RAQUEL REIS LIMA

STUDY OF MODEL EMULSION (O/W) STABILIZED BY VEGETABLE PROTEINS AND POSSIBLE APPLICATIONS IN VEGETABLE BEVERAGES

Thesis submitted to the Graduate Program in Food Science and Technology of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Advisor: Antônio Fernandes de Carvalho
Co-advisors: Rodrigo Stephani
Ítalo Tuler Perrone

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Antônio Fernandes de Carvalho
Adviser

“Do not be overcome by evil,
but overcome evil with good’’.

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ABSTRACT

LIMA, Raquel Reis, D.Sc., Universidade Federal de Viçosa, March, 2023. **Study of model emulsion (O/W) stabilized by vegetable proteins and possible applications in vegetable beverages.** Advisor: Antônio Fernandes de Carvalho. Co-advisors: Rodrigo Stephani and Ítalo Tuler Perrone.

Interest in switching diets to plant-based products instead of animal-based foods is high among people who seek quality of life, maintain healthy habits and are committed to sustainable diets and reducing pollution, land use and water consumption. The growing demand for plant-based products is changing the way global industry produces food. Modern consumers are increasingly interested in the ingredients that manufacturers use in their food. Food manufacturers are therefore trying to remove ingredients that consumers find problematic or are unfamiliar with from the ingredient list and replace them with simple and familiar ingredients. Plant-based beverages are one of the products that have entered the market to meet consumer demand for plant-based alternatives in their daily diet. From a physicochemical point of view, plant-based beverages are defined as water-in-oil emulsions, consisting essentially of water, oil and vegetable protein. In order to formulate vegetable beverages and understand their behaviour, it is necessary to know separately the behaviour of oils and proteins used in beverage formulations beforehand. The aim of this work is to investigate models of oil-in-water emulsions, focusing on emulsions stabilised by different vegetable protein sources and emulsions prepared with oils whose composition is predominantly medium-chain triacylglycerol.

Keywords: Modification of plant-based protein. Food emulsion. Pea protein. Lentil protein. Fava bean protein. Coconut oil.

RESUMO

LIMA, Raquel Reis, D.Sc., Universidade Federal de Viçosa, março de 2023. **Estudo de emulsão modelo (O/A) estabilizada por proteínas vegetais e possíveis aplicações em bebidas vegetais**. Orientador: Antônio Fernandes de Carvalho. Coorientadores: Rodrigo Stephani e Ítalo Tuler Perrone.

O interesse em mudar as dietas para produtos à base de plantas em vez de alimentos de origem animal é alto entre as pessoas que buscam qualidade de vida, mantêm hábitos saudáveis e estão comprometidas com dietas sustentáveis e com a redução da poluição, do uso da terra e do consumo de água. A crescente demanda por produtos à base de plantas está mudando a maneira como a indústria global produz alimentos. Os consumidores modernos estão cada vez mais interessados nos ingredientes que os fabricantes usam em seus alimentos. Os fabricantes de alimentos estão, portanto, tentando remover ingredientes que os consumidores acham problemáticos ou não estão familiarizados com a lista de ingredientes e substituí-los por ingredientes simples e familiares. As bebidas à base de plantas são um dos produtos que entraram no mercado para atender à demanda do consumidor por alternativas à base de plantas em sua dieta diária. Do ponto de vista físico-químico, as bebidas à base de plantas são definidas como emulsões de água em óleo, consistindo essencialmente de água, óleo e proteína vegetal. Para formular bebidas vegetais e entender seu comportamento, é necessário conhecer separadamente o comportamento dos óleos e proteínas utilizados nas formulações de bebidas de antemão. O objetivo deste trabalho é investigar modelos de emulsões óleo-em-água, com foco em emulsões estabilizadas por diferentes fontes de proteína vegetal e emulsões preparadas com óleos cuja composição é predominantemente triacilglicerol de cadeia media.

Palavras-chave: Modificação de proteína vegetal. Emulsão alimentícia. Proteína de ervilha. Proteína de lentilha. Proteína de fava. Óleo de coco.

