Disponível em: <http://ec.europa.eu/health/ph_risk/risk_en.htm>

GANSKE, F.; BORNSCHEUER, U. T. Optimization of lipase-catalyzed glucose fatty acid ester synthesis in a two-phase system containing ionic liquids and t-BuOH. **Journal of Molecular Catalysis B: Enzymatic**, v. 36, p. 40–42, 2005.

GUISAN, J. M. et al. **Immobilization of Enzymes and Cells Methods and Protocols**. Fourth Edition ed. [s.l.] Springer Protocols, Humana Press, 2020.

GUMEL, A. M. et al. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, v. 46, n. 11, p. 2079–2090, 2011.

INPRAKHON, P. et al. Lipase-catalyzed synthesis of sucrose monoester: Increased productivity by combining enzyme pretreatment and non-aqueous biphasic medium. **Journal of Biotechnology**, v. 259, p. 182–190, 2017.

JIA, C. et al. A simple approach for the selective enzymatic synthesis of dilauroyl maltose in organic media. **Journal of Molecular Catalysis B: Enzymatic**, v. 62, p. 265–269, 2010.

LIESE, A.; HILTERHAUS, L. Evaluation of immobilized enzymes for industrial applications. **Chemical Society Reviews**, v. 42, n. 15, p. 6236–6249, 2013.

LINDEQUE, R. M.; WOODLEY, J. M. Reactor selection for effective continuous biocatalytic production of pharmaceuticals. **Catalysts**, v. 9, n. 3, 2019.

MEENA, J. et al. Recent advances in nano-engineered approaches used for enzyme immobilization with enhanced activity. **Journal of Molecular Liquids**, v. 338, 2021.

MENG, Y.-C. et al. Effect of sucrose fatty acid esters on pasting, rheological properties and freezeethaw stability of rice flour. **Food Hydrocolloids**, v. 40, p. 64–70, 2014.

MONTEIRO, R. R. et al. Liquid lipase preparations designed for industrial production of biodiesel. Is it really an optimal solution? **Renewable Energy**, v. 164, p. 1566–1587, 2021.

MORA VARGAS, J. A. et al. Natural sucrose esters: Perspectives on the chemical and physiological use of an under investigated chemical class of compounds. **Phytochemistry**, v. 177, 2020.

NGUYEN, P. C. et al. A novel maltoheptaose-based sugar ester having excellent emulsifying properties and optimization of its lipase-catalyzed synthesis. **Food Chemistry**, v. 352, 2021.

NIETO, S. et al. Ultrasound-assisted enzymatic synthesis of xylitol fatty acid esters in solvent-free conditions. **Ultrasonics Sonochemistry**, v. 75, 2021.

PAPPALARDO, V. M. et al. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, v. 433, p. 383–390, 2017.

PEREIRA GONÇALVES, M. C. et al. Trends on enzyme immobilization researches based on bibliometric analysis. **Process Biochemistry**, v. 76, p. 95–110, 2019.

RAFIEE, F.; REZAEE, M. Different strategies for the lipase immobilization on the chitosan based supports and their applications. **International Journal of Biological Macromolecules**, v. 179, p. 170–195, 2021.

REMONATTO, D. et al. Utilization of clay materials as support for aspergillus japonicus lipase: An eco-friendly approach. **Catalysts**, v. 11, n. 10, 2021.

REMONATTO, D. et al. Applications of immobilized lipases in enzymatic reactors: A review. **Process Biochemistry**, v. 114, p. 1–20, 2022.

RODRIGUES, R. C. et al. Immobilization of lipases on hydrophobic supports: immobilization mechanism, advantages, problems, and solutions. **Biotechnology Advances**, v. 37, p. 746–770, 2019.

SALEM, J. H. et al. Effect of acyl donor chain length on isoquercitrin acylation and biological activities of corresponding esters. **Process Biochemistry**, v. 45, n. 3, p. 382–389, 2010.

TOMKE, P. D.; RATHOD, V. K. Lipase-catalyzed synthesis of propyl-phenyl acetate: a kinetic and thermodynamic study. **Bioprocess and Biosystems Engineering**, v. 43, n. 9, p. 1659–1670, 2020.

VAN DEN BROEK, L. A. M.; BOERIU, C. G. Enzymatic synthesis of oligo-and polysaccharide fatty acid esters. **Carbohydrate Polymers**, v. 93, p. 65–72, 2013.

ZAGO, E. et al. Enzymatic synthesis of amphiphilic carbohydrate esters: Influence of physicochemical and biochemical parameters. **Biotechnology Reports**, v. 30, 2021.

ZAIDAN, U. H. et al. Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. **Food Chemistry**, v. 131, n. 1, p. 199–205, 2012.

CAPÍTULO 1 - KINETIC STABILITY OF THE OIL-IN-WATER EMULSIONS AND DYNAMIC INTERFACIAL PROPERTIES OF MIXTURES OF SUCROSE ESTERS AND POLYSACCHARIDES

ABSTRACT

Sucrose esters were enzymatically synthesized using immobilized *Candida antarctica* lipase B in different organic solvents and substrate molar ratios. The results showed that 2-methyl-2-butanol and the 1:3 molar ratio (sucrose: stearic acid) provided the best yields. The esters purified by solvent extraction were identified by thin layer chromatography and infrared spectroscopy. The critical micellar concentration for the biosurfactant was determined to be approximately 10 mg/L and the equilibrium surface tension was 45 mN/m. The effect of interaction between sucrose esters and polysaccharides (guar gum or xanthan gum) on interfacial characteristics and emulsion properties were investigated. It was observed an increase in the equilibrium interfacial tension when the polymers were added to the medium, probably due to complex formation in the *bulk*. Nevertheless, emulsified systems in the presence or absence of polysaccharides, presented great kinetic stability along 28 days, due to the predominance of the elastic character of the interfacial region.

Keywords: Sugar ester; Biosurfactants; Interfacial properties; Interaction; Emulsion.

1. Introduction

Traditionally, food industries have been using synthetic oil-derived surfactants in the areas of baking, confectionery and dairy products. As a result of increased consumer interest in environmental and health issues, the focus on product development has shifted steadily towards the use of safe and biodegradable raw materials. Sugar esters are products with surfactant properties derived from cheap renewable raw materials (sugars and fatty acids). Its use for emulsion, foam and dispersion applications has received considerable attention due to the advantages over traditional surfactants, such as biodegradability, low toxicity, biocompatibility, and good efficacy under extreme temperature and pH conditions (FOLEY et al., 2012; YIN et al., 2009).

Among various sugar esters, sucrose esters are non-ionic surfactants formed from sucrose (acyl acceptor) and fatty acids (acyl donor). Since sucrose contains eight hydroxyl groups, compounds with varying degrees of esterification ranging from mono to octa esters can be produced. These surfactants can be obtained by either chemical or enzymatic esterification. In the enzymatic process, the reactions are conducted at lower temperatures as compared to the chemical process. In addition, enzymatic reactions are quite selective, with fewer by-products (GUMEL et al., 2011; VAN KEMPEN et al., 2013). Sucrose esters have great applicability and are mainly used as emulsifiers. For some applications, these sugar esters still have some advantages over surfactants synthesized by chemical route from petroleum derivatives, due to their antibacterial and crystallization inhibiting properties (CHANSANROJ; BETZ, 2010; CHOI et al., 2011).

Understanding the association of surfactants and polysaccharides in fluid interfaces is an important issue for many fundamental studies and technological applications of common industrial processes where they can be employed, such as foaming and emulsification (GUZMÁN et al., 2016; MCCLEMENTS, 2016). In general, surfactants and polymers can interact through various mechanisms, with hydrophobic and electrostatic interactions being the most frequent (SINGH; NILSSON, 1999). The properties of these mixed systems are dependent on various factors, such as polymer and surfactant charge, polymer structure, surfactant chain length, and surfactant and polymer concentrations (GODDARD, 2002; NEGM et al., 2015). In most applications, surfactants and polymers are added to products for rheological behavior control, kinetic stabilization or modification of their adsorption on surfaces.

Although enzymatic synthesis of sugar esters is well understood and mixed surfactant and polymer systems have been the subject of researches (CAO et al., 2013; GUZMÁN et al.,

2016; KRSTONOŠIĆ; MILANOVIĆ; DOKIĆ, 2019; SOHRABI et al., 2013), until the present moment studies on the effect of sugar ester and polysaccharide interactions on fluid interfaces are scarce. Furthermore, studies of dynamic interfacial properties are essential for understanding emulsification (KARBASCHI et al., 2014).

In this study, sucrose esters were synthesized by enzymatic esterification of sucrose and stearic acid using immobilized *Candida antarctica* lipase B (CALB) and purified by solvent extraction. Our hypothesis is that the surfactant-polymer interactions at the oil-water interface can impact the interfacial film properties and, thus, affect the stability of emulsified systems. Therefore, mixtures of sucrose esters and polysaccharides (guar and xanthan gums) were prepared and the dynamic interfacial properties were determined. Oil-in-water (O/W) emulsions containing these mixtures were also prepared and kinetic stability were analyzed along the 28 days.

2. Materials and methods

2.1. Materials

Candida antarctica lipase B (CALB) immobilized on acrylic resin (≥ 5000 units (U) propyl laurate per gram enzyme) was purchased from Sigma-Aldrich (Saint Louis, USA) along with the reagents: acetone, acetic acid, stearic acid, chloroform, absolute ethanol, sucrose, sodium hydroxide, molecular sieve (4 Å), methanol, 2-methyl-2-butanol (2M2B). Guar gum extracted from the seeds of *Cyamopsis tetragonoloba* (CAS No. 9000-30-0) is a non-ionic polysaccharide and Xanthan gum from *Xanthomonas campestris* (CAS No. 11138-66-2) is an anionic polysaccharide, both gums were also purchased of Sigma-Aldrich (Saint Louis, USA). Acetonitrile was purchased from Merck (Darmstadt, Germany). Sodium azide and phenolphthalein were purchased from Synth (Diadema, Brazil). All chemical reagents used were of analytical grade. Activated charcoal and sunflower oil were obtained from local market. Solutions were prepared using deionized water (Milli Q - Merck Millipore, Germany).

2.2. Enzymatic esterification reactions

Sucrose (1.5 mM) and stearic acid ($C_{18}H_{36}O_2$) at different molar ratios (1:1, 1:2, 1:3) were added to glass vials containing 5 mL of solvent (acetone, acetonitrile or 2M2B) previously dehydrated in activated molecular sieves 4 Å (20% w/v) for 24 h before use. Esterification reactions were initiated by adding 60 mg of immobilized CALB to the vials, keeping them under constant agitation at 150 rpm in an orbital oven at 65 °C for 72 h. To prevent solvent

evaporation, the vials were sealed with screw caps. Control experiments were performed, where only sucrose, stearic acid and solvent were incubated under the respective evaluated experimental conditions.

2.3. Quantitation of fatty acid conversion

The residual free fatty acid in the reaction mixture was determined by the volumetric method (NETA et al., 2012; ZAIDAN et al., 2012). Thus, 0.1 g of sample of reaction mixture was diluted in 20 mL of 0.1% (w/v) phenolphthalein solution in absolute ethanol. The mixture was then titrated with standardized sodium hydroxide solution (0.1 M). The amount of free fatty acid (A, in mol/g) in the sample was calculated for both, test and control samples. The esterification yield (Y, %), expressed as equivalent to acid conversion, was calculated using equations (1) to (3).

$$A_1 = \frac{(V_s \times 0.1 \times fc)}{M_s} \quad (1)$$

$$A_2 = \frac{(V_c \times 0.1 \times fc)}{M_c} \quad (2)$$

$$Y\ (\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100 \quad (3)$$

where V_s and V_c (mL) are the volumes of sodium hydroxide spent on titration of sample and control, respectively, fc is the correction factor for the NaOH solution and its value was 0.9908, M_s and M_c (g) are the mass of sample and control, respectively. All measurements were performed in triplicate.

2.4. Purification of sucrose esters by solvent extraction

Sucrose esters were produced in greater amount under conditions that presented higher yield and were then purified according to the methodology adapted from Enayati et al. (2018). The reaction mixture was filtered using Whatman filter paper to remove the catalyst and molecular sieves. The filtered reaction mixture was then dried in rotary evaporator. After solvent evaporation, ethanol-water solution (50:50, v/v) was added. The extraction of the esters was performed for 5 hours at 55 °C, under agitation. The mixture was then centrifuged

(Centrifuge 5804R, Eppendorf) at 1000 × g for 10 min at 25 °C and the supernatant was reserved. The extraction process was repeated twice. Sucrose esters were obtained after the complete evaporation of ethanol and water from the supernatant in oven at 70 °C.

2.5. Thin Layer Chromatography (TLC)

Purified sucrose esters were qualitatively identified by thin layer chromatography (TLC) using glass plates coated with a 0.25 mm layer of silica gel (Whatman, USA). The mobile phase used was chloroform: methanol: acetic acid: water (75:15:8:2, v/v). The esters and stearic acid samples were prepared in chloroform and the sucrose sample in deionized water. Subsequently, the plates were sprayed with sulfuric acid solution in 50% methanol (v/v) and heated at 110 °C for 10 min to develop the stains of sugar, acid and sugar esters (adapted from Cao et al. (1996)).

2.6. Infrared Spectroscopy

A sample of purified sucrose esters was analyzed by infrared spectroscopy without prior preparation, according to the adapted methodology of Bayada et al. (1995). Fourier transform infrared attenuated total reflectance (ATR-FTIR) was recorded on an FT-IR spectrometer (Varian 600-IR, equipped with a Pike GladiATR accessory). A sample of approximately 100 µg was deposited on the sample holder. To obtain the infrared spectra, 64 scans in the range of 4000 - 400 cm^{-1} and 4 cm^{-1} resolution were accumulated.

2.7. Determination of the critical micellar concentration (CMC)

The CMC of purified sucrose esters was determined using a pendant drop tensiometer (PAT-1M, SINTERFACE Tensiometer vers. 8.01, Germany), measuring the surface tension of a series of aqueous solutions containing the esters in varying concentrations. All solutions were prepared 24 h before the first measurement of surface tension in order to obtain a complete solubilization of the surfactant. For the preparation of the sucrose ester solutions, deionized water preheated at 70 °C was used. A drop of the aqueous solution containing the esters (20 mm^2) was automatically formed at the tip of the capillary and inserted into an airway space. The image of the drop formed was captured and digitalized by the CCD camera of the equipment and the surface tension at 25 °C was determined as a function of time (10800 s), adjusting the Gauss-Laplace equation using the specific software of the equipment. Before all measurements, the surface tension of deionized water at 25 °C was checked (about 72 ± 0.5

mN/m). The measurements of all solutions were performed in triplicate and the results expressed as mean values.

2.8. Determination of interfacial properties influenced by guar and xanthan gums

2.8.1 Purification of the sunflower oil

Sunflower oil was used as an oily phase. The oil was previously purified with extraction of free fatty acids in an aqueous medium, followed by adsorption with activated carbon. A mass of 250 g of oil was mixed with 50 g of deionized water and the mixture was kept under magnetic stirring for 30 min at 25 °C. The mixture was then centrifuged (Centrifuge 5804R, Eppendorf) at $1000 \times g$ during 10 min at 25 °C and the aqueous phase was discarded. Subsequently, activated carbon was added to the oil (25%, w/w) and the mixture was maintained at 35 °C under constant agitation of 200 rpm for at least 12 h. Afterwards, the activated carbon was removed by centrifugation at $1000 \times g$ for 5 min at 25 °C and vacuum filtration was performed using a 0.45 μm pore diameter filter. Sunflower oil was considered acceptable only when the interfacial tension value between the oil and deionized water remained above 25 mN/m for 1 h (after reaching dynamic equilibrium), since interfacial tension values between 25 to 32 mN/m are typical for pure oil and water systems (FINGAS, 2017).

2.8.2 Preparation of sucrose esters and sucrose esters-polysaccharides solutions

Aqueous stock solutions of guar gum (0.5% w/w) and xanthan gum (0.5% w/w) were previously prepared by dissolving the appropriate mass of each gum in deionized water. In order to promote complete hydration of the biopolymers, the solutions were prepared at least 12 h before starting the experiments. Sodium azide (0.02%, w/w) was added as a preservative. The final composition of the treatments was obtained by diluting the stock solutions of the gums in aqueous solution containing the purified sucrose esters (10 mg/L). Solutions of pure sucrose esters (E) were prepared by dissolving the esters in deionized water preheated at 70 °C. All solutions were equilibrated 30 min before use.

The biopolymer concentration values evaluated were chosen based on the apparent viscosity values of their solutions measured at 100 s^{-1} using a HAAKE MARS rheometer (Modular Advanced Reometer System, Thermo Electron Corp., Germany) equipped with a thermostatic bath (Phoenix 2C30P, Thermo Electron Corp., Germany) at 25 °C. It was intended to evaluate usual concentrations of gums in food emulsions. For the purpose of comparison between the two different polysaccharides, concentrations of both gums that provide the same

viscosity were chosen. Thus, for the apparent viscosity values of 0.02 and 0.04 Pa.s, the concentrations of guar gum were 0.31% w/w (G31) and 0.35% w/w (G35) and the concentrations of xanthan gum were 0.20% w/w (X20) and 0.25% w/w (X25), respectively.

