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Exploring tannic acid-whey protein isolate microgels as Pickering stabilizers:

From microscale studies to conventional homogenization techniques

São José do Rio Preto

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

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“I’ve done my best, and I begin to understand what is meant by ‘the joy of strife’. Next to trying and winning, the best thing is trying and failing.”

Lucy M. Montgomery (2019, p. 311)

RESUMO

Emulsões de Pickering têm recebido crescente atenção devido à sua alta estabilidade contra coalescência. Contrastando com emulsificantes convencionais, partículas sólidas adsorvem irreversivelmente na interface óleo-água. O conhecimento fundamental sobre estabilização de Pickering se concentra no comportamento de adsorção de partículas de origem inorgânica, de modo que mais entendimento a respeito de emulsões de Pickering de grau alimentício se faz necessário a fim de explorar a aplicação desse sistema vantajoso em alimentos. Em vista disso, nós propomos microgéis de proteínas do soro de leite (WPI) obtidas a partir da reticulação com ácido tânico como uma nova partícula de grau alimentício a ser utilizada para estabilizar emulsões de Pickering. Nossos resultados mostram que a etapa de reticulação pôde ajustar as propriedades físicas dos microgéis de WPI, conferindo-lhes menos polidispersidade, menor tamanho de partícula e maior ângulo de contato, o que refletiu em emulsões de Pickering mais estáveis. Em seguida, exploramos o comportamento de adsorção dos microgéis de WPI reticulados com ácido tânico por meio de experimentos de Langmuir trough. Monocamadas formadas pelos microgéis e WPI nativo foram comparadas, fornecendo entendimentos sobre a atividade de superfície dos microgéis e a respectiva elasticidade da monocamada. Esses entendimentos foram combinados com a estabilidade física de emulsões de Pickering produzidas por dispositivos de microfluídica, o que permitiu determinar os principais parâmetros para a produção de gotas de emulsão estáveis utilizando esses microgéis. Tendo em mente a composição complexa de produtos alimentícios, a influência de surfactantes de baixa massa molar nas propriedades físicas de emulsões de Pickering também foi avaliada. Microgéis de WPI reticulados por ácido tânico demonstraram ser melhores estabilizantes que o WPI nativo, principalmente pela supressão de coalescência causada pela floculação em ponte. Ao contrário de partículas de Pickering convencionais, onde somente a força de cisalhamento aplicada é relevante para a adsorção de partículas na interface, observamos que um determinado intervalo de tempo é requerido pelos microgéis para promover a cobertura completa das gotas, devido à sua atividade superficial. A presença de surfactantes de baixa massa molar não comprometeu a estabilidade física de emulsões de Pickering ou removeu quantidade considerável de microgéis da interface ao longo do tempo. Sendo assim,

nós oferecemos uma nova partícula de origem biológica com oportunidade de ser aplicada como estabilizante de Pickering em sistemas alimentícios.

Palavras-chave: Microgéis de WPI. Comportamento de adsorção. Microfluídica. Floculação. Estabilização sinergística.

ABSTRACT

Pickering emulsions have received increased attention due to their high stability against coalescence. Contrasting to conventional stabilizers, solid particles adsorb irreversibly at the oil-water interface. The fundamental knowledge about Pickering stabilization is concentrated on the adsorption behaviour of particles from inorganic origin. More understanding about food-grade Pickering emulsions is needed in order to explore the application of such advantageous system into foodstuff. In view of that, we propose whey protein (WPI) microgels obtained from crosslinking with tannic acid as a novel food-grade particle for stabilizing Pickering emulsions. Our results showed that the crosslinking step was able to tune physical properties of WPI microgels, conferring to them small polydispersity, smaller particle size and increased contact angle, which reflected to more stable Pickering emulsions. Later, we explored the adsorption behaviour of tannic acid-crosslinked WPI microgels by means of Langmuir trough experiments. Monolayers formed by microgels and native WPI were compared, giving insights about the surface activity of microgel particles and the elasticity of the formed monolayer. Those understandings were combined with the physical stability of Pickering emulsions produced through a microfluidic device, enabling to determine the main parameters for producing stable droplets using those microgels. Keeping in mind the complex composition of foodstuff, the influence of low molecular weight surfactant on the physical properties of Pickering emulsions was also addressed. Tannic acid-crosslinked WPI microgels showed to be a better stabilizer than native WPI mainly by supressing droplet coalescence by bridging flocculation. Due to their surface-active character, enough adsorption time is required to promote completely coverage of emulsion's droplets, as opposed to conventional Pickering particles, in which only the shear force plays a role on particle adsorption. The presence of low molecular weight surfactants did not impair the physical stability of Pickering emulsions nor desorbed considerable amounts of microgels from the interface over time. Thus, we offered here a new food-grade particle as opportunity to be applied as Pickering stabilizer into food systems.