SUMMARY

1. GENERAL INTRODUCTION

Food emulsions are the result of an emulsification process that involves mixing two immiscible liquids, one of which is dispersed in the other by mechanical action such as mixing, stirring or shearing (Chang et al., 2015; Mohan & Narsimhan, 1997). Oil-in-water emulsions are of great importance in the food industry as they are used not only as functional ingredients but also for various applications such as milk, cream, sauces, desserts, beverages, ice cream, margarine, salad dressings, soups, infant formula and others (M. Chen & Sun, 2021; Ding et al., 2021; McClements, 2006).

Vegetable beverages meet the definition of oil-in-water emulsions, although there is still no legislation in Brazil defining the identity and quality standards of these beverages. In general, vegetable drinks are made from oil, water and protein. Consumers' growing interest in natural products and concern for the environment are forcing the industry to reduce ingredients on labels so that beverages can be presented on the market as naturally as possible, with ingredients that are familiar to consumers (Jeske et al., 2017).

Therefore, the search for natural emulsifiers has increased, as only oil and water are not stable and do not withstand storage (Kinsella, 1976) .Vegetable proteins are an excellent source of natural emulsifiers as they possess technological properties in addition to their nutritional properties. Proteins can act as emulsifiers due to their ability to form and stabilise small droplets. Proteins are natural amphiphilic molecules with interfacial activity and stabilising properties (Pearce & Kinsella, 1978). When adsorbed at the oil-water interface in the emulsion, they reduce the interfacial tension and thus promote droplet formation. They form an interfacial layer (viscoelastic film) that stabilises the droplets against flocculation or coalescence by electrostatic repulsion and imparts physical stability to the emulsion during subsequent processing and storage (Bergfreund et al., 2021; M. Kumar et al., 2021)

The emulsifying ability of proteins depends on their molecular structure and physicochemical properties (Ng et al., 2020; Pearce & Kinsella, 1978). The stabilisation of emulsions by plant proteins depends on several factors related to the type of protein (Cui et al., 2020), structure (Du et al., 2018), flexibility (R. Li et al., 2019) extraction methods (Wang et al., 2019; Sun et al., 2020), drying (Gong et al., 2015; Shen et al., 2021), heating (Zhang, et al., 2020) and ionic strength (L. Cui et al., 2020). New studies are constantly being conducted with various plant sources to improve, modify and enhance the emulsifying properties of plant proteins, e.g. by using ultrasound(X. Sun et al., 2020b; F. Wang et al., 2020a; Y. Zhang et al., 2018a), enzymatic hydrolysis(Mokni Ghribi et al., 2015; Zang et al., 2019), complexation with polysaccharides (Ribeiro et al., 2021; Yan et al., 2021)and polyphenols (X. Liu et al., 2023; Yan, Yao, et al., 2022), with the aim of expanding their use and completely replacing synthetic emulsifiers.

Another fundamental ingredient for the production of oil-in-water emulsions in the formulation of vegetable beverages are vegetable oils. Oils are energetic lipid sources that change the texture, taste and colour of emulsions (Gulão et al., 2018). As interest in healthy foods grows, so does interest in oils, which can play a dual technological and nutritional role. Oils whose composition is predominantly medium-chain solids are ideal for the production of oil-in-water emulsions. In addition to the nutritional aspects, the chemical structure of these prepared foods also has technological properties, such as the low melting point, which causes these oils to solidify at temperatures below 25 °C and return to a liquid state at higher temperatures without the oils losing their technological or nutritional properties (Kumar & Krishna, 2015; Ferrari & Soler 2015; Kiliç & Oezer, 2019).

Based on these reports, Chapter 1 therefore addresses the main chemical, physical and enzymatic methods listed in the current literature as promising for improving the technofunctional properties of various plant protein sources and their use in food formulations through a literature review. Chapter 2, building on Chapter 1, addresses the synergistic effect of different plant proteins as a way to improve and/or enhance the properties of emulsifying proteins in legume proteins, and Chapter 3 addresses the physico-chemical and nutritional aspects of plant oils with predominantly medium-chain fatty acids and their use in oil-in-water emulsions.

- The papers below are in the format required by the various journals in which they were published or submitted.