2.8.3 Measurement of dynamic interfacial properties

The dynamic interfacial properties between the aqueous solution (containing sucrose esters and whether or not containing the polysaccharides) and purified oil were determined by drop profile analysis tensiometry (PAT-1M, SINTERFACE Tensiometer vers. 8.01, Germany) at 25 °C, as described by Simiqueli et al. (2019a), with modifications. A drop of aqueous solution (20 mm^2) was automatically formed at the tip of the capillary inserted into the purified oil. The droplet image was captured and digitized by a CCD camera and the interfacial tension was determined by analyzing the droplet profile and adjusting to it the Gauss-Laplace equation using the specific software of the equipment. Measurements were performed up to the dynamic equilibrium condition (approximately 5400 s). After this time, sinusoidal oscillations were applied to the drop volume with a 3% amplitude to remain within the linear viscoelastic region, and the frequency ranged from 0.0025 to 0.02 Hz (NEUMANN et al., 2018). Dilatational parameters were calculated using a Fourier transform algorithm implemented in the software package. Thus, it was possible to obtain the elastic modulus (E') and viscous modulus (E") and the phase angle (ϕ), calculated by $\tan \phi = E'' / E'$. Lower values of ϕ (<45°) indicate a predominantly elastic interface region and greater ϕ values (>45°) a predominantly viscous interface. All measurements were made in triplicate.

2.9. Preparation of emulsions

The oily and aqueous phases were prepared similarly to the solutions off the interfacial experiments. Oil-in-water (O/W) emulsions were prepared by dispersing purified sunflower oil (20%, w/w) in aqueous solution of sucrose esters (0.5%, w/w) containing different concentrations of polysaccharides, the same concentrations described in section 2.8.2. Pre-emulsions were prepared using a high-speed homogenizer (Omni Macro ES Digital Programmable Homogenizer, Kennesaw, USA) at 15000 rpm for 3 min at room temperature. Subsequently, the pre-emulsions were subjected to 6 homogenization cycles in a high-pressure homogenizer (Emulsiflex-C5, 39 Avestin, Ottawa, Canada) operating at 15000 psi. The pH of the emulsions was measured and, if necessary, adjusted to pH 7 using HCl and/or NaOH (1 M).

2.10. Instrumental analysis of emulsified systems

2.10.1 Rheological properties

The rheological properties were determined using a HAAKE MARS oscillating dynamic rheometer (Modular Advanced Reometer System, Thermo Electron Corp., Germany), equipped with a thermostatic bath (Phoenix 2C30P, Thermo Electron Corp., Germany) at 25 °C. The coaxial cylinder sensor (DG41) was used with strain rate ranging from 0.1 to 200 s^{-1} (ascending, descending and ascending ramp) during 6 min (Steffe, 1996).

Characterization of viscoelasticity was obtained by means of the oscillatory dynamic test. Measurements were performed with the cone and plate geometry sensor (cone angle = 1°, diameter = 60 mm, gap = 0.051 mm) (C60/1). First, the region of linear viscoelasticity was determined by submitting the samples to the shear sweep test (1 to 1000 mPa, 1.0 Hz). Next, the solutions were submitted to the frequency sweep test (0.1 to 10.0 Hż, 20.0 mPa) to obtain the parameters of the elastic (G') and viscous (G") modules.

2.10.2 Zeta Potential, Particle Diameter and Emulsion Polydispersity Index (PDI)

The zeta potential (or ζ potential) was obtained based on measurements of the electrophoretic mobility of the oil droplets, which move under the action of an electric field. The average diameter and polydispersity index were determined by the dynamic light scattering (DLS) technique. All measurements were performed using ZetasizerNano-ZS equipment (Malvern Instruments Inc., Southborough, MA). Samples were diluted in deionized water ($1:10^{-4}$) and measurements were taken at 25 °C and pH 7. Measurements were made with at least 3 sequential readings immediately after preparation and also over the storage period (28 days) in order to characterize the systems and evaluate the kinetic stability of the emulsions.

2.10.3 Microstructure and macroscopic stability

The microstructure of the emulsions was analyzed by a light microscope (OLYMPUS BX-60), with a magnification of 1000 x. Emulsion aliquots without prior preparation were placed on slides and carefully covered with glass coverslips.

The extent of phase separation of the emulsions was quantified in terms of the system creaming index. To facilitate visualization of phase separation, the oil was stained with Sudam III prior to the homogenization step. Thus, immediately after the homogenization process, 3 mL aliquots of the emulsions were transferred to glass tubes. Photographs of the emulsions were taken shortly after emulsion preparation and after a twenty-eight day storage period, at 25 °C.

2.11. Statistical analysis

The effects of substrate molar ratio (sugar: fatty acid) (X_1) and organic solvent type (X_2) on the synthesis of sucrose esters were studied following a completely randomized design (CRD) in factorial scheme 3^2, with 3 replications. The data obtained were evaluated by analysis of variance (ANOVA), considering the main effect of each independent variable and the interaction between them. The first order model was adjusted and its adequacy was judged by the significance of the regression parameters ($p \leq 0.05$).

Comparison of the different emulsified systems was performed via the analysis of variance, followed by the Tukey test ($p \leq 0.05$). All measurements were performed in triplicate. Statistical analyses were performed using the SAS® software (Statistical Analysis System - SAS), version 9.3, licensed by the Federal University of Viçosa.

3. Results and discussions

3.1. Synthesis and identification of sucrose esters

For each organic solvent used, the influence of the substrate molar ratio variable on the yield of the esterification reaction was statistically evaluated (Table 1).

Table 1. Regression models for fatty acid conversion yield (Y) as a function of substratemolar ratio (X1) for reactions carried out in different solvents, their coefficients of determination (R^2) and probability levels (p)

Solvent	Regression models	R^2	p-value
Acetone	$Y = 15.175 - 1.6259X_1$	0.9600	0.0301
Acetonitrile	$Y = 4.5744 - 0.7850\ X_1$	0.9849	0.0291
2M2B	$Y = 26.012 + 9.3133\ X_1$	0.9904	0.0483

The increase of substrate molar ratio provided a significant reduction ($p \leq 0.05$) in the yield of fatty acid conversion to sucrose esters when using acetone and acetonitrile solvents (Figure 1). However, the opposite was verified when the 2M2B solvent was employed. In general, lipase-catalyzed conversion of fatty acids into sugar esters can be improved by providing an excess of fatty acid, since the excess substrate in the reaction mixture can shift the balance toward product synthesis of esterification. However, the solubility of reagents in the reaction medium is a limiting factor of reaction progress and is dependent on the type of solvent

used (AN et al., 2019; GUMEL et al., 2011). It was observed that the solubilization of the reagents using 2M2B was higher compared in relation to acetone and acetonitrile, as well as the reaction yield, which in turn increased with the increase of substrate molar ratio. The higher yield (53.4%) obtained in fatty acid conversion is comparable to that of other esterification reactions for the synthesis of sucrose esters in which lipases were used as catalyst (INPRAKHON et al., 2017; NETA et al., 2012).

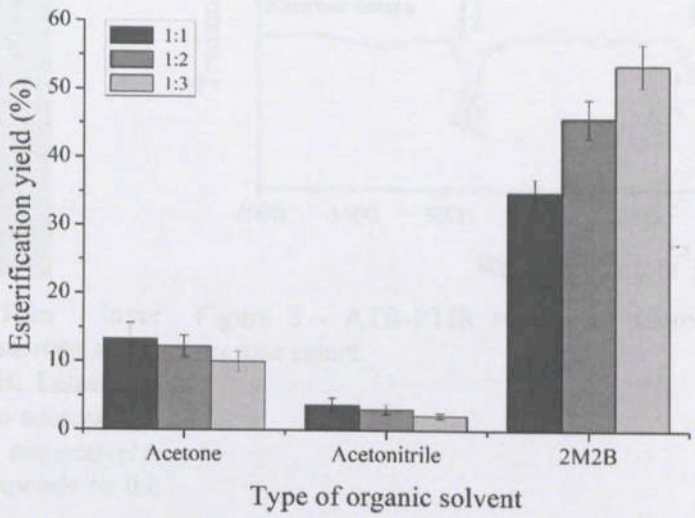

Figure 1- Fatty acid conversion yields as a function of organic solvent type for different substrate molar ratios (1:1, 1:2 and 1:3).

The synthesis of sucrose esters was confirmed by TLC (Figure 2), which shows in the band A a spot referring to the sucrose pattern, presenting an Rf value of 0.01, and in band B the stearic acid pattern that followed the solvent front with an Rf of 0.9. Two intense dark spots next to each other for the esterification product after purification were observed in range C, with a Rf value of 0.45 and 0.50. Thus, it can be inferred from this analysis that sucrose esters were likely produced.

Characteristic ATR-FTIR spectra of sucrose, stearic acid and purified product samples after enzymatic esterification are shown in Figure 3. The spectrum obtained for sucrose esters indicated a decrease in the characteristic bandwidth for the free hydroxyl group (O-H) at 3300-3400 cm^{-1} due to esterification of the sucrose OH groups. The intensification in the product spectrum signals around 2915 cm^{-1} indicated the presence of the alkyl group. The new band around 1750 cm^{-1}, corresponding to the wavelength for infrared absorption of a carbonyl ester bond (PAVIA et al., 2008), confirms that the esterification of sucrose was successful. Intense

signals in the 1000 to 1150 cm^{-1} wavelength range assigned to the carbon-oxygen single bond (-C-O-) present in sugars also indicated that the product was a sucrose ester.

Figure 2 - Thin layer chromatography of sucrose esters synthesized in 2M2B. Lanes (A) and (B) correspond to sucrose and stearic acid samples, respectively. The range (C) corresponds to the synthesized product.

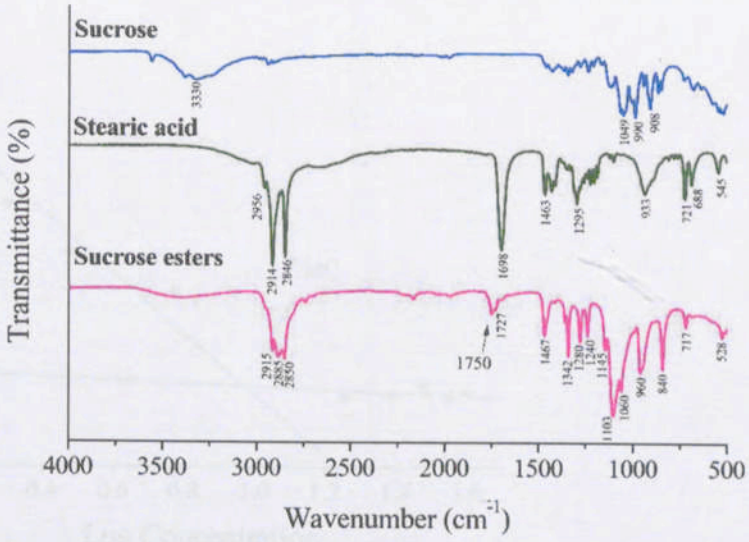

Figure 3 - ATR-FTIR spectra of sucrose, stearic acid and sucrose esters.

3.2. Critical Micellar Concentration (CMC)

In the graphic representation (Figure 4) of the dynamic surface tension (γ) versus logarithm of surfactant concentration (log C), there are two regions (below and above the CMC). Below the CMC the reduction in surface tension is dependent on the increase in surfactant concentration. On the other hand, at surfactant concentrations above CMC the surface tension becomes almost constant. The point of intersection between the horizontal and angular lines corresponds to the critical micellar concentration (ALLEN; TAO, 2002; GAUDIN et al., 2018).

The sucrose esters synthesized in this study showed a low CMC (approximately 10 mg/L), characteristic of non-ionic surfactants. This result is close to that obtained by Soultani et al. (2003) for a commercial sucrose stearate (CMC 14 mg/L). The surface tension (γ_{cmc}) after reaching the CMC obtained for sucrose esters was 45 ± 1 mN/m. This value is close to the values reported in the literature (35-45 mN/m) for sugar esters at the same temperature (25 °C) (ALLEN; TAO, 2002; BECERRA et al., 2008; SOULTANI et al., 2003). It is noted that

concentrations of sugar esters required to achieve the minimum surface tension are very low and may be indicated in applications where limited amounts of surfactants are desirable.

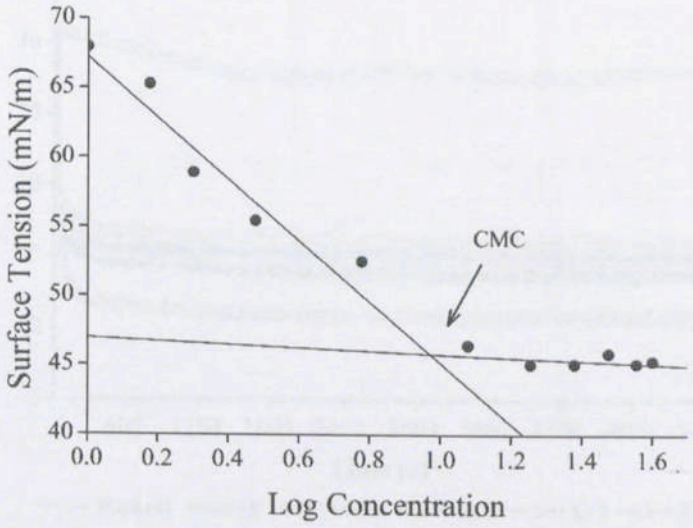

Figure 4 - Dynamic surface tension variation *versus* log concentration of sucrose esters.

3.3. Characterization of interfacial properties

3.3.1. Interfacial tension

In this study, tensiometry in conjunction with kinetic stability analyses of emulsified systems composed of polymer-surfactant mixtures provided a good understanding of the interactions between them. The interfacial tension profiles as a function of time between purified oil and deionized water and between purified oil and aqueous solutions composed solely of sucrose esters or mixed with gums (guar or xanthan) are shown in Figure 5. It was observed that equilibrium interfacial tension (γ_{eq}) remained practically constant (28 ± 1 mN/ m) for the oil/water system without the addition of surfactant, demonstrating that the oil purification process was efficient. Addition of sucrose esters in the aqueous medium reduced the interfacial tension from 28 to 11 mN/m. For the solutions with polysaccharides addition, the equilibrium interfacial tension values were higher, being around 14 to 16 mN/m.

Therefore, it was observed that a possible formation of sucrose esters-polysaccharides intermolecular complexes in *bulk* resulted in a different interfacial behavior compared to the pure surfactant solution. With the occurrence of the interaction in *bulk*, the content of free sucrose ester monomer was decreased in the solution and, consequently, the interfacial tension increased. That is, the sucrose esters-guar gum and sucrose esters-xanthan gum intermolecular

complexes led to the less exposition of hydrophobic groups of sucrose esters to the oil-water interface.

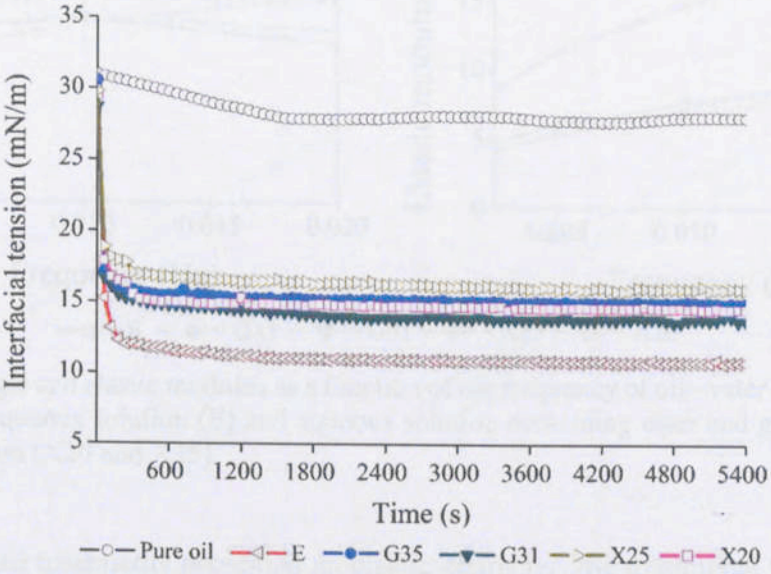

Figure 5 - Interfacial tension decay profiles obtained for the purified oil, pure esters aqueous solution (E) and aqueous solution containing esters and guar gum (G31 and G35) or xanthan gum (X20 and X25).