Keywords: WPI microgels. Adsorption behaviour. Microfluidics. Bridging flocculation. Synergistic stabilization.

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1 INTRODUCTION

Pickering emulsions (PICKERING, 1907) or particle-stabilized emulsions are well known for their high stability against coalescence. In these systems, particles with partial wettability are able to place themselves at the oil-water interface, giving rise to a rigid physical barrier that protects neighbouring droplets from merging upon collision. Besides the high stability, Pickering emulsions present an asset for application in the food industry due to their surfactant-free character, enabling the design of clean label products that have been increasingly demanded by consumers around the world (BERTON-CARABIN; SCHROËN, 2019; CHEVALIER; BOLZINGER, 2013).

Considerable knowledge has been collected about the interaction of stabilizing particles from inorganic sources at the oil-water interface and its impact on emulsion's stability (YANG et al., 2017). However, the production of suitable Pickering stabilizer particles using food components as starting materials remains a challenge, mainly because food materials possess a very complex composition, which lead to the obtention of heterogeneous particles (i.e., high polydispersity, rugous surface, non-homogeneous shape) (SAN-MIGUEL; BEHRENS, 2012; ZANINI et al., 2017). In addition, particles made from food materials are often accompanied by surface-active molecules that can adsorb at the interface, thus leading to synergistic or competitive interactions with the particles and moving the system away from a classic Pickering stabilization (BERTON-CARABIN; SCHROËN, 2015). Both reasons make it very difficult for scientists to connect emulsion characteristics with particle attributes but, in spite of this, some food materials have already been explored for producing Pickering particles, such as starch (MAREFATI et al., 2015), cellulose (HU et al., 2016b), chitosan (RIBEIRO et al., 2020), soy protein isolate (BENETTI; SILVA; NICOLETTI, 2019), zein (WANG et al., 2020a), fat (SCHRÖDER et al., 2019), and gelatin (TAN et al., 2014). Nonetheless, the explanation about mechanisms involved in the formation of such emulsions remains supported by the knowledge on adsorption of inorganic particles.

With the increasing interest on Pickering emulsions by the scientific community, a new class of stabilizing particles has emerged. The so-called microgel particles are composed by soft swollen polymer networks that differ considerably from hard particles in their interfacial functionality. Microgels can deform upon adsorption, assuming a fried-egg like structure that is able to cover a larger interfacial area than hard Pickering

particles, conferring even higher desorption energy (DICKINSON, 2017). Among food materials, whey protein is regarded as a widely available and accepted protein source that can be successfully used as starting material for microgel's production. The high surface activity of protein molecules is an asset to produce microgel particles because they combine the high desorption energy of Pickering particles with the ability of protein molecules to adsorb at any interface (LI et al., 2018; MURRAY, 2019). Thus, protein microgels represent an interesting option to be applied as stabilizer particles in food-grade Pickering emulsions.

Additionally, the physical properties of microgel particles can be tuned in order to improve even more their ability as Pickering stabilizer. The crosslinking reaction is claimed as a method to modify physical and mechanical properties of materials, such as chemical and thermal stability, structural rigidity, and permeability (Gonsalves, Araújo, Soares, Goulart, & Abreu, 2011). In proteins, the crosslinking is mostly performed by reactions with aldehydes, mainly glutaraldehyde and formaldehyde. However, these substances have toxic potential and are not allowed for use in food (Azeredo & Waldron, 2016), making necessary to search for alternative reactants.