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2. CHAPTER 1

Plant-based proteins: A review of factors modifying the protein structure and affecting emulsifying properties

Abstract

Oil-in-water emulsions are widely used in the food industry and form the basis for various products such as milk, sauces and ice cream. With increasing environmental awareness and consumer appreciation for healthy food and clean labels, the industry has increased demand for natural emulsifiers, often limiting the use of synthetic emulsifiers. Plant-based proteins have been investigated for use as natural emulsifiers; however, most of these plant proteins have lower emulsifying activity than animal proteins. physical, chemical and enzymatic methods are being explored to alter the structure of proteins and improve their emulsifying properties, but the changes can have opposite effects depending on the concentration, type of agent and type of protein. The aim of this work was to understand the effects of the main methods already tested in the literature on the emulsification properties of different plant protein sources and to extend their application as natural emulsifiers for the preparation of oil-in-water emulsions.

Keywords: Emulsion, solubility, hydrophobicity, modification of plant-based protein, flexibility

1. Introduction

Food emulsions are the result of the emulsification process, being a unitary operation involving a mixture of two immiscible liquids, one of which is dispersed in the other through the mechanical action of mixing, stirring, or shearing (Mohan, 1997; Chang et al., 2015). Oil-in-water emulsions are of great importance in the food industry as they are used not only as functional ingredients but also for various applications such as milk, cream, sauces, desserts, beverages, ice cream, margarine, salad dressings, soups, infant formula and others. to improve the solubility, bioavailability, stability and sensory properties of other foods (M. Chen & Sun, 2021; Ding et al., 2021; McClements, 2006). However, emulsions consisting of only water and oil are not stable because the dispersed droplets tend to coalesce, resulting in phase separation. To improve the stability of emulsions, emulsifiers and stabilisers are usually used

(Figure 1). The emulsifier aids dispersion formation reduces the interfacial tension between the two immiscible liquids and creates a larger surface area between the two phases. (Kinsella 1976; S. Xu et al., 2019; Ng et al., 2020).

Today's consumers are increasingly aware of and concerned about environmental and health issues and demand from industry products made from natural ingredients, clean labels, high nutritional value and lower environmental impact (Hu et al., 2013; Du et al., 2018; Zhu et al., 2020), motivating the replacement of synthetic emulsifiers with natural emulsifiers in foods and beverages. (Y. Wang et al., 2017) .Proteins are often used as emulsifiers to stabilise O/W emulsions due to their ability to form and stabilise small droplets. Proteins are natural amphiphilic molecules with interfacial activity and colloidal stabilising properties. Proteins are adsorbed by the oil-water interface in the emulsion, which reduces the interfacial tension and (Kinsella, 1978) forms an interfacial layer between droplets; this stabilises the droplets against flocculation or coalescence electrostatic repulsion and imparts physical stability to the emulsion during subsequent processing and storage (Bergfreund et al., 2021; Kumar et al., 2021).

Plant proteins are promising sources of natural emulsifiers but are not yet widely used as emulsifiers because they have poorer emulsifying properties than animal proteins (Tang, 2017). The emulsification properties of proteins are always related to their structure. One of the crucial properties of the protein for use as an emulsifier is its instantaneous or potential amphiphilicity. The position and composition of the individual amino acids along the polypeptide chain determines the shape and position of the hydrophobic and hydrophilic parts in the structure, which defines the amphiphilicity. Other factors such as protein structure, flexibility and isoelectric point can affect protein solubility and hydrophobicity - parameters that directly affect protein emulsifiability - by altering the rate of structural reorganisation of the protein adsorbed from the interface, which in turn affects steric hindrance between droplets and alters protein emulsion stability. Most plant proteins have rigid structures, which also contributes to lower solubility and limits their use as food ingredients (Tang & Shen, 2013; Y. Liu et al., 2011; R. Li et al., 2019; Meenmanee et al., 2022)

Various methods have been explored to improve the physicochemical, functional and physiological properties of plant-based proteins (T. Li et al., 2021; Zhu et al., 2020), including physical, chemical and enzymatic modifications (Boye et al., 2020). Sun et al., 2020; Shen et al., 2021). Modifications of plant-based proteins can improve their technological performance and expand their use as natural emulsifiers.Xu et al., (2016) observed that after enzymatic treatment, aromatic residues were more exposed due to cross-linking. It was reported that changes in pH and oxidation made the structures of pea and rice proteins more flexible, making them easier to adsorb at the oil-water interface (L. Cui et al., 2020; H. Li et al., 2021). Zhang (2020) pointed out that heat treatment can destroy the compact structure of the proteins, which improves their adsorption at the interface.