A similar result was observed for the commercial surfactant Tween 80 in the presence of xanthan gum at air-water interface (KRSTONOŠIĆ; MILANOVIĆ; DOKIĆ, 2019). The authors concluded that the pronounced increase in surface tension in mixed solutions of these substances was due interaction in *bulk*, occurred by hydrophobic interaction and/or hydrogen bonds for the xanthan gum-Tween 80 complexes, resulting in a decreased concentration of free surfactant monomers and leading to the assumption that the complexes formed could not adsorb at the interface. Therefore, can infer for the present research, for the conditions evaluated, that the amount of sucrose esters is adsorbed at the interface is dependent on the presence of polysaccharides.

3.3.2. Interfacial Viscoelasticity

Figure 6 shows the phase angle and elastic modulus of the different aqueous solutions inserted into the purified oil as a function of frequency. In all treatments the phase angle was less than 45°, indicating that the interfacial films were predominantly elastic. The trend of storage and loss modules was similar for all treatments, that is, surface elasticity increased and viscosity decreased with increasing frequency. By increasing the frequency, faster changes occur in the surface area. Therefore, in order to restore system equilibrium, surfactant adsorption and desorption kinetics are higher (NEUMANN et al., 2018).

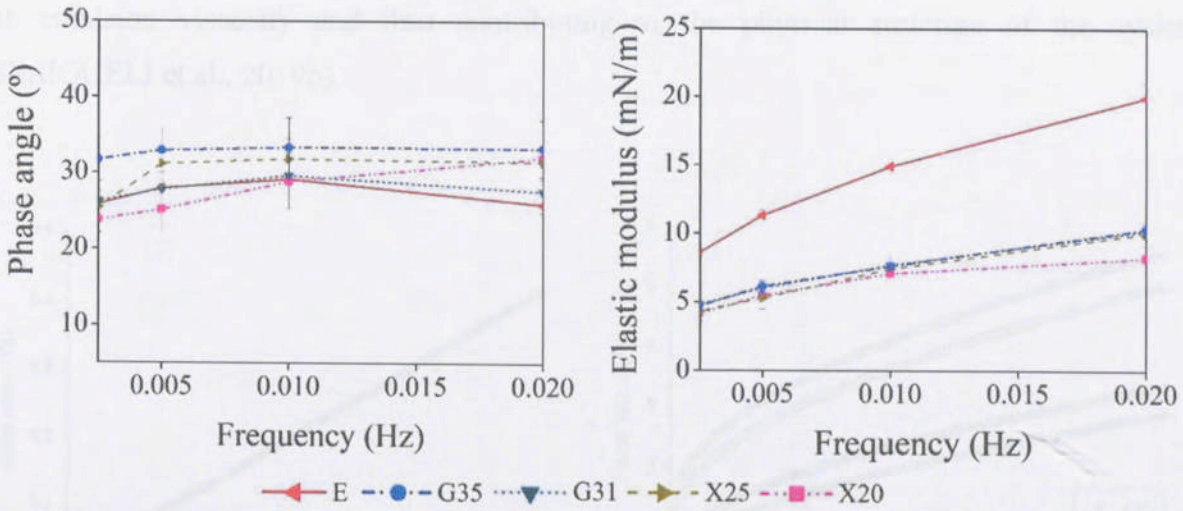

Figure 6 - Phase angle and elastic modulus as a function of the frequency of oil–water interfaces obtained for the pure ester aqueous solution (E) and aqueous solution containing ester and guar gum (G31 and G35) or xanthan gum (X20 and X25).

Although all treatments presented an elastic character, the treatments with addition and increased polysaccharide concentration resulted in lower elasticity of the interfacial layer, since they presented higher phase angle values compared to the treatment containing only the esters in the aqueous solution. This result reaffirms the conclusion obtained in the previous analysis of interfacial tension, in which probably the polysaccharides and sucrose esters interacted with each other (hydrophobic interaction or/and hydrogen bonds), leading to the formation of a complex in *bulk*.

3.4. Physical characterization of the emulsified systems

3.4.1. Rheological properties

The results of the flow behavior analysis (Figure 7 and Table 2) showed that emulsions containing polysaccharides presented a non-Newtonian pseudoplastic behavior. The emulsions without polysaccharides had a Newtonian behavior. None of the emulsions showed thixotropic behavior. Viscoelastic characteristics of emulsions containing polysaccharides were also measured. These systems were predominantly elastic (Figure 8) as the relationship between rheological modules was less than 1 (tg δ <1) (Steffe, 1996).

The predominance of elastic character tends to favor the reduction of *brownian* motion of oil droplets, as well as the frequency and intensity of collisions between them, contributing to the kinetic stability of the system (CAI et al., 2018). The elasticity of the emulsified systems is probably related to the presence of xanthan gum and guar gum thickeners. These polysaccharides have the functional capacity to attract water molecules to their chain, increasing

the emulsion viscosity and thus contributing to the physical structure of the system (SIMIQUELI et al., 2019b).

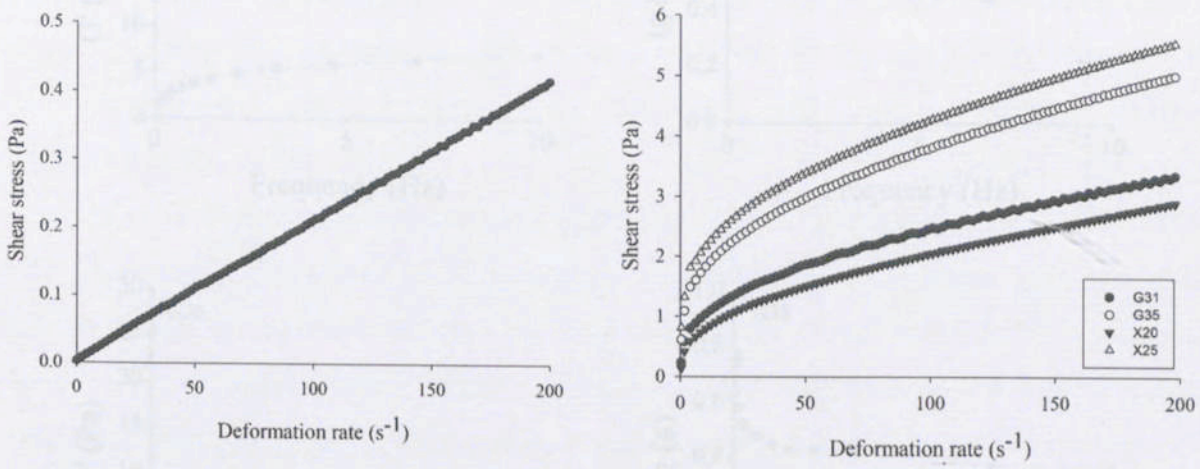

Figure 7 - Rheograms of the emulsified systems with and without polysaccharides.

Table 2. Average parameters of the Newton model used for rheological characterization of the emulsions without polysaccharides (E), average parameters of the Ostwald-de- Waele model used for rheological characterization of the emulsions containing polysaccharides (G31, G35, X20 and X25) and mean values of absolute (μ) and apparentviscosity (η_{100})

Newton Model				
Treatments		**R^2**		**μ (Pa.s)**
E		0.999		0.002 ± 0.000
Ostwald-de-Waele Model				
Treatments	**K (Pa.s^n)**	**N**	**R^2**	**η_{100} (Pa.s)**
G31	0.376 ± 0.001	0.410 ± 0.001	0.999	0.025 ± 0.000
G35	0.742 ± 0.010	0.724 ± 0.009	0.999	0.041 ± 0.001
X20	0.255 ± 0.004	0.456 ± 0.003	0.999	0.021 ± 0.000
X25	0.921 ± 0.015	0.332 ± 0.002	0.997	0.042 ± 0.001

[1] Values represent means ± SD of triplicates. η_{100}: apparent viscosity at 100 s^{-1}.

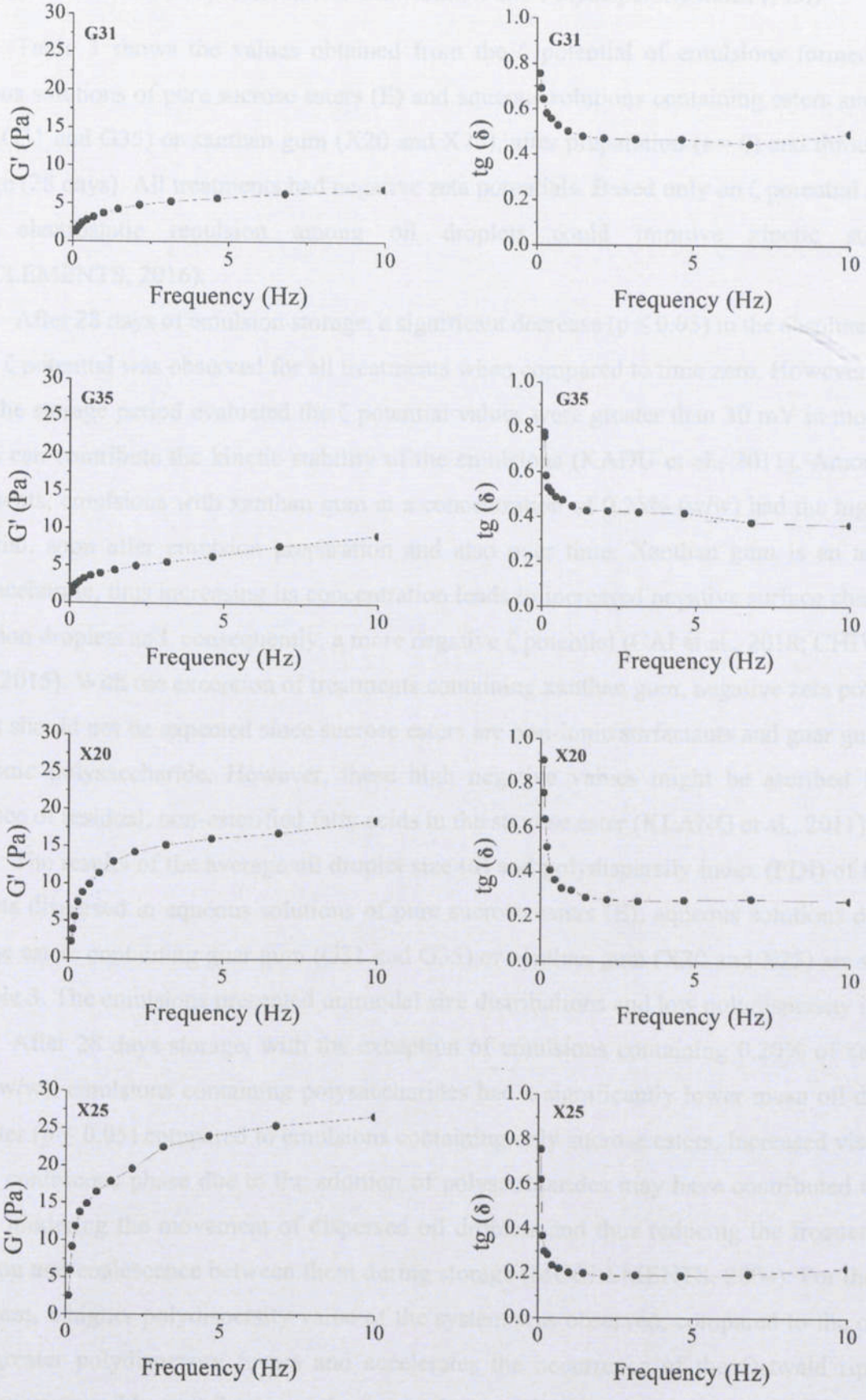

Figure 8 - Modulus of elasticity (G') and loss angle (tg δ) as a function of frequency for the O/W emulsion systems.

3.4.2. Zeta Potential, Droplet Diameter Distribution and Polydispersity Index (PDI)

Table 3 shows the values obtained from the ζ potential of emulsions formed with aqueous solutions of pure sucrose esters (E) and aqueous solutions containing esters and guar gum (G31 and G35) or xanthan gum (X20 and X25), after preparation (t = 0) and throughout storage (28 days). All treatments had negative zeta potentials. Based only on ζ potential (< -30 mV), electrostatic repulsion among oil droplets could improve kinetic stability (MCCLEMENTS, 2016).

After 28 days of emulsion storage, a significant decrease ($p \leq 0.05$) in the absolute value of the ζ potential was observed for all treatments when compared to time zero. However, even after the storage period evaluated the ζ potential values were greater than 30 mV in modulus, which can contribute the kinetic stability of the emulsions (KADU et al., 2011). Among the treatments, emulsions with xanthan gum at a concentration of 0.25% (w/w) had the highest ζ potential, soon after emulsion preparation and also over time. Xanthan gum is an anionic polysaccharide, thus increasing its concentration leads to increased negative surface charge of emulsion droplets and, consequently, a more negative ζ potential (CAI et al., 2018; CHIVERO et al., 2015). With the exception of treatments containing xanthan gum, negative zeta potential values should not be expected since sucrose esters are non-ionic surfactants and guar gum is a non-ionic polysaccharide. However, these high negative values might be ascribed to the presence of residual, non-esterified fatty acids in the sucrose ester (KLANG et al., 2011).

The results of the average oil droplet size (d) and polydispersity index (PDI) of the oil droplets dispersed in aqueous solutions of pure sucrose esters (E), aqueous solutions of pure sucrose esters containing guar gum (G31 and G35) or xanthan gum (X20 and X25) are shown in Table 3. The emulsions presented unimodal size distributions and low polydispersity index.

After 28 days storage, with the exception of emulsions containing 0.20% of xanthan gum (w/w), emulsions containing polysaccharides had a significantly lower mean oil droplet diameter ($p \leq 0.05$) compared to emulsions containing only sucrose esters. Increased viscosity of the continuous phase due to the addition of polysaccharides may have contributed to this result, hindering the movement of dispersed oil droplets and thus reducing the frequency of collision and coalescence between them during storage (MCCLEMENTS, 2004). For the X20 treatment, a higher polydispersity value of the system was observed, compared to the others. This greater polydispersity favors and accelerates the occurrence of the Ostwald ripening mechanism, possibly contributing to the greater increase in the average droplet diameter.

Table 3. Zeta potential of emulsions oil droplets, average oil droplets diameter (d) and emulsion polydispersity index (PDI) immediately after preparation and after 28 days of storage of the O/W emulsions

Treatments	*t = 0 day*	*t = 7 days*	*t = 14 days*	*t = 21 days*	*t = 28 days*
	ζ potential (mV)				
E	-45.60 ± 1.73^{bA}	-46.68 ± 0.89^{aA}	-42.27 ± 0.55^{bB}	-37.33 ± 1.12^{cC}	-32.92 ± 0.60^{dD}
G31	-42.27 ± 0.79^{cdA}	-42.15 ± 2.14^{bA}	-40.73 ± 0.96^{bAB}	-40.1 ± 0.43^{bB}	-35.70 ± 2.25^{bcC}
G35	-43.90 ± 1.17^{bcA}	-42.55 ± 0.82^{bAB}	-40.60 ± 0.87^{bC}	-40.82 ± 1.70^{bBC}	-37.08 ± 0.84^{bD}
X20	-40.57 ± 0.88^{dA}	-37.33 ± 0.68^{cB}	-36.57 ± 1.16^{cB}	-37.43 ± 1.88^{cB}	-34.57 ± 0.72^{cdC}
X25	-52.35 ± 0.55^{aA}	-47.08 ± 0.66^{aB}	-45.75 ± 0.76^{aBC}	-44.85 ± 0.93^{Ac}	-42.73 ± 0.82^{aD}
Treatments	*d (nm)*				
E	155.65 ± 0.80^{cA}	264.27 ± 3.79^{aB}	267.68 ± 2.72^{aB}	270.17 ± 6.83^{aB}	280.82 ±13.62^{aC}
G31	184.97 ± 1.20^{bA}	227.62 ± 1.69^{cB}	225.12 ± 1.63^{cB}	248.95 ± 12.17^{cC}	261.07 ± 5.77^{bD}
G35	202.28 ± 2.08^{aA}	250.85 ± 2.85^{bB}	256.43 ± 2.15^{bBC}	259.35 ± 13.26^{bBC}	262.73 ±1.84^{bC}
X20	192.58 ± 2.21^{bA}	256.40 ± 7.58^{abB}	261.77 ±2.70^{abBC}	267.02 ± 5.02^{abC}	284.00 ± 5.37^{aD}
X25	190.35 ± 2.49^{bA}	215.92 ± 1.05^{dB}	224.13 ± 1.74^{cB}	248.83 ± 1.72^{cC}	259.55 ± 2.98^{bD}
Treatments	*PDI*				
E	0.09 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.18 ± 0.03	0.21± 0.03
G31	0.07 ± 0.02	0.07 ± 0.03	0.07 ± 0.02	0.15 ± 0.04	0.20 ± 0.02
G35	0.06 ± 0.03	0.16 ± 0.02	0.17 ± 0.02	0.21 ± 0.03	0.17 ± 0.03
X20	0.10 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.24 ± 0.01	0.24 ± 0.03
X25	0.085 ± 0.02	0.087 ± 0.01	0.09 ± 0.01	0.17 ± 0.01	0.19 ± 0.02

[1] Means followed by the same letter do not differ statistically from each other at the 5% probability level by Tukey's test. Lowercase letters in the column (same day) and uppercase letters in the row (same treatment). Values represent means ± SD of triplicates.