In this thesis, we propose the use of whey protein microgels crosslinked by organic acids as an alternative for Pickering emulsions stabilizer. Firstly, the microgel particles were produced, characterized regarding their physical properties, and evaluated as stabilizer of emulsions containing roasted coffee oil as disperse phase. Later, an extensive investigation about the interfacial behaviour of whey protein microgels prepared by crosslinking with tannic acid was implemented. Subsequently, emulsions stabilized by these microgels were produced using a microfluidic device, which enabled to determine the main parameters that affect the physical stability of these emulsions. Finally, the influence of the presence of low molecular weight molecules in Pickering emulsions stabilized by the microgel particles was investigated.

8 GENERAL CONCLUSIONS

In the present thesis, we demonstrated that the crosslinking of WPI microgels by organic acids is a feasible strategy to tune their physical properties. Such approach enabled the formation of particles more suitable for Pickering stabilization, besides being food-grade and complying with the “clean label” classification of food products. An improved performance was observed for tannic acid-crosslinked WPI microgels, in which emulsions stabilized by them showed neither creaming nor sedimentation after 7 days of storage.

A deeper investigation about the adsorption behaviour of tannic acid-crosslinked WPI microgels enabled us to verify their soft and surface-active nature. At the interface, the soft core of microgels flatten, covering a larger interfacial area than non-deformable particles. Experiments on microfluidics device revealed that the adsorption time has an important role on the stability of emulsions produced by these microgels. This contrasts with the former knowledge about Pickering particles, in which only the shear force is told to have a role on emulsion stability. This different behaviour is related to the surface-activeness of microgel particles and their ability to deform, which allow them to reach/reorganize at the interface over time. Also, we identified that microgel particles supress droplet coalescence by bridging flocculation, providing emulsions with enhanced stability when compared to their conventional counterparts (stabilized by native WPI).

Despite our efforts, the 10 step-ultrafiltration procedure was not able to remove all the non-reacted native WPI from the microgel dispersion, which means that a pure Pickering system could not be achieved in our work. However, by evaluating emulsions stabilized by blends of tannic acid-crosslinked WPI microgels and native WPI, we verified that native protein molecules assisted on disrupting flocs over time or in the formation of smaller droplets during homogenization, besides not being able to promote the desorption of microgels from the interface. This points to a synergistic mechanism of stabilization rather than competition for adsorption.

To conclude, a new food-grade particle with increased ability to stabilize Pickering emulsions is proposed in the present thesis, in addition to explanations on the mechanisms by which they adsorb at the interfaces and confer improved stability to emulsions. Even though the production of these microgel particles is accompanied by remaining non-reacted native proteins, we demonstrated that the presence of such

molecules does not impair either microgel adsorption or emulsion physical stability, which shows that tannic-acid crosslinked WPI microgels can be successfully applied to the production of food-grade Pickering emulsions.

9 SUGGESTIONS FOR FUTURE STUDIES

Investigate the long-term stability of emulsions stabilized by tannic-acid crosslinked WPI microgels, including microbiological growth over time.

Verify the performance of tannic-acid crosslinked WPI microgels on protecting bioactive compounds from stress conditions (i.e., exposure to light, heating, and presence of enzymes).

Study the impact of different crosslinking mechanisms (chemical, physical, and enzymatic) on microgels' physical properties.

Investigate the adsorption behaviour of WPI microgels with different crosslinking densities and evaluate the morphological aspect of the formed monolayers by using atomic force microscopy.

Propose a new methodology to purify the WPI microgel dispersion and produce/characterize a pure Pickering system – emulsions stabilized solely by microgel particles.

Propose a methodology to determine the interfacial composition of droplets stabilized by blends of WPI microgels and native WPI that enables to accompany the interfacial composition of droplets over storage time.

Compare hard particles obtained from proteins with protein microgels regarding to their performance as Pickering stabilizers.

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