To this end, the present review aims to elucidate and illustrate the changes in the structure of various plant protein sources due to physical, chemical and enzymatic processing, the mechanisms underlying these changes and the effects of these changes on the functional properties of proteins, especially their emulsifying and stabilising capacity in oil-in-water emulsions. It is expected to provide a reference for potential applications of plant proteins in the food industry.

2. Chemical modifications affecting the emulsifying properties of plant-based proteins.

2.1 pH Changes

The pH affects the emulsifying ability, primarily through changing the charge distribution in the protein molecules. At extreme pH, proteins tend to denature or disassociate, which exposes a greater part of their hydrophobic nucleus, allowing better adherence to the interface. In denatured proteins, previously hidden sulfhydryl groups may form disulphide bonds within themselves or with other proteins (Wu et al., 2011; X. Xu et al., 2016)

When disulphide bonds are formed at the oil-water interface, emulsion stability is achieved (Wu et al., 2011). In a study where sulphydryl groups on proteins were chemically blocked one by one, the emulsions formed resulted in less stable emulsions. It was found that the interfacial protein layer was ruptured and the oil content changed to the continuous phase (Wu et al., 2011). In contrast, a study with lentil protein isolates showed that emulsion stability improved when disulphide bonds were reduced (Joshi et al., 2011). The reduction of disulphide bonds reduced the amount of interfacial bonding leading to coalescence (Joshi et al., 2011). This suggests that although interfacial bonding is important to improve the viscoelastic properties of the interface (which increases the stability of the emulsion), the bonds formed can also occur between two droplet interfaces, which is detrimental to the stability of the emulsion (Lam & Nickerson, 2013).

At low or high pH, proteins carry positive or negative net charges, resulting in an electrostatic repulsive force that helps to keep protein molecules separate, disrupting the structure of the native protein, shifting the equilibrium towards the unfolded form and subsequently exposing the functional groups hidden in protein molecules, ultimately increasing protein solubility. Near the isoelectric point, on the other hand, proteins aggregate due to strong intermolecular interactions, resulting in less interaction with water and thus lower protein solubility. The enormous effect of pH change was demonstrated in the solubility of chia protein isolates, which were almost completely soluble at a pH of 12.0 and had low solubility (40%) at a pH of 1.0. Studies on globulins and flaxseed albumin showed that albumin had the best effect on emulsification at pH 5 and 7, while globulin was a more effective emulsifier at pH 9.0. In general, globulin isolate proved to be a better emulsifier under alkaline conditions, while albumin isolate proved to be a better emulsifier under acidic conditions (Nwachukwu & Aluko, 2018; Yuliana et al., 2014)

Protein solubility can increase or decrease depending on its intrinsic characteristics. For instance, extreme pH (e.g. pH 12) increased the solubility of pea protein isolate but decreased the solubility of soybean protein isolate; however, the solubility of pea isolates did not change when extracted at pH 10. Moreover, all samples of pea protein isolates exhibited lower solubility at pH 4 and 5, with the highest solubility at pH 8.0. This trend of solubility curve could be explained by decreased liquid surface load at pH near the isoelectric point protein (pH 4 and 5) and increased positive/negative net charge at pH 3 and 8, as evidenced by ζ-potential measurements (Jiang et al., 2013; L. Cui et al., 2020; Zhu et al., 2020) .