3.4.3. Microstructure

Figure 9 shows micrographs of all emulsions immediately after preparation. Observation of the emulsions by optical microscopy showed that the formulation containing only sucrose esters had uniform droplets.

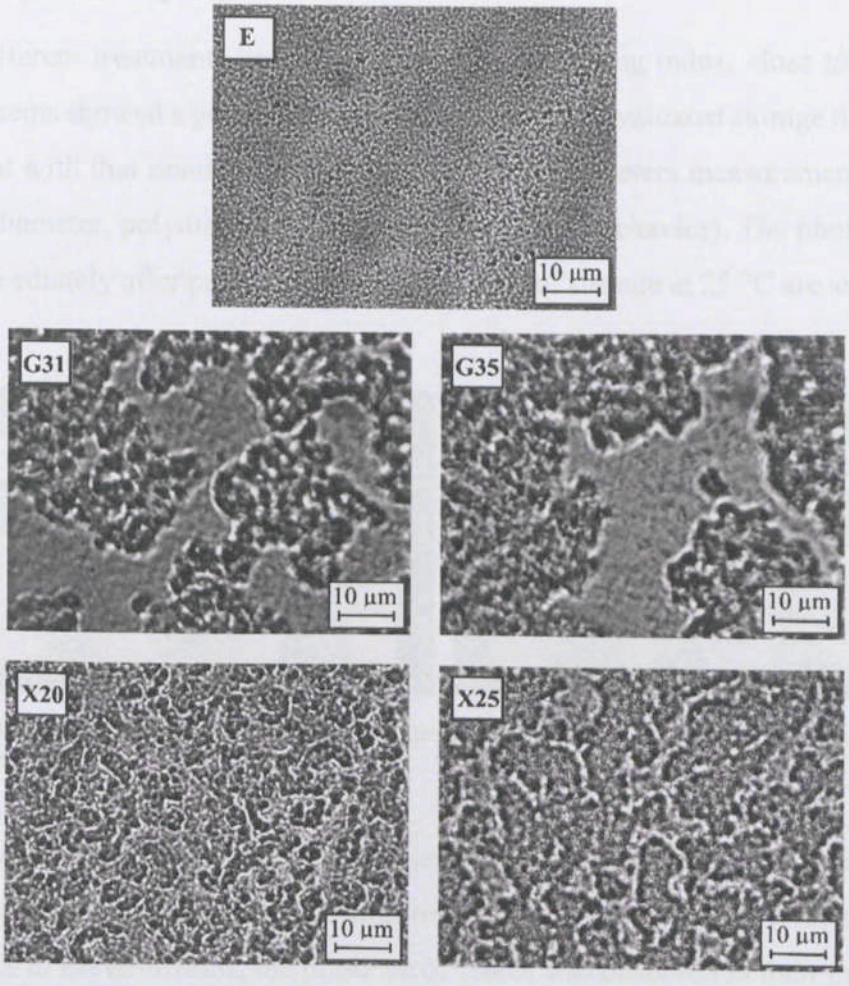

Figure 9 - Micrographs of emulsions containing sucrose ester (E), mixtures of sucrose esters-guar gum (G31 and G35) and mixtures of sucrose esters-xanthan gum (X20 and X25).

However, the addition of polysaccharides changed the emulsion morphology to a flocculated droplets pattern. In systems containing hydrocolloids, flocculation is an omnipresent phenomenon (DICKINSON, 2003). Flocculation occurs through a depletion mechanism and is caused by the presence of non-adsorbent polysaccharides (such as guar gum and xanthan gum) in the *bulk* of the emulsion (CAO; DICKINSON; WEDLOCK, 1990). Kaltsa et al. (2013) also observed flocculated droplets in emulsion microstructure containing 20% of the oil phase and different concentration of guar gum or xanthan gum in aqueous phase. The authors reported that the gaps formed between the flakes seemed to be more extended with the

addition of xanthan gum and stated that this behavior is characteristic due to its high molecular weight compared to guar gum. Chivero et al. (2015) also observed the presence of flocculated droplets in O/W emulsion containing guar and xanthan gum, attributing this phenomenon to the depletion mechanism.

3.4.4. Macroscopic Stability

The different treatments presented a very low creaming index, close to 0%. Thus, all emulsified systems showed a good kinetic stability during the evaluated storage time. This result is in agreement with that obtained by physicochemical parameters measurements (ζ potential, mean droplet diameter, polydispersity index and rheological behavior). The photographs of the emulsions immediately after preparation and at 28 days of storage at 25 °C are shown in Figure 10.

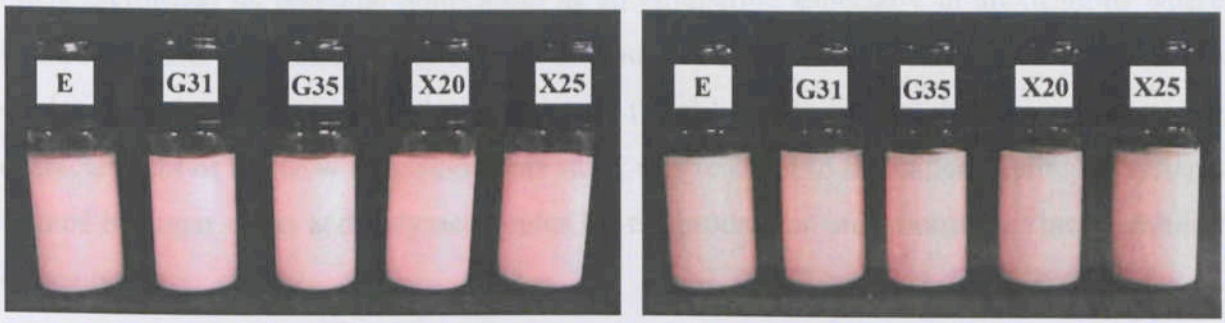

Figure 10 - Photographs of emulsified systems immediately after preparation (left) and with 28 days of storage (right) at 25 °C.

Emulsions prepared without the addition of polysaccharides showed uniform droplets observed by optical microscopy, in addition to a low creaming index. With the addition of polysaccharides in the emulsions, the presence of flakes was observed in their microstructures, even though creaming has been very low. Chivero et al. (2015) evaluated emulsions containing 0.2% and 0.3% (w/w) of polysaccharides (guar gum or xanthan gum) and did not observe creaming during 30 days storage at 25 °C, even with flocculation having been observed by microscopy. When very low concentrations of polysaccharides are used, the entropy loss linked to particle aggregation overcome the depletion mechanism and the emulsion remains stable (DICKINSON, 2003).

4. Conclusions

In the present study, the sucrose fatty acid esters were synthesized using immobilized CALB lipase. The highest yield for the synthesis of sucrose esters was obtained in a molar ratio

of 1:3 (sugar: fatty acid) in 2-methyl-2-butanol. TLC and ATR-FTIR confirmed the synthesis of the sucrose esterification product with stearic acid. The sucrose esters produced showed low values of surface and interfacial tension and low value of CMC (10 mg/L), characteristic of non-ionic surfactants.

Addition of polysaccharides in the sucrose ester solution increased interfacial tension as well as the *bulk* viscosity, compared to the solution without polysaccharides. Regardless, all the emulsions presented great stability with no creaming formation. However, the presence of polysaccharides resulted in flocculated droplets probably due to a depletion mechanism. The kinetic stability of the O/W emulsions was favored by the sum of several factors, which are: low interfacial tension, elastic interfacial film, small drops and viscosity of the continuous phase (for emulsions with polysaccharides).

The low values of surface and interfacial tension obtained with the produced sucrose esters confirmed its potential application as an emulsifier, especially in applications where limited amounts of surfactants are desirable. In addition, based on the association of the results of the analysis of the interfacial properties of the solutions and the physical and chemical characteristics of the O/W emulsions, this study was relevant to the understanding of systems formed by sugar esters and polysaccharides for the production and control of kinetic stability of emulsions.

5. References

ALLEN, D. K.; TAO, B. Y. Synthesis and characterization of maltose fatty acid monoesters as biosurfactants. **Journal of Surfactants and Detergents**, v. 5, n. 3, p. 245–255, 2002.

AN, D. et al. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 577, p. 257–264, 2019.

BAYADA, A. et al. ATR-IR Spectroscopy for the Investigation of Solution Reaction Kinetics: Hydrolysis of Trimethyl Phosphate. **Applied Spectroscopy**, v. 49, n. 12, p. 1789–1792, 1995.

BECERRA, N. et al. Characterization of micelles formed by sucrose 6-O-monoesters. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 327, n. 1–3, p. 134–139, 2008.

CAI, Y. et al. Effect of xanthan gum on walnut protein/xanthan gum mixtures, interfacial adsorption, and emulsion properties. **Food Hydrocolloids**, v. 79, p. 391–398, 2018.

CAO, C. et al. Effect of gum arabic on the surface tension and surface dilational rheology of trisiloxane surfactant. **Food Hydrocolloids**, v. 30, n. 1, p. 456–462, 2013.

CAO, L. et al. Lipase-Catalyzed Solid Phase Synthesis of Sugar Fatty Acid Esters. **Biocatalysis and Biotransformation**, v.4, n.4, p. 269–283, 1996.

CAO, Y.; DICKINSON, E.; WEDLOCK, D. J. Creaming and flocculation in emulsions containing polysaccharide. **Food Hydrocolloids**, v. 4, n. 3, p. 185–195, 1990.

CHANSANROJ, K.; BETZ, G. Sucrose esters with various hydrophilic-lipophilic properties: Novel controlled release agents for oral drug delivery matrix tablets prepared by direct compaction. **Acta Biomaterialia**, v. 6, n. 8, p. 3101–3109, 2010.

CHIVERO, P. et al. Effect of xanthan and guar gums on the formation and stability of soy soluble polysaccharide oil-in-water emulsions. **Food Research International**, v. 70, p. 7–14, 2015.

CHOI, S. J. et al. Formulation and properties of model beverage emulsions stabilized by sucrose monopalmitate: Influence of pH and lyso-lecithin addition. **Food Research International**, v. 44, n. 9, p. 3006–3012, 2011.

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

ENAYATI, M. et al. Synthesis and characterization of lactose fatty acid ester biosurfactants using free and immobilized lipases in organic solvents. **Food Chemistry**, v. 266, p. 508–513, 2018.

FINGAS, M. **Oil Spill Science and Technology**. Gulf Professional Publishing, Capítulo 3, 2017.

FOLEY, P. et al. Derivation and synthesis of renewable surfactants. **Chemical Society Reviews**, v. 41, n. 4, p. 1499–1518, 2012.

GAUDIN, T. et al. Investigating the impact of sugar-based surfactants structure on surface tension at critical micelle concentration with structure-property relationships. **Journal of Colloid and Interface Science**, v. 516, p. 162–171, 2018.

GODDARD, E. D. Polymer/surfactant interaction: Interfacial aspects. **Journal of Colloid and Interface Science**, v. 256, n. 1, p. 228–235, 2002.

GUMEL, A. M. et al. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, v. 46, n. 11, p. 2079–2090, 2011.

GUZMÁN, E. et al. Polymer-surfactant systems in bulk and at fluid interfaces. **Advances in Colloid and Interface Science**, v. 233, p. 38–64, 2016.

INPRAKHON, P. et al. Lipase-catalyzed synthesis of sucrose monoester: Increased productivity by combining enzyme pretreatment and non-aqueous biphasic medium. **Journal of Biotechnology**, v. 259, p. 182–190, 2017.

KADU, P. J. et al. Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS). **Pharmaceutical Development and Technology**,

v. 16, n. 1, p. 65–74, 2011.

KALTSA, O. et al. Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers. **Ultrasonics Sonochemistry**, v. 20, n. 3, p. 881–891, 2013.

KARBASCHI, M. et al. Rheology of interfacial layers. **Current Opinion in Colloid and Interface Science**, v. 19, n. 6, p. 514–519, 2014.

KLANG, V. et al. Development of sucrose stearate-based nanoemulsions and optimisation through γ-cyclodextrin. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 79, n. 1, p. 58–67, 2011.

KRSTONOŠIĆ, V.; MILANOVIĆ, M.; DOKIĆ, L. Application of different techniques in the determination of xanthan gum-SDS and xanthan gum-Tween 80 interaction. **Food Hydrocolloids**, v. 87, n. 2018, p. 108–118, 2019.

MCCLEMENTS, D. J. Protein-stabilized emulsions. **Current Opinion in Colloid and Interface Science**, v. 9, n. 5, p. 305–313, 2004.

MCCLEMENTS, D. J. **Food emulsions: principles, practice and techniques**. CRC Press, 2016.

NEGM, N. A. et al. Polymer-cationic surfactant interaction: 1. Surface and physicochemical properties of polyvinyl alcohol (PVA)-S-alkyl isothiouronium bromide surfactant mixed systems. **Journal of Surfactants and Detergents**, v. 18, n. 2, p. 245–250, 2015.

NETA, N. DO A. S. et al. Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions. **Food Hydrocolloids**, v. 27, n. 2, p. 324–331, 2012.

NEUMANN, S. M. et al. Interactions in water in oil in water double emulsions: Systematical investigations on the interfacial properties and emulsion structure of the outer oil in water emulsion. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 537, p. 524–531, 2018.

PAVIA, D. L. et al. **Introduction to spectroscopy**. Cengage Learning, 2008.

SIMIQUELI, A. A. et al. Ovalbumin and guar gum foam and its surface properties as influenced by sucrose and sorbitol. **International Journal of Biological Macromolecules**, v. 135, p. 226–232, 2019a.

SIMIQUELI, A. A. et al. W/O/W emulsions applied for conveying FeSO4: Physical characteristics and intensity of metallic taste perception. **LWT - Food Science and Technology**, v. 100, p. 278–286, 2019b.

SINGH, S. K.; NILSSON, S. Thermodynamics of interaction between some cellulose ethers and SDS by titration microcalorimetry. II. Effect of polymer hydrophobicity. **Journal of Colloid and Interface Science**, v. 213, n. 1, p. 152–159, 1999.

SOHRABI, B. et al. The study of polymer-surfactant interaction in catanionic surfactant mixtures. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 436, p. 890–897, 2013.

SOULTANI, S. et al. Comparative study of some surface active properties of fructose esters and commercial sucrose esters. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 227, n. 1–3, p. 35–44, 2003.

VAN KEMPEN, S. E. H. J. et al. Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification. **Food Chemistry**, v. 138, n. 2–3, p. 1884–1891, 2013.

YIN, H. et al. Characteristics of biosurfactant produced by Pseudomonas aeruginosa S6 isolated from oil-containing wastewater. **Process Biochemistry**, v. 44, n. 3, p. 302–308, 2009.

ZAIDAN, U. H. et al. Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. **Food Chemistry**, v. 131, n. 1, p. 199–205, 2012.

CAPÍTULO 2 - DEVELOPMENT OF AN ENZYMATIC BIOREACTOR FOR CONTINUOUS SYNTHESIS OF SUCROSE ESTERS AND EVALUATION OF THE INTERFACIAL PROPERTIES OF THE SURFACTANT

ABSTRACT

An enzymatic bioreactor was developed for the continuous synthesis of sucrose esters. *Candida rugosa* lipase (CRL) was immobilized on a polyacrylamide cryogel column. Response surface methodology with a central composite rotational design was implemented to optimize three experimental operating conditions (substrate molar ratio, temperature and proportion of organic solvents). The maximum percentage of esterification predicted was 93.01% using a molar ratio of 1:3.13 (sucrose: stearic acid), temperature of 55.8 °C and proportion of 7.44: 92.56 (DMSO: *tert*-butanol). Even after 10 successive cycles, the bioreactor was able to maintain a high conversion rate. The synthesis of sucrose esters was confirmed by thin layer chromatography (TLC) and infrared spectroscopy (FTIR). The equilibrium surface tension (γ_{eq}) and critical micellar concentration (CMC) of these esters were obtained, and their values (44 ± 1 mN/m and 14.5 mg/L, respectively) were characteristic of a non-ionic surfactant. The study of interfacial viscoelasticity showed that sucrose esters form predominantly elastic films at the oil-water interface. The esters produced were surfactants capable of adsorbing at the interface and promoting a significant decrease in interfacial tension, demonstrating a potential application of these compounds as emulsifiers for industry.

Keywords: Sugar esters; *Candida rugosa* lipase; Cryogel; Optimization; Interfacial viscoelasticity.

1. Introduction

In recent years, the industrial scale use of lipases has grown considerably. These enzymes stand out for the various reactions they catalyze, being used in several biotechnological segments, such as in the production of food, detergents, paper, medicines, cosmetics, biodiesel, in the treatment of effluents, among others (CASTIGLIONI et al., 2020; GUMEL et al., 2011; NIETO et al., 2021). Among the processes catalyzed by lipases, the synthesis of esters stands out as a promising aspect, due to the importance of numerous esters as additives in obtaining industrialized products. An example of this are sucrose esters, which are compounds obtained from sucrose and fatty acids linked by ester bonds, which exhibit surfactant properties, that is, they are capable of reducing surface and interfacial tension and promoting, for example, the emulsification of immiscible liquids.

Sucrose esters can be obtained from chemical or enzymatic processes, however, enzymatic reactions have the advantage of being conducted under milder temperature conditions, in addition to offering a high degree of chemo-, regio-, enantio- and diastereoselectivity; while the chemical route is less selective and leads to the production of a heterogeneous mixture of products with different degrees of esterification and different acylation positions (FERNANDES et al., 2021; FERRER et al., 2000).

Although several studies describe the synthesis of sugar esters from lipases, there is an ongoing need to develop new enzyme technologies for the production of these compounds and to optimize the reaction conditions in order to increase the yield of the esterification process and make it economically viable. Thus, the use of immobilized enzymes is a suitable alternative to achieve these goals. Immobilization, in addition to making the enzyme easily separable from the reaction medium, also promotes retention of enzymatic activity and allows its reuse for continuous operations (LIESE; HILTERHAUS, 2013; MEENA et al., 2021; RAFIEE; REZAEE, 2021).

As a solid support, supermacroporous cryogels are low-cost matrices, with potential for the development of enzymatic bioreactors. Cryogels have large pores (approximately 10 to 100 μm), which promote greater permeability to flow and low resistance to mass transfer. In this way, bioreactors produced from immobilization of lipases in cryogel can have a configuration that allows a continuous process, with the advantage of basically convective mass transfer (ARMUTCU et al., 2020; ERTÜRK; MATTIASSON, 2014).

In the present study, a commercial *Candida rugosa* lipase (CRL) was used for the development of an enzymatic bioreactor in order to increase the operational stability in

esterification reactions for the synthesis of sucrose esters. Although the enzymatic synthesis of sugar esters is well understood, studies investigating the continuous synthesis of these compounds from enzymatic bioreactors are still scarce. For industrial applications, continuous operation is preferable to batch operation due to its greater efficiency, greater process control, the possibility of enzyme reuse without the need for separation of the reaction medium, the minimization of damage to the biocatalyst due to the lower shear stress and, consequently, lower operating costs (RAKMAI; CHEIRSILP, 2016). Thus, the study of the continuous enzymatic synthesis of sucrose esters, on a laboratory scale, is important mainly to predict the behavior of the bioreactor under different conditions and verify if the support is suitable for possible use on a large scale.

Therefore, the objectives of this study were to develop an enzymatic bioreactor, through the covalent linkage of CRL in a cryogel matrix, optimize the enzymatic production of sucrose esters and characterize these surfactants in terms of their surfactant properties, by the analysis of surface and interfacial tension and the modulus of expansion of the interfacial layers, obtained by dynamic tests. The effects of three variables (molar ratio of substrates, temperature and proportion of solvents) on esterification yield were evaluated using the central composite rotational design (CCRD) as the experimental project for response surface methodology (RSM) and, then, optimal reaction conditions were proposed.

2. Materials and methods

Lipase from *Candida rugosa* (Type VII, ≥700 unit/mg solid) was purchased fromSigma-Aldrich (Saint Louis, USA) along with the reagents: acrylamide (AM), N, N'-methylenebisacrylamide (MBAM), ammonium persulfate (APS), allylglycidyl ether (AGE, 99%), N, N, N ', N'-tetramethyl-ethylenediamine (TEMED), glutaraldehyde (GA, 50% aqueous solution), ethylenediamine (EDA, 99%), sodium borohydride ($NaBH_4$), bovine serum albumin (BSA), *p*-nitrophenyl palmitate (*p*-NPP), *p*-nitrophenol, Triton X-100, gum arabic, acetone, acetic acid, stearic acid, chloroform, absolute ethanol, sodium hydroxide, sucrose, methanol, dimethyl sulfoxide (DMSO) and *tert*-butanol. Phenolphthalein were purchased from Synth (Diadema, Brazil).

All chemical reagents used were of analytical grade. Activated carbon and sunflower oil were obtained from local market. Solutions were prepared using deionized water (Milli Q - Merck Millipore, Germany).

2.1. Synthesis of polyacrylamide (PAM) cryogel

The cryogel synthesis process was adapted from the methodology proposed by Mól et al. (2017). Monomers were weighed (1.185 g AAm, 0.3175 g MBAAm) and dissolved in 20 mL of deionized water. Soon after, 1% (w/w) of AGE was added and the volume were completed to 25 mL with water. Then, the mixture was degassed for about 10 minutes in an ultrasonic bath and cooled to 0-5 °C. Polymerization was initiated by the addition of 0.0275 g of APS and 23.8 µL of TEMED. There action mixture was poured into glass columns and frozen at -12 °C for 24 h. The cryogel obtained was thawed at 25 °C and then washed with 100 mL of deionized water with the aidof a peristaltic pump (BT/F Series Intelligent Displaying) at a flow rate of 1 mL/min. Subsequently, cryogels were stored under refrigeration until the moment of use.

2.2. CRL immobilization in PAM cryogel

The cryogel column was connected to a peristaltic pump and methanol (50 mL) was recirculated at a flow rate of 1 mL/min for 2 h. Then, ethylenediamine (0.5 M in 0.2 M Na_2CO_3; 50 mL) was pumped to the column at a flow rate of 1 mL/min in the recycling mode for 10 h. Afterwards, the column was washed with 100 mL of 0.1 M sodium phosphate buffer, pH 5.5, at 1 mL/min. Soon after, a solution of glutaraldehyde (5% v/v; 100 mL) in 0.1 M sodium phosphate buffer, pH 5.5, was pumped to the column at a flow rate of 1 mL/min in a recycling mode for 6 h. All previous steps were performed at 25 °C.

The derivatized matrix with functional aldehyde groups was used to immobilize the CRL. First, the column was washed with 0.1 M sodium phosphate buffer, pH 5.5 for 1 h. Then, the enzyme solution (8 mg/mL; 25 mL) in 0.1 M sodium phosphate buffer, pH 5.5 was recycled through the column at a flow rate of 0.25 mL/min at 25 °C for 24 h. Finally, the freshly prepared $NaBH_4$ solution (0.1 M in sodium carbonate buffer, pH 9.2) was applied to the column at a flow rate of 0.25 mL/min for 1.5 h in recycling mode. Then, the column was washed with 100 mL of water at a flow rate of 0.25 mL/min and stored at 5 °C until use.

2.3. Characterization of the enzyme bioreactor

2.3.1. Swelling capacity, expansion degree, porosity and scanning electron microscopy

Swelling capacity (S) was determined according to Savina et al. (2005). The dehydrated bioreactor (m_d, g) was placed in a container with water until complete immersion, for 24 h. The

excess water was removed and the wet bioreactor (m_w, g) was weighed again and the swelling capacity was calculated according to Eq.(1):

$$S = \frac{m_w - m_d}{m_d} \quad (1)$$

The degree of expansion (ED) was determined according to Gonçalves et al. (2016). A sample of the hydrated bioreactor was immersed in a graduated cylinder containing 20 mL of deionized water, and the difference in volume after that was measured. The degree of expansion (mL/g) was calculated as the ratio between the hydrated bioreactor volume (V_w, mL) and its dehydrated mass (m_d, g), as shown by Eq. (2):

$$ED = \frac{V_w}{m_d} \quad (2)$$

The total porosity (φ_T), fraction of macropores (φ_M), fraction of meso and micropores (φ_m), bound water fraction (φ_{wb}) and fraction of dry polymer (φ_d) were determined with methodology proposed by Plieva et al. (2004). The morphology of the enzymatic bioreactor was also evaluated by Scanning Electron Microscopy (SEM). Samples of dried bioreactor were broken in half, fixed in stubs and sprayed with a thin layer of gold. Then, the samples were analyzed in a scanning electron microscope (Carl Zeiss LEO EVO 40 XVP, Oberkochen, Germany), with an acceleration voltage of 20kV.

2.3.2. Porosity and Axial dispersion

Residence time distribution (RTD) was measured on a preparative chromatographic system (Åkta Pure, GE Healthcare, Sweden) using a pulse of acetone (100 µL, 1% v/v in water) as a tracer at 25 °C. The mobile phase (deionized water) was injected into the system at different flow velocities (U = 1,06E^{-04} to 6,37E^{-04} m/s), and the peak signal was monitored at the column outlet by a UV detector at 280 nm. All analyzes were performed in triplicate. To compensate for the dispersive effects generated by the equipment itself, the same procedure described above was carried out, but without the use of the column, but with a pipe segment used with the same length of the column in its place. The mean residence time (t_R) and variance (σ^2) were obtained from the RTD curves by the momentum method, using Eq. (3) and (4) (CHAVES et al., 2020). Exit age distribution, E_θ, is the RTD of the fluid in the reactor. The E_θ function was determined

according to Eq. (5) (CHAVES et al., 2020):

$$t_R = \frac{\int_0^\infty t\, Abs(t)dt}{\int_0^\infty Abs(t)dt} \quad (3)$$

$$\sigma^2 = \frac{\int_0^\infty t^2\, Abs(t)dt}{\int_0^\infty Abs(t)dt} - t_R^2 \quad (4)$$

$$E_\theta = t_R \times \frac{Abs(t)}{\int_0^\infty Abs(t)dt} \quad (5)$$

where t and Abs represent the time (s) and absorbance, respectively.

Porosity (ε_T) was determined by linear regression of Eq. (6) (LEVENSPIEL, 1999).

$$t_R = \frac{L}{U} \times \varepsilon_T \quad (6)$$

where: L is the column length (m), U is the flow velocity of the mobile phase (m/s) and ε_T is the total porosity of the column.

The apparent axial dispersion coefficient for each flow rate studied was calculated by non- linear regression, solving Eq. (7) and using the Solver tool of the MS-Excel 2016 software (Microsoft, Redmond, USA) (FURUSAWA; SMITH; SUZUKI, 1976).

$$\frac{\sigma^2}{t_R^2} = 2\left(\frac{D_{ax}}{uL}\right) - 2\left(\frac{D_{ax}}{uL}\right)^2\left[1 - exp\left(\frac{-uL}{D_{ax}}\right)\right] \quad (7)$$

where D_{ax} is the axial dispersion coefficient (m²/s) and u (m/s) is the interstitial fluid velocity ($u = U/\varepsilon_T$) through the column.

The height equivalent of theoretical plates (HETP) were determined according to Eq. (8) (GUIOCHON, 2006):

$$HETP = L\frac{\sigma_t^2}{t_R^2} \quad (8)$$

2.3.3. *Hydraulic permeability*

The hydrostatic pressure drop (ΔP_w) in the column was analyzed using a liquid chromatograph (Åkta Pure, GE Healthcare, Sweden) using deionized water at different flow velocities. The hydraulic permeability (K_w) was determined by linear regression of the Darcy equation (Eq. (9)) (CHAVES et al., 2020):

$$\frac{\Delta P_W}{L} = \frac{-\mu_W}{K_W} \times U \tag{9}$$

where ΔP_w (Pa) is the pressure drop through the column, μ_w (Pa.s) is the water viscosity at 25 °C, and K_w (m^2) is the hydraulic permeability.

To set-off the pressure drop due to equipment and column, the experiment was also conducted using an empty column.

2.4. *Determination of the immobilized enzyme content in the cryogel matrix*

The total content of immobilized enzyme in the cryogel was indirectly determined from the difference between the amount of protein in the solution before and after immobilization using the Bradford method (BRADFORD, 1976). Absorbance was measured at 595 nm on a UV-Vis spectrophotometer (Biomate 3, Thermo Scientific, USA) and an analytical curve was determined using bovine serum albumin (BSA) as standard.

2.5. *Bioreactor activity assay*

The enzymatic activity of the bioreactor was determined by the hydrolysis method of *p*-nitrophenyl palmitate (*p*-NPP) (GUPTA; RATHI; GUPTA, 2002). Thus, the substrate solution was prepared under continuous stirring, consisting of 6 mL of the *p*-NPP solution (7.95 mM in isopropanol) and 54 mL of Tris HCl buffer (50 mM, pH 7, containing 0.4% w/v Triton X-100 and 0.1% w/v gum arabic). Soon after, the substrate solution was pre-incubated at 37 °C for 15 min and then pumped through the enzymatic bioreactor at a flow rate of 0.25 mL/min at 37 °C. After the process reached steady state conditions, samples were collected at the exit of the bioreactor and were analyzed for the concentration of *p*-nitrophenol in a UV-Vis spectrophotometer (Biomate 3, Thermo Scientific, USA), at 410 nm.

An analytical curve was prepared using *p*-nitrophenol in different concentrations. It was

defined that an unit of enzymatic activity (1 U) corresponds to the amount of enzyme capable of producing 1 μmol of *p*-nitrophenol per minute.

2.6. Determination of immobilization efficiency

The immobilization efficiency was calculated in terms of the recovered hydrolytic activity (R_{HA}) and immobilization efficiency (η) (MILAŠINOVIĆ et al., 2012), according to the Eq. (10) and (11):

$$R_{HA}(\%) = \frac{A_{biorreator}}{P_c \times A_0} \times 100 \quad (10)$$

$$\eta(\%) = \frac{C_1V_1 - C_2V_2}{C_1V_1} \times 100 \quad (11)$$

where A_0 is the specific hydrolytic activity of the free lipase (U/mg_{enzyme}), Pc is the mass of immobilized enzyme (mg) per gram of cryogel, $A_{bioreactor}$ is the apparent hydrolytic activity of the bioreactor (U) per gram of cryogel, C_1 and C_2 are the initial and final enzyme concentration (mg/mL), respectively, and V_1 and V_2 the volumes (mL) of the enzyme solution used for immobilization and the supernatant after immobilization, respectively.

2.7. Optimization of continuous synthesis of sucrose esters

Sucrose esters were synthesized from sucrose and stearic acid in a mixture of two organic solvents (DMSO/*tert*-butanol). The substrate solution was pumped through the bioreactor at a continuous flow rate of 0.25 mL/min for 6 h, according to the experimental conditions that were designed. The effects of substrate molar ratio, temperature (°C) and solvent proportion (DMSO: *tert*-butanol) in the reaction medium were evaluated.

Samples of the reaction product were collected at the exit of the bioreactor and the residual free fatty acid was determined by the volumetric method (FERNANDES et al., 2021). Thus, the reaction product was titrated with standard sodium hydroxide solution (0.1 M). The amount of free fatty acid (A, in mol/g) in the sample was calculated for both, test and control samples. The esterification yield (%), expressed as equivalent to acid conversion, was calculated using Eq. (12) to (14).

$$A_1 = \frac{(V_s \times 0.1 \times fc)}{M_s} \quad (12)$$

$$A_2 = \frac{(V_c \times 0.1 \times fc)}{M_c} \quad (13)$$

$$Esterification\ yield\ (\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100 \quad (14)$$

where V_s and V_c (mL) are the volumes of sodium hydroxide consumed on titration of sample and control, respectively, fc is the correction factor for the NaOH solution (0.9910), M_s and M_c (g) are the mass of sample and control, respectively. All measurements were made in triplicate.

2.8. Experimental design and statistical analysis

The response surface methodology (RSM) was adopted to optimize the reaction conditions for the sucrose esters synthesis. The independent variables substrate molar ratio (sugar: fatty acid) (X_1), temperature (X_2) and proportion of solvents (DMSO: *tert*-butanol) (X_3), were selected for the optimization study. The response or dependent variable was the esterification yield of a reaction (Y).

The effects of independent variables on the esterification yield (%) were carried out based on a Central Composite Rotational Design (CCRD), whose coded levels and actual values of the variables are shown in Table 1. The CCRD consisted of a 2^3 factorial design with 8 assays, 6 axial points and 3 central points, totaling 17 experimental runs.

Table 1 - Coded and actual levels of variables used in the experimental design

Factors	Variables	Levels				
		-1.68	-1	0	+1	+1.68
X_1	Substrate molar ratio (sugar: fatty acid)	1:1.32	1:2	1:3	1:4	1:4.68
X_2	Temperature (°C)	46.6	50	55	60	63.4
X_3	Proportion of solvents (DMSO: *tert*-butanol)	1.6:98.4	5:95	10:90	15:85	18.4:81.6

The experimental results obtained were analyzed by the regression procedure using the

following second order polynomial equation (Eq. (15)):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + e \quad (15)$$

where Y is the response variable (%); β_0 is the model intercept; X_i and X_j are the levels of the independent variables; e is the error; and β_i, β_{ii} and β_{ij} are regression coefficients.

Statistical significance of the model was evaluated by analysis of variance (ANOVA) and the suitability of the model was assessed using Fisher's statistical test (F-test) by testing for significant differences between sources of variation in experimental results, i.e. the significance of the regression, the lack of fit, and the coefficient of multiple determination (R^2). Statistical analyses were performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria). Differences were considered to be significant when $p < 0.05$.