The isoelectric point of any protein depends on several factors, including the content of hydrophobic and aromatic amino acids and the significantly higher proportion of negatively charged amino acids and low molecular weight polypeptides, which increase structural flexibility and solubility compared to larger polypeptides (L. Cui et al., 2020). When the pH of emulsions stabilised with rice bran protein approaches its isoelectric point, the solubility of the protein is minimal and the content of effective components that can act as emulsifiers is minimised. At pH 7-9, which is above the isoelectric point, the average droplet size remained small, especially at pH 9 where no evidence of droplet aggregation was observed. This phenomenon may be attributed to negatively charged carboxyl groups and neutral amino groups, which imparted a net negative charge to the droplets. Therefore, electrostatic repulsion may be the most appropriate mechanism to prevent droplet aggregation in protein-stabilised emulsions (Zang et al., 2019).

2.2 Oxidation

Protein oxidation is a structural modification induced directly by reactive oxygen species or indirectly via reaction with lipid peroxidation by-products. Oxidative modification can trigger a series of changes in the amino acid side chains and structure of the protein polypeptide, resulting in protein fragmentation, crosslinking, unfolding, and conformational changes. Free radicals attack almost all types of amino acid side chains; in particular, aromatic amino acid and sulphur-containing side chains are vulnerable to oxidation (Zhang et al., 2013; Li et al., 2021; Zhang et al., 2013).

Lipids are sensitive to oxidation, and lipid peroxidation produces free radicals. In addition, lipid hydroperoxides and reactive aldehydes can oxidise proteins. The lipidoxygenase remaining in the protein catalyses lipid peroxidation (Chen et al., 2013; Wu et al., 2011). In soy protein, oxidation had either positive or negative effects on the gelling properties depending on the oxidant. In addition, lipid peroxidation was confirmed to occur during storage of emulsions and its by-products can cause protein oxidation. Oxidation can alter the secondary and tertiary structure of proteins, leading to a change in their physicochemical and functional properties (Chen et al., 2013; Vera et al., 2019; Wu et al., 2011). Moderate oxidation of rice bran proteins increased their flexibility, exposing the hydrophobic and loaded amino acids buried within the protein molecule. The exposed hydrophobic amino acids interacted with oil droplets to facilitate the adsorption of the protein at the oil–water interface and the formation of a viscoelastic film that reduced the tension at the interface, causing the droplets to divide into smaller sizes. Electrostatic repulsion between droplets can be promoted by the exposure of amino acids loaded on the surface of the protein; thus, flocculation and coalescence of droplets can be inhibited, increasing their dispersion. In rice bran protein, moderate oxidation weakened the degree of flocculation and coalescence of the emulsion droplets and increased emulsion stability under the action of gravity and centrifugal force (H. Li et al., 2021; Tang, 2017; Chen et al., 2013; H. Li et al., 2021).

Excessive oxidation can increase the content of protein adsorbed at the interface, inducing aggregation (Chen et al., 2013). In addition, decrease in emulsion stability under excessive oxidation can promote the formation of a hard interface film of protein adsorbed at the interface; this is not desirable because under moderately oxidising conditions, the formation of interface membranes that are more flexible and viscoelastic by proteins adsorbed at the interface increases the stability of emulsions. In rice bran protein, excessive oxidation led to the re-burial of hydrophobic and charged amino acids, which reduced electrostatic repulsion between emulsion droplets, leading to flocculation and coalescence, reduction in emulsion uniformity (Tang, 2017; H. Li et al., 2021), and decrease in protein flexibility, making it difficult for protein adsorbed at the oil–water interface to form a viscoelastic interface film. Therefore, with increase in the degree of oxidation, the degree of flocculation and coalescence of emulsion droplets increases, in addition to the reduction in the stability of the emulsion under the action of gravity and centrifugal force (Li et al., 2021; Zhu et al., 2020; Chen et al., 2013; Li et al., 2021).

2.3 Glycosylation

Glycosylation is a type of chemical modification based on the basic principle of the Maillard reaction, which involves an aminocarbonyl reaction. Because the reaction is triggered spontaneously by heating without the addition of any chemical reagents, it is a relatively ideal and promising chemical modification method to effectively improve functional properties of proteins, such as solubility, emulsification, foaming, gelling, and heat stability (R. Li et al., 2019; Pirestani et al., 2017; Y. Wang et al., 2017). Improvements in these properties may be due to alteration or increased flexibility of the secondary structure of the protein isolate; this promotes the expansion and redistribution of protein emulsion at the oil–water interface and improves the emulsifying activity of the protein and the Maillard reaction, which alters the protein structure to expose hydrophobic groups and increase hydrophobicity (Molina et al., n.d.).