2.9. Operational stability of the enzymatic bioreactor

The operational stability of the bioreactor was evaluated by successive esterification reactions of stearic acid and sucrose, using the optimal reaction conditions, at a continuous flow rate of 0.25 mL/min for 6 h. The esterification yield was monitored by determining the conversion of stearic acid to sucrose esters. After each run, the column was washed with 80 mL of hexane at a flow rate of 0.5 mL/min. Then, the bioreactor was used in a new cycle using new substrates. The initial activity of the bioreactor was considered 100%.

2.10. Sucrose ester characterization

2.10.1. Ester purification

Sucrose esters were produced under conditions that presented higher yield and were then purified according to Fernandes et al. (2021). The reaction mixture was then concentrated in rotary evaporator. After solvent evaporation, ethanol-water solution (50:50, v/v) was added. The extraction of the esters was performed for 5 h at 55 °C, under agitation. The mixture was then centrifuged (Centrifuge 5804R, Eppendorf) at 1000 × g for 10 min at 25 °C and the supernatant was collected. The extraction was repeated twice and the sucrose esters were

obtained after evaporation of ethanol and water in oven at 70 °C.

2.10.2. Thin layer chromatography (TLC) and Infrared spectroscopy (ATR-FTIR)

The synthesis of sucrose esters was confirmed qualitatively by thin layer chromatography (TLC) using glass plates coated with a 0.25 mm layer of silica gel (Whatman, USA). The mobile phase used was chloroform: methanol: acetic acid: water (75:15:8:2, v/v). The purified esters and stearic acid samples were prepared in chloroform and the sucrose sample in deionized water. Subsequently, the plates were sprayed with sulfuric acid solution in 50% methanol (v/v) and heated at 110 °C for 10 min to develop the stains of sugar, fatty acid and purified sugar esters (adapted from Cao et al. (1996)).

Also, the purified sucrose esters were analyzed by infrared spectroscopy without prior preparation. Fourier transform infrared attenuated total reflectance (ATR-FTIR) was recorded on an FT-IR spectrometer (Varian 600-IR, equipped with a Pike GladiATR accessory). A sample of approximately 100 μg was deposited on the sample holder. To obtainthe infrared spectra, 64 scans in the range of 4000 - 400 cm^{-1} and 4 cm^{-1} resolution were accumulated.

2.10.3. Surface properties

The critical micelle concentration (CMC) of purified sucrose esters and the dynamic interfacial properties were determined by drop profile analysis tensiometry (PAT-1M, SINTERFACE Tensiometer vers. 8.01, Germany) at 25 °C. To determine the CMC, surface tension measurements were taken of a series of aqueous solutions containing the esters in varying concentrations. The solutions were prepared 24 h before the first measurement of surface tension in order to obtain a complete solubilization of the surfactant. For the preparation of the sucrose ester solutions, deionized water preheated at 70 °C was used (FERNANDES et al., 2021). A drop of the aqueous solution containing the esters (20 mm^2) was automatically formed at the tip of the capillary and inserted into an airway space or in purified sunflower oil. The image of the drop formed was captured and digitalized by the CCD camera of the equipment and the surface tension at 25 °C was determined as a function of time, adjusting the Gauss-Laplace equation using the specific software of the equipment.

Sinusoidal oscillations were also applied to the drop volume with a 3% amplitude to remain within the linear viscoelastic region, and the frequency ranged from 0.0025 to 0.02 Hz (NEUMANN et al., 2018). Dilatational parameters were calculated using a Fourier transform algorithm implemented in the software package. Thus, it was possible to obtain the elastic

modulus (E') and viscous modulus (E") and the phase angle (ϕ), calculated by $\tan \phi = E'' / E'$. Lower values of ϕ (<45°) indicate a predominantly elastic interface region and greater ϕ values (>45°) a predominantly viscous interface. All measurements were made in triplicate.

3. Results and discussions

3.1. Bioreactor characterization

In this study, an enzymatic bioreactor for the synthesis of sugar esters was successfully developed. The PAM cryogel column (12 cm length and 1 cm in diameter) was functionalized with glutaraldehyde and the immobilization of CRL occurred by the formation of covalent bonds between the amino groups (-NH_2) of the lipase and the aldehyde groups (-CHO) present in the modified cryogel. The efficiency of the immobilization (η) was approximately 66.6 % of the CRL and the recovered hydrolytic activity (R_{HA}) determined as a ratio between the apparent activity of the bioreactor containing the immobilized lipase and the specific activity of the free lipase was 73.1 %. The morphological characterization of the cryogel with the parameters swelling capacity (S) and expansion degree (ED), after immobilization of the CRL, are shown in Table 2.

Table 2. Morphological characteristics of the bioreactor

Morphological parameter	Value
S (g/g)	14.79 ± 0.71
ED (mL/g)	17.64 ± 0.96
φM	0.7528 ± 0.0482
φm	0.0272 ± 0.0032
φwb	0.1473 ± 0.0089
φd	0.0726 ± 0.0023
φT	0.783 ± 0.012

[1]Where S: swelling capacity; ED: expansion degree; φM: fractions of macropores; φm: meso- and micropores; φwb: water-bound; φd: dry polymer; φT total porosity.

The macroporous fraction of the cryogels was dominant in the structure, indicating that

most pores are large and demonstrating that lipase immobilization did not alter the supermacroporous structure of the cryogel matrix, which is interesting when the reaction substrates are generally macromolecule compounds (ARVIDSSON et al., 2003). The values of swelling capacity, expansion degree and total porosity are close to the observed in the literature (YAO et al., 2006) and reinforce the porous nature of its structure. Figure 1 shows the distribution of a tracer (acetone) for the different conditions evaluated. The residence time distribution curves exhibited symmetrical peaks independent of fluid velocity, indicating reduced dispersion. According to the method of moments, the average residence time and the variance for each flow velocity were determined, from these values the apparent axial dispersion coefficients and the HEPT were obtained.

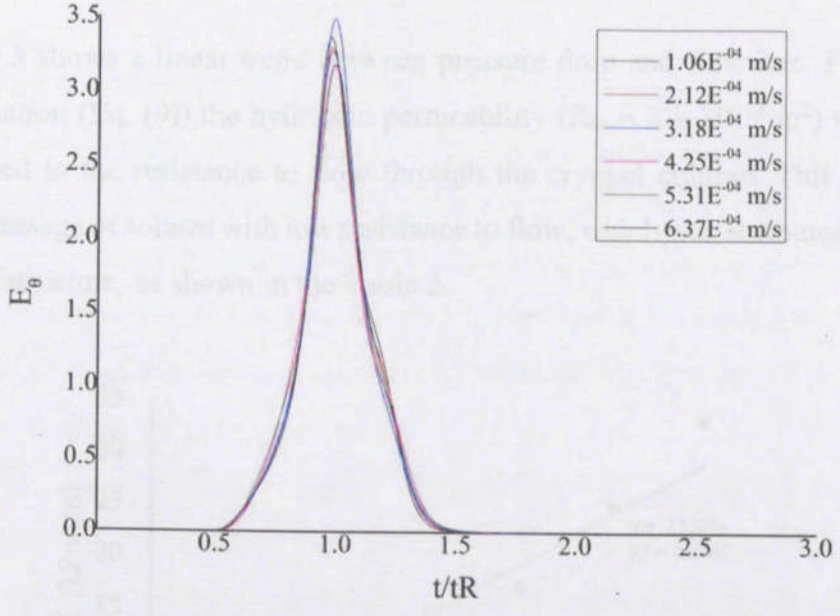

Figure 1 - Residence time distribution curves.

The bed porosity was also determined by linear regression of Eq. (7) and a value of 0.78 was obtained, which indicates that about 78% of the total pores were filled with free water. The axial dispersion coefficients presented values between 10^{-7} and 10^{-6} m^2/s, increasing with increments of flow velocity. This result indicates low axial dispersion and high cryogel column efficiency. HEPT values ranged from 0.26 to 0.29 cm and did not vary according to flow velocity (Figure 2) which shows its convective mass transfer nature. These results are comparable to those reported by other authors for PAM cryogels (FONTAN et al., 2018; MÓL et al., 2019). This means that, during the reactions of esterification, mass transfer occurs predominantly by convection, improving column performance and contributing to high yields.

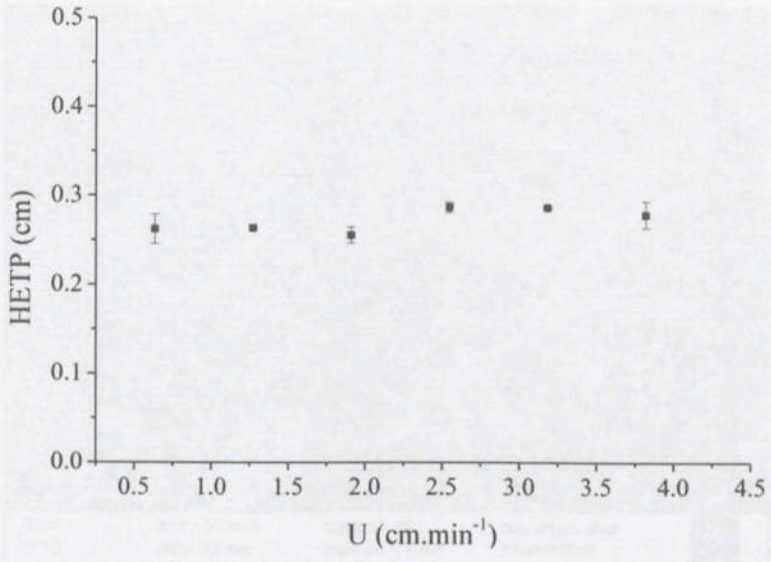

Figure 2 - Height Equivalent to Theoretical Plate (HETP) for the cryogel.

Figure 3 shows a linear trend between pressure drop and flow rate. From the Darcy-Weisbach equation (Eq. (9)),the hydraulic permeability ($K_w = 2 \times 10^{-13}$ m^2) was determined, which is related to the resistance to flow through the cryogel column. This value was low, allowing the passage of solutes with low resistance to flow, which was attributed to the cryogel's highly porous structure, as shown in the Table 2.

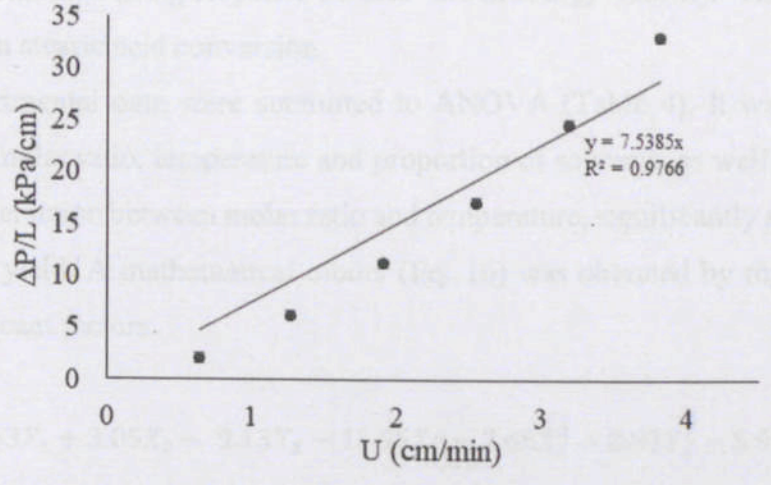

Figure 3 - Experimental data of ΔP/L at different liquid superficial velocities (•) and fitted linear regression (–) to determine cryogel permeability.

The bioreactor, developed by immobilizing the CRL on the cryogel, consisted of a spongy matrix with interconnected monolithic channels, with diameters ranging from 7 μm to 105 μm (Figure 4). Large pores of the cryogel structure were confirmed by a porosity value of 78%. The presence of macropores ensure a large surface area and allows the free passage of virtually any solute, characteristics that are important for enzymatic bioreactors.

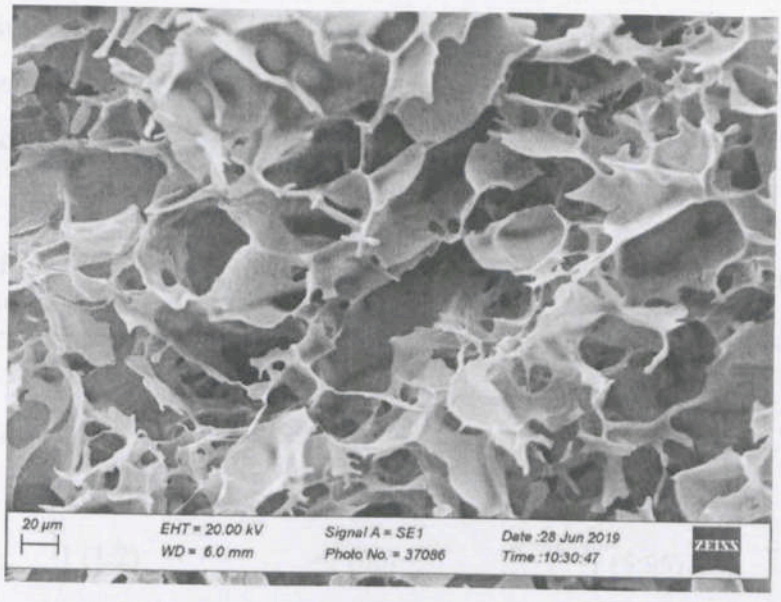

Figure 4 - A scanning electron microscope photograph of the bioreactor of CRL immobilized on cryogel.

3.2. Optimization of continuous sucrose esters synthesis

Several factors can affect the esterification yield and therefore process optimization is very important. In this work, the optimization of the synthesis of sucrose esters in a continuous production system was made using 2^3 CCRD to evaluate the effects of substrate molar ratio, temperature and proportion of solvents (DMSO: *tert*-butanol). The esterification capacity of bioreactor was optimized using response surface methodology (RSM). Table 3 shows the results of yield in stearic acid conversion.

The experimental data were submitted to ANOVA (Table 4). It was shown that the factors substrate molar ratio, temperature and proportion of solvents, as well as their quadratic effects and the interaction between molar ratio and temperature, significantly affected ($p < 0.05$) the esterification yield. A mathematical model (Eq. 16) was obtained by regression analysis, using only significant factors.

$$Y = 90.06 + 5.63X_1 + 3.05X_2 - 9.13X_3 - 15.95X_1^2 - 7.66X_2^2 - 8.83X_3^2 - 5.99X_1X_2 \qquad (16)$$

where Y is the percentage of esterification yield and X_1, X_2 and X_3 are the coded values of substrate molar ratio, temperature and proportion of solvents, respectively.

The statistical analysis indicated an appropriate adjustment of the model to the experimental results, as confirmed by the high determination coefficient obtained ($R^2 = 0.98$). The statistical insignificance for the lack of fit ($p > 0.05$) and the low pure error value, confirmed

that the model was suitable for prediction within the interval selected in the experimental design. The observed values vs. predicted values plot (Figure 5) also show almost linear distribution, which is indicative of a good model.

Table 3. Matrix of the Central Composite Rotatable Experimental Design 2^3 (coded and real values) with responses in terms of the esterification yield

Assay	Molar ratio X_1	Temperature (°C) X_2	Proportion of solvents X_3	Yield (%) Y
1	-1 (1:2)	-1 (50)	-1 (5:95)	55.7
2	-1 (1:2)	+1 (60)	-1 (5:95)	75.1
3	+1 (1:4)	-1 (50)	-1 (5:95)	77.2
4	+1 (1:4)	+1 (60)	-1 (5:95)	71.3
5	-1 (1:2)	-1 (50)	+1 (15:85)	34.5
6	-1 (1:2)	+1 (60)	+1 (15:85)	51.3
7	+1 (1:4)	-1 (50)	+1 (15:85)	58.2
8	+1 (1:4)	+1 (60)	+1 (15:85)	52.4
9	-1.68 (1:1.32)	0 (55)	0 (10:90)	32.2
10	+1.68 (1:4.68)	0 (55)	0 (10:90)	52.6
11	0 (1:3)	-1.68 (46.6)	0 (10:90)	60.7
12	0 (1:3)	+1.68 (63.4)	0 (10:90)	70.9
13	0 (1:3)	0 (55)	-1.68 (1.6: 98.4)	74.9
14	0 (1:3)	0 (55)	+1.68 (18.4: 81.6)	50.1
15	0 (1:3)	0 (55)	0 (10)	91.2
16	0 (1:3)	0 (55)	0 (10)	87.6
17	0 (1:3)	0 (55)	0 (10)	92.3

[1] Numbers in parenthesis represents the uncoded experimental values.

Table 4. ANOVA performed on the esterification yield results

Source	DF[a]	SS[b]	MS[c]	F- *value*	p-*value*
X_1	1	431.955	431.955	71.4763	0.013704
$(X_1)^2$	1	2861.299	2861.299	473.4637	0.002105
X_2	1	127.049	127.049	21.0230	0.044422
$(X_2)^2$	1	660.094	660.094	109.2267	0.009031
X_3	1	1137.150	1137.150	188.1661	0.005272
$(X_3)^2$	1	876.908	876.908	145.1034	0.006821
X_1X_2	1	286.801	286.801	47.4575	0.020428
X_1X_3	1	6.301	6.301	1.0427	0.414608
X_2X_3	1	0.781	0.781	0.1293	0.753600
Lack of Fit	5	101.511	20.302	0.2495	
Pure Error	2	12.087	6.043		

[a] DF: degrees of freedom; [b] SS: sum of squares; [c] MS: mean of squares.

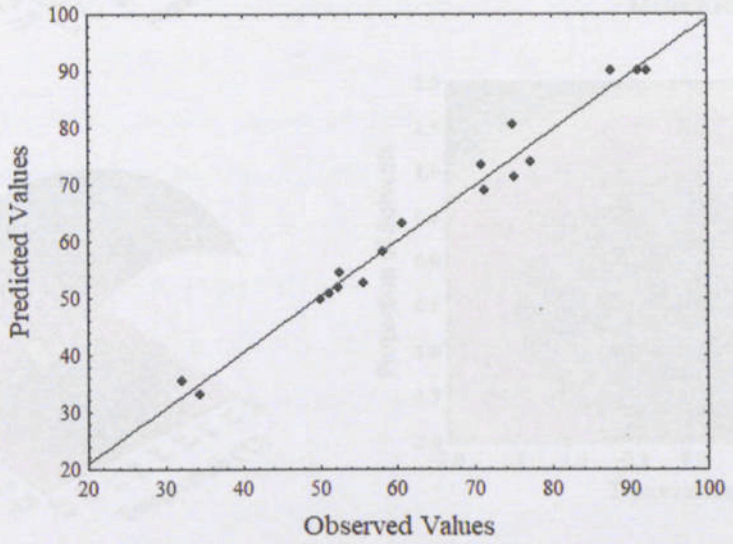

Figure 5 - Observed values *versus* values predicted by the model. The almost linear distribution of the experimental numbers is indicative of a good model.

The relationship between factors that affect the esterification reaction and its yield can be better understood by examining the response surface and contour plots (Figure 6) generated from the predicted model. The response surfaces for the synthesis of sucrose esters showed a maximum point, indicating that their production was optimized within the evaluated ranges.

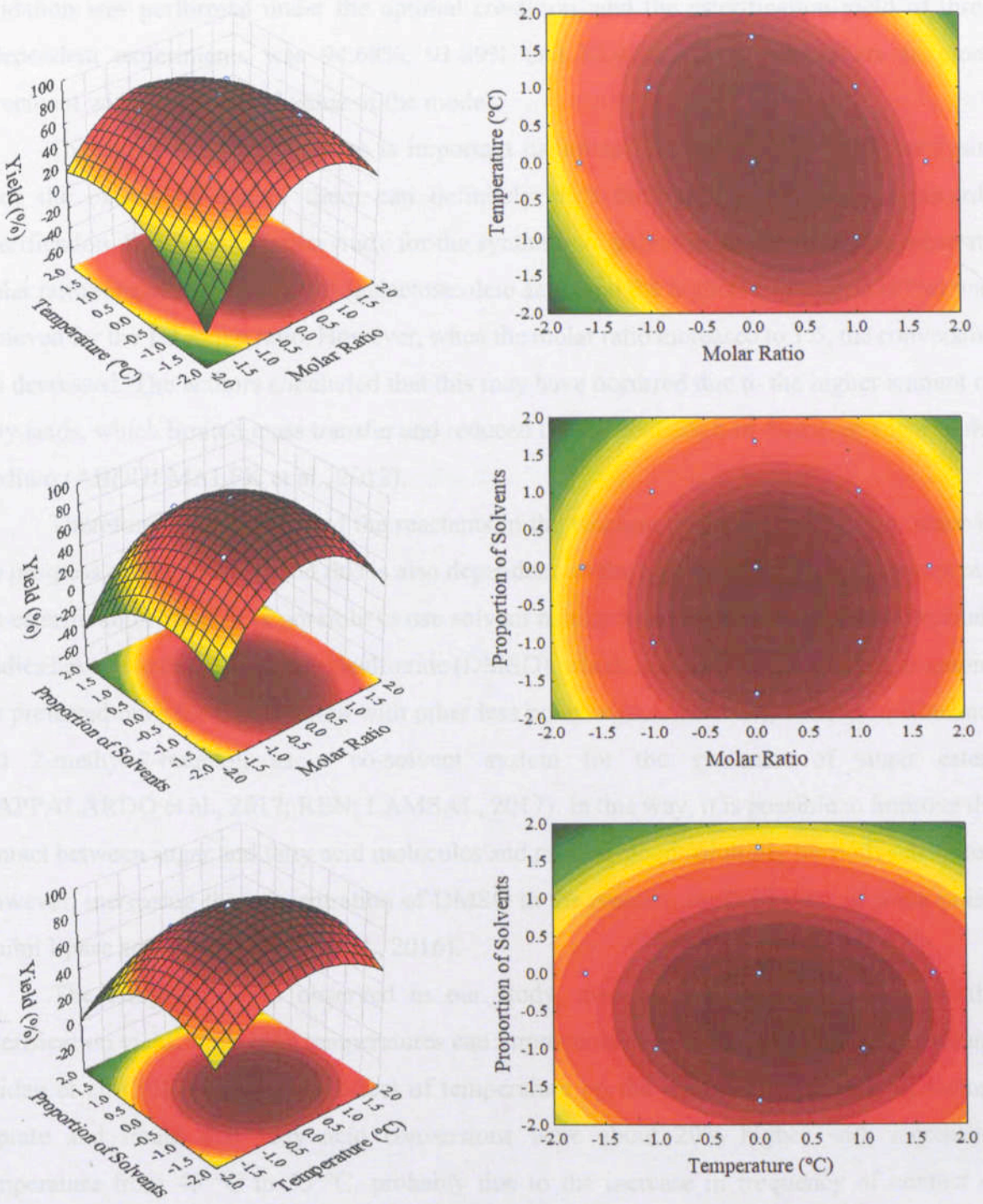

Figure 6 - Response surface (left) and contour (right) plots of the predicted model equation.

It was observed that the esterification yield increased with increasing temperature, as well as the molar concentration of stearic acid and the proportion of DMSO in the medium, until reaching an optimal point, where a further increase resulted in a decrease in the production of esters of sucrose. The maximum percentage of esterification predicted by the adjusted model was equal to 93.01% (±0.4%) for a molar ratio of 1:3.13 (sucrose: stearic acid), temperature of 55.8 °C and a proportion of organic solvent of 7.44: 92.56 (DMSO: *tert*-butanol). The model

validation was performed under the optimal condition, and the esterification yield of three independent experiments was 94.68%, 91.89% and 92.47%. These results are in close agreement with the predicted value of the model.

The molar ratio of substrates is important parameter that affects enzymatic catalysis, since the excess of one of them can definitely shift the reaction equilibrium towards esterification. In an optimization study for the synthesis of galactose oleate ester, the substrate molar ratio ranged from 1:1 to 1:5 (galactose:oleic acid) and the highest conversion (87%) was achieved for the 1:3 molar ratio. However, when the molar ratio increased to 1:5, the conversion has decreased. The authors concluded that this may have occurred due to the higher amount of fatty acids, which limited mass transfer and reduced the concentration of dissolved sugar in the medium (ABDULMALEK et al., 2012).

Therefore, the solubility of the reactants in the reaction medium is a limiting factor in the progress of the reaction, and this is also dependent on the type of solvent used. To improve the esterification yield, it is possible to use solvent mixtures in suitable proportions. Previous studies have revealed that dimethylsulfoxide (DMSO), which is a highly polar solvent, is among the preferred ones for combination with other less polar organic solvents, such as *tert*-butanol and 2-methyl-2-butanol, as a co-solvent system for the synthesis of sugar esters (PAPPALARDO et al., 2017; REN; LAMSAL, 2017). In this way, it is possible to improve the contact between sugar and fatty acid molecules and provide good solubility for both substrates. However, increasing the concentration of DMSO in the reaction medium may, at some point, inhibit lipase activity (KUMAR et al., 2016).

The temperature, as observed in our study, also has an important effect on the esterification yield, since high temperatures can improve mass transfer in the reaction medium. Zaidan et al. (2012) studied the effect of temperature on the enzymatic synthesis of lactose caprate and found that fatty acid conversions were about 20% higher with increasing temperature from 45 °C to 55 °C, probably due to the increase in frequency of contact of molecules in the reaction medium caused by the application of higher energy levels to the system. However, at 60 °C, a decrease in the conversion rate was observed, which occurs as a result of the loss of enzyme activity, due to thermal denaturation.

3.3. Bioreactor reusability

The main purpose of using immobilized enzymes is to retain the activity during subsequent cycles, in addition, reuse is one of the main qualities of the immobilized enzyme

system for financial viability. The recycling efficiency of the enzymatic bioreactor was determined by measuring the esterification yield in 10 continuous cycles. The bioreactor containing immobilized CRL was washed with n-hexane to remove any substrate or product at the end of each 6 h cycle. Thus, it was found that the relative activity of the bioreactor was retained by up to 80.7% after 10 successive esterification cycles (Figure 7). This result suggests a strong interaction and stabilization of the lipase structure on the cryogel surface which acts as a suitable support to prevent the lipase from being inactivated after repeated esterification cycles.

The high operational efficiency of the biocatalyst reuse suggests that the bonds between the enzyme and the cryogel matrix must have provided an increase in the rigidity of the lipase structure and protected the enzyme from denaturation during its repetitive use, thus increasing its catalytic performance. It is also worth mentioning that washing the biocatalyst after each cycle with a nonpolar solvent, such as hexane, removes the water produced in the reaction and, especially, removes the products that accumulate around the enzyme, contributing to greater retention of the enzymatic activity (Zago et al., 2021).

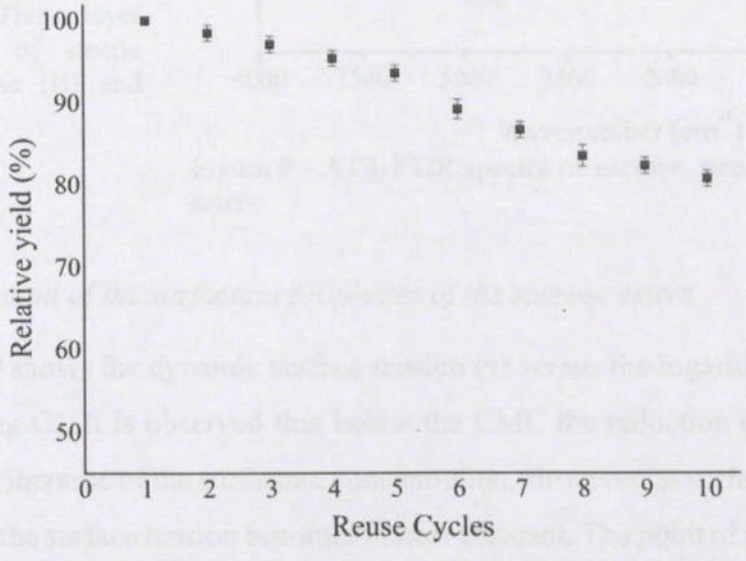

Figure 7 - Reusability of the enzymatic bioreactor.

3.4. Sucrose esters identification

TLC analysis confirmed the synthesis of sucrose esters. As shown in Figure 8 band A shows the stearic acid pattern that went ahead of the solvent with a Rf of 0.9 while band B shows a spot referring to the sucrose pattern presenting a Rf value of 0.02. A dark spot for the esterification product after purification was observed in lane C with a Rf value of 0.45.

The infrared spectra obtained for sucrose, stearic acid and the purified esterification product are shown in Figure 9, where is observed the presence of the characteristic carbonyl band of the ester linked to the aromatic ring at 1725 cm^{-1} confirming that the acyl group was successfully added to the sucrose molecule. Furthermore, the spectrum obtained for sucrose esters indicated a decrease in the characteristic bandwidth for the free hydroxyl group(OH) at 3300-3400 cm^{-1} due to the esterification of the sucrose OH groups. Intense signals inthe 1000 to 1150 cm^{-1} wavelength range attributed to the single carbon-oxygen bond (-C-O-) present in sugars also indicated that the product was a sucrose ester (PAVIA et al., 2008).

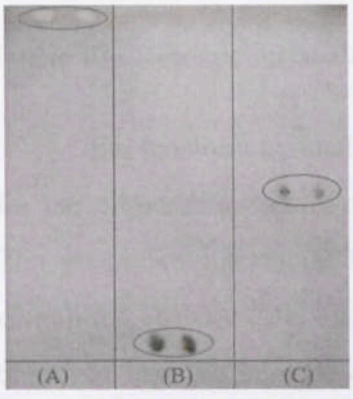

Figure 8 – Thin layer chromatography of stearic acid (A), sucrose (B) and sucrose ester (C).

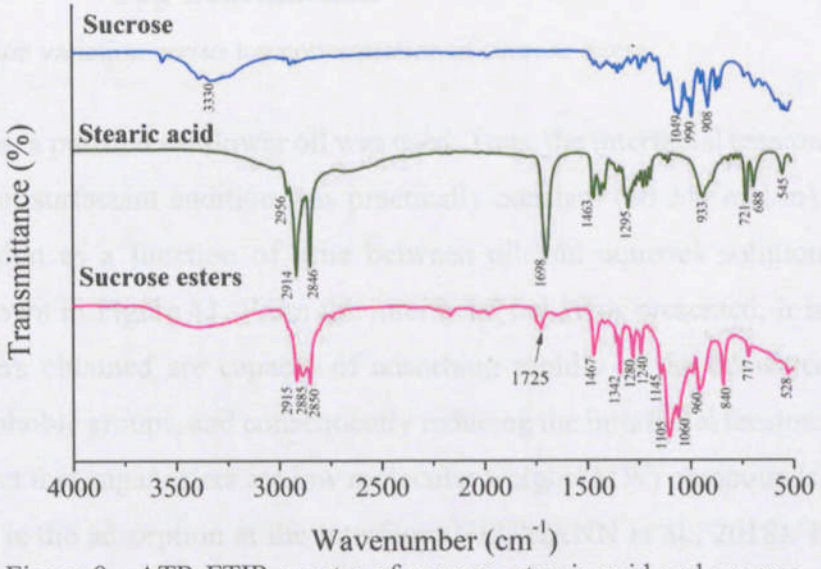

Figura 9 - ATR-FTIR spectra of sucrose, stearic acid and sucrose esters.

3.5. *Characterization of the surfactant properties of the sucrose esters*

Figure 10 shows the dynamic surface tension (γ) *versus* the logarithm of sucrose esters concentration (log C). It is observed that below the CMC the reduction of surface tension is dependent on the increase of the surfactant concentration. However, at surfactant concentrations above the CMC, the surface tension becomes almost constant. The point of intersection between the horizontal and angular lines corresponds to the CMC (FERNANDES et al., 2021). In this study, the sucrose esters synthesized had low CMC (approximately 14.5 mg/L), characteristic of nonionic surfactants. This result is close to that obtained by Soultani et al. (2003) for a commercial sucrose stearate (CMC 14 mg/L). The surface tension (γ_{cmc}) after reaching the CMC obtained for sucrose esters was 44 ± 1 mN/m. These results are also similar to those reported in the literature (35–45 mN/m) for sugar esters at the same temperature (25 °C) (BECERRA et al., 2008; FERNANDES et al., 2021).

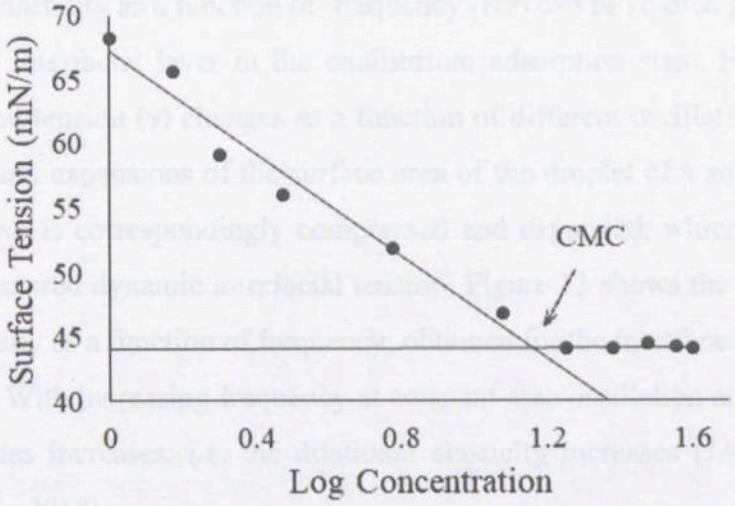

Figure 10 - Dynamic surface tension variation *versus* log concentration of sucrose esters.

For tensiometry analyses, a purified sunflower oil was used. Thus, the interfacial tension for the oil/water system without surfactant addition was practically constant (30 ± 1 mN/m). The profile of interfacial tension as a function of time between oil and aqueous solution containing sucrose esters is shown in Figure 11. From the interfacial behavior presented, it is observed that the sucrose esters obtained are capable of adsorbing rapidly at the oil-water interface, exposing their hydrophobic groups, and consequently reducing the interfacial tension. This can be explained by the fact that sugar esters are low molecular weight (MW) compounds. The lower the MW, the faster is the adsorption at the interface (NEUMANN et al., 2018). It was observed that the addition of sucrose esters in the aqueous medium reduced the interfacial tension from 30 to 11 ± 1 mN/m. This result is similar to that found for sucrose esters in our previous study (FERNANDES et al., 2021).

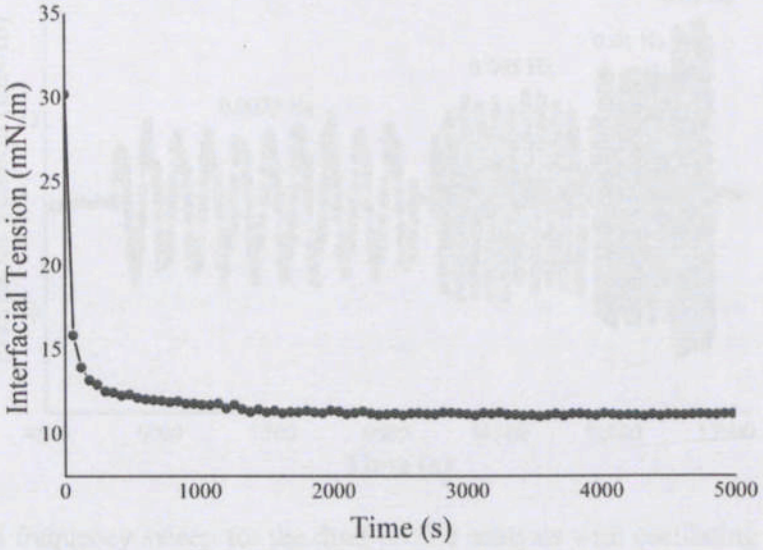

Figure 11 - Interfacial tension decay profile between sunflower oil and aqueous sucrose ester solution.

The viscoelasticity as a function of frequency (Hz) can be studied generating harmonic disturbances in a interfacial layer in the equilibrium adsorption state. Figure 12 shows the dynamic interfacial tension (γ) changes as a function of different oscillation frequencies. Due to compressions and expansions of the surface area of the droplet of a sucrose ester solution, the interfacial layer is correspondingly compressed and expanded, which can be seen in the change of the measured dynamic interfacial tension. Figure 13 shows the phase angle and the modulus of elasticity as a function of frequency, obtained for the interface oil-aqueous solution of sucrose esters. With increasing frequency at constant area oscillation amplitude, the change in the γ amplitudes increases, i.e. the dilational elasticity increases (JAVADI et al., 2013; NEUMANN et al., 2018).

Under dynamic conditions, phase differences (described by phase angle, θ) are characteristic of the interface viscoelastic behavior. In this study, the phase angle was <45° indicating that the interfacial films formed from sucrose esters were predominantly elastic. The interfacial elasticity is associated with the ability of a system to establish a new surface tension value due to a timely area variation. According discussed previously, changes in interfacial tension occur either as a consequence of a sudden compression/ expansion of the interface or, also, due to a concentration variation of the surfactant. As the elasticity values are associated with the processes of diffusion, adsorption/desorption, as well as the rearrangement of molecules at the interface, they depend on the frequency in which the perturbation is applied. Thus, interfacial elasticity is directly related to the stability of emulsions and foam films (CASELI et al., 2005; FERNANDES et al., 2021).

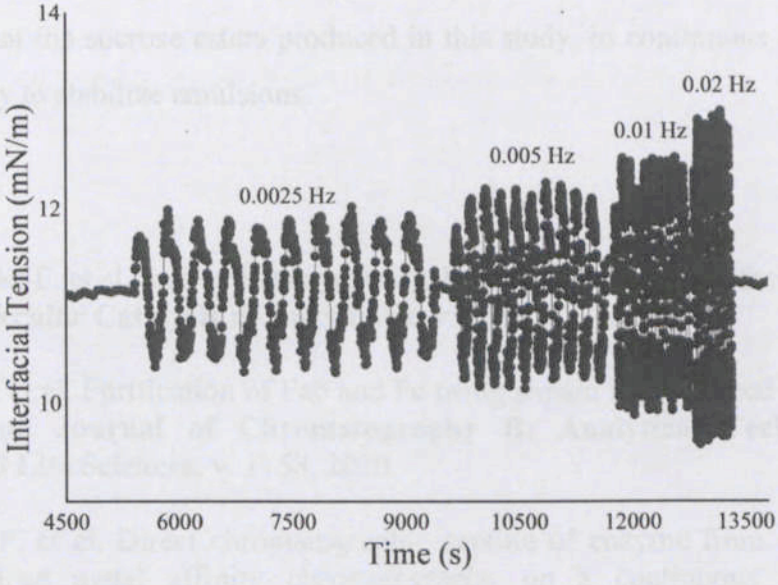

Figure 12 - Typical frequency sweep for the drop profile analysis with oscillating drop of the sucrose ester solution.

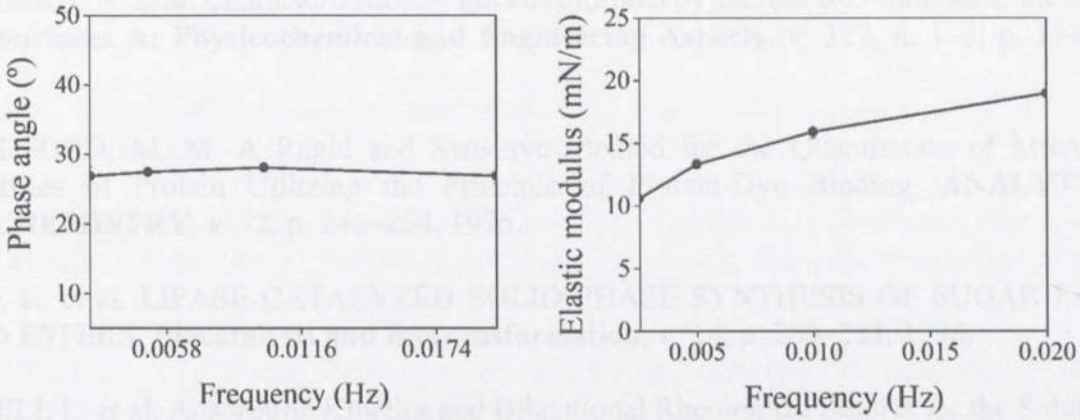

Figura 13 - Phase angle and modulus of elasticity as a function of frequency for the oil-aqueous sucrose ester solution interface.

4. Conclusions

A supermacroporous bioreactor for the continuous synthesis of sucrose esters was successfully produced from the immobilization of CRL in PAM cryogel and the optimization of the esterification reaction was performed using a response surface methodology, based on a central composite rotational design (R^2 = 98%). The predicted maximum percentage of esterification after 6 hours of reaction was 93.01% using a molar ratio of 1:3.13 (sucrose: stearic acid), temperature of 55.8 °C and ratio of 7.44:92,56 (DMSO: *tert*-butanol). The synthesis of sucrose esters was confirmed by TLC and FTIR and the surfactant properties of these produced compounds were evaluated. Sucrose esters had a low CMC (14.5 mg/L), characteristic of non-ionic surfactants, and caused a significant decrease in surface and interfacial tensions. In addition, sucrose esters produced predominantly elastic interfacial films. Thus, it was demonstrated that the sucrose esters produced in this study, in continuous operations, have a promising ability to stabilize emulsions.

5. References

ABDULMALEK, E. et al. Improved enzymatic galactose oleate ester synthesis in ionic liquids. **Journal of Molecular Catalysis B: Enzymatic**, v. 76, p. 37–43, 2012.

ARMUTCU, C. et al. Purification of Fab and Fc using papain immobilized cryogel bioreactor separator system. **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, v. 1158, 2020.

ARVIDSSON, P. et al. Direct chromatographic capture of enzyme from crude homogenate using immobilized metal affinity chromatography on a continuous supermacroporous adsorbent. **Journal of Chromatography A**, v. 986, n. 2, p. 275–290, 2003.

BECERRA, N. et al. Characterization of micelles formed by sucrose 6-O-monoesters. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 327, n. 1–3, p. 134–139, 2008.

BRADFORD, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. **ANALYTICAL BIOCHEMISTRY**, v. 72, p. 248–254, 1976.

CAO, L. et al. LIPASE-CATALYZED SOLID PHASE SYNTHESIS OF SUGAR FATTY ACID ESTERS. **Biocatalysis and Biotransformation**, v. 14, p. 269–283, 1996.

CASELI, L. et al. Adsorption Kinetics and Dilatational Rheological Studies for the Soluble and Anchored Forms of Alkaline Phosphatase at the Air/Water Interface. **Journal of the Brazilian Chemical Society**, v. 16, n. 5, p. 969–977, 2005.

CASTIGLIONI, G. Z. et al. Production of volatile compounds by yeasts using hydrolysed grape seed oil obtained by immobilized lipases in continuous packed-bed reactors. **Bioprocess and Biosystems Engineering**, v. 43, n. 8, p. 1391–1402, 2020.

CHAVES, G. L. et al. Hydrodynamics and dynamic capacity of cryogels produced with different monomer compositions. **Journal of Applied Polymer Science**, v. 137, n. 13, 2020.

ERTÜRK, G.; MATTIASSON, B. Cryogels-versatile tools in bioseparation. **Journal of Chromatography A**, v. 1357, p. 24–35, 2014.

FERNANDES, R. N. et al. Kinetic stability of the oil-in-water emulsions and dynamic interfacial properties of mixtures of sucrose esters and polysaccharides. **Food Chemistry**, v. 357, 2021.

FERRER, M. et al. Chemical versus enzymatic catalysis for the regioselective synthesis of sucrose esters of fatty acids. **Studies in Surface Science and Catalysis**, v. 130 A, p. 509–514, 2000.

FONTAN, R. DA C. I. et al. Alternatives for characterizing macroporous polyacrylamide monolithic ion exchanger columns. **Polymer Engineering and Science**, v. 58, n. 10, p. 1717–1725, 2018.

FURUSAWA, T.; SMITH, J. M.; SUZUKI, M. Rate Parameters in Heterogeneous Catalysis by Pulse Techniques. **Catalysis Reviews**, v. 13, n. 1, p. 43–76, 1976.

GONÇALVES, G. R. F. et al. Development of supermacroporous monolithic adsorbents for purifying lectins by affinity with sugars. **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, v. 1033–1034, p. 406–412, 2016.

GUIOCHON, G. The limits of the separation power of unidimensional column liquid chromatography. **Journal of Chromatography A**, v. 1126, n. 1–2, p. 6–49, 2006.

GUMEL, A. M. et al. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, v. 46, n. 11, p. 2079–2090, 2011.

GUPTA, N.; RATHI, P.; GUPTA, R. Simplified para-nitrophenyl palmitate assay for lipases and esterases. **Analytical Biochemistry**, v. 311, n. 1, p. 98–99, 2002.

JAVADI, A. et al. Characterization methods for liquid interfacial layers. **European Physical Journal: Special Topics**, v. 222, n. 1, p. 7–29, 2013.

KUMAR, A. et al. Impacts of ceramic filler and the crystallite size of polymer matrix on the ionic transport properties of lithium triflate/poly (vinylidene fluoride-co-hexafluoropropene) based polymer electrolytes. **Electrochimica Acta**, v. 215, p. 1–11, 2016.

LEVENSPIEL, O. Chemical reaction engineering. In: **Industrial and Engineering Chemistry Research**. American Chemical Society, 1999. v. 38, p. 4140–4143.

LIESE, A.; HILTERHAUS, L. Evaluation of immobilized enzymes for industrial applications. **Chemical Society Reviews**, v. 42, n. 15, p. 6236–6249, 2013.

MEENA, J. et al. Recent advances in nano-engineered approaches used for enzyme immobilization with enhanced activity. **Journal of Molecular Liquids**, v. 338, 2021.

MILAŠINOVIĆ, N. et al. Synthesis of n-amyl isobutyrate catalyzed by *Candida rugosa* lipase immobilized into poly(N-isopropylacrylamide-co-itaconic acid) hydrogels. **Chemical Engineering Journal**, v. 181–182, p. 614–623, 2012.

MÓL, P. C. G. et al. Development of an affinity cryogel for one step purification of lysozyme from chicken egg white. **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, v. 1044–1045, p. 17–23, 2017.

MÓL, P. C. G. et al. Production and capture of β-glucosidase from Thermoascus aurantiacus using a tailor made anionic cryogel. **Process Biochemistry**, v. 82, p. 75–83, 2019.

NEUMANN, S. M. et al. Interactions in water in oil in water double emulsions: Systematical investigations on the interfacial properties and emulsion structure of the outer oil in water emulsion. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 537, p. 524–531, 2018.

NIETO, S. et al. Ultrasound-assisted enzymatic synthesis of xylitol fatty acid esters in solvent-free conditions. **Ultrasonics Sonochemistry**, v. 75, 2021.

PAPPALARDO, V. M. et al. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, v. 433, p. 383–390, 2017.

PAVIA, D. L. et al. **Introduction to spectroscopy**. Cengage Learning, 2008.

PLIEVA, F. M. et al. Characterization of polyacrylamide based monolithic columns. **Journal of Separation Science**, v. 27, n. 10–11, p. 828–836, 2004.

RAFIEE, F.; REZAEE, M. Different strategies for the lipase immobilization on the chitosan based supports and their applications. **International Journal of Biological Macromolecules**, v. 179, p. 170–195, 2021.

RAKMAI, J.; CHEIRSILP, B. Continuous production of β-cyclodextrin by cyclodextrin glycosyltransferase immobilized in mixed gel beads: Comparative study in continuous stirred tank reactor and packed bed reactor. **Biochemical Engineering Journal**, v. 105, p. 107–113, 2016.

REN, K.; LAMSAL, B. P. Synthesis of some glucose-fatty acid esters by lipase from *Candida antarctica* and their emulsion functions. **Food Chemistry**, v. 214, p. 556–563, 2017.

SAVINA, I. N.; MATTIASSON, B.; GALAEV, I. Y. Graft polymerization of acrylic acid onto macroporous polyacrylamide gel (cryogel) initiated by potassium diperiodatocuprate. **Polymer**, v. 46, n. 23, p. 9596–9603, 2005.

YAO, K. et al. Preparation of polyacrylamide-based supermacroporous monolithic cryogel beds under freezing-temperature variation conditions. **Chemical Engineering Science**, v. 61, n. 20, p. 6701–6708, 2006.

ZAIDAN, U. H. et al. Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. **Food Chemistry**, v. 131, n. 1, p. 199–205, 2012.

CONCLUSÕES GERAIS

Neste trabalho, ésteres de ácidos graxos de sacarose foram sintetizados enzimaticamente a partir de sacarose e de ácido esteárico (C18: 0) em diferentes condições operacionais (proporções molares de substrato, solventes orgânicos e temperatura). No primeiro estudo, utilizou-se o sistema em batelada para a síntese destes surfactantes a partir de lipases imobilizadas de *Candida antarctica* B. Os resultados mostraram um rendimento de até 53,4% usando 2-metil-2-butanol e razão molar de 1:3 (sacarose:ácido esteárico). O produto da reação foi purificado e a síntese foi confirmada por cromatografia em camada delgada e espectroscopia no infravermelho. As propriedades superficiais e interfaciais dos ésteres de sacarose e sua capacidade emulsificante foram avaliadas na ausência e presença de polissacarídeos, goma guar ou goma xantana. Os resultados confirmaram a pontecialidade de aplicação destes surfactantes em sistemas emulsionados.

No segundo estudo, foi desenvolvido um biorreator enzimático constituído de lipases de *Candida rugosa* imobilizadas em criogéis supermacroporosos. Os criogéis são matrizes de baixo custo e apresentam uma configuração que permite um processo contínuo, superior aos reatores em leito fixo, uma vez que a transferência de massa é basicamente convectiva. Assim, o biorreator produzido foi caracterizado em termos das suas propriedades morfológicas e eficiência de imobilização e, sua pontecialidade de aplicação na síntese contínua de ésteres de sacarose foi confirmada. Após a otimização das condições operacionais, a porcentagem máxima de esterificação prevista foi de 93,01% utilizando uma razão molar de 1:3,13 (sacarose: ácido esteárico), temperatura de 55,8 °C e proporção de 7,44:92,56 (DMSO: *tert*-butanol). Os ésteres de sacarose produzidos eram surfactantes capazes de adsorver na camada interfacial e promover uma diminuição significativa nas tensões superficial e interfacial.