R. Li et al. (2019) reported that when stabilised with soybean and dextran protein conjugates, the emulsion stability first increased and then significantly decreased, peaking in 5 h. This increase may be explained as follows: when glucose is connected to the peptide chain of soy protein isolate via covalent binding, steric protein impediment increases and polymerisation of the interfacial protein becomes difficult (Zang et al., 2017). Simultaneously, the viscosity of the protein solution may increase when glucose is added. Presumably, the blockage of amine groups following conjugation with the carbohydrate part may increase the liquid negative load and hydrophilicity in general, causing repulsion between proteins and intensified solvation and contributing to the formation of a macromolecular stabilising film around oil droplets rather than modifying the continuous phase rheology (R. Li et al., 2019).

The same emulsion behaviour was observed in another study (Y. Liu et al., 2012) using peanut proteins conjugated with dextran. The emulsifying properties of peanut protein associated with polysaccharides were improved compared with those of the native protein and intensified after heating. Emulsion stability increased on the first day, followed by a decrease. Dry heat-induced conjugation improved protein solubility and stability, ultimately improving its emulsification properties. As a non-active hydrophilic polysaccharide on the surface, dextran does not exhibit a strong absorption tendency at the air–water interface, although it can markedly increase the stability of protein foams, acting as a thickening or gelling agent (Dickinson, 1999).

Solubility is a determining factor for the ability to form emulsions. The solubility of wheat protein isolate was significantly improved after association with dextran. This was because the protein gained more hydrophilic groups after grafting with dextran, and binding with dextran inhibited non-covalent interactions between proteins, increasing solubility. The formation of protein–polysaccharide conjugates has been proven to integrate the characteristic of proteins to strongly adsorb at the oil–water interface with the characteristic of polysaccharides to isolate in an aqueous phase medium, conferring better emulsification activity and stabilisation of wheat protein emulsions (Xue et al., 2017). Similarly, other studies have shown that glycosylation modification reduces protein hydrophobicity, increasing hydrophilic groups and partially protecting hydrophobic groups, which may be related to the type of protein and degree of Maillard reaction (C. Li et al., 2014; Qu et al., 2018; R. Li et al., 2019).

Whilst the addition of dextran alone did not affect the hydrophobicity of the protein surface, the hydrophobicity of heated β-conglycinin was significantly higher than that of native β-conglycinin, suggesting that more hydrophobic clusters were exposed on the molecular surface of the protein following heat treatment (X. Zhang et al., 2012). Meanwhile, glycosylation can reduce the exposure of intramolecular hydrophobic groups, and the binding of hydrophilic linear dextran chains increases hydrophilicity at the molecular surface. Thus, the hydrophobicity of the conjugate reduces in the macromolecular agglomeration environment. These results indicate that the molecular structure of the soy protein isolate was unfolded and the hydrophobic groups were exposed as a result of increased surface hydrophobicity due to the Maillard reaction and heat treatment. Furthermore, the variations in surface hydrophobicity and flexibility were consistent.

3. Physical modifications affecting the emulsifying properties of plant-based proteins

3.1 Heat treatment

Heat treatment is a unitary operation widely used in the food industry and can modify the structural and functional properties of globular proteins. Heat treatment causes conformational changes and promotes intermolecular bond formation in the protein adsorption layers as well as hydrophobic interactions and protein aggregation through disulphide bond formation (A. Liu et al., 2018). Heat-denatured proteins or aggregates have been used for foam and emulsion stabilisation (J. M. Wang et al., 2012).

Heat treatments have been used to improve protein functionalities (Cui et al., 2014; Keerati-u-rai & Corredig, 2009; Peng et al., 2016; Shao & Tang, 2014; J. M. Wang et al., 2012; Zhang et al., 2020)In soy protein isolates, heat treatment at 95°C for 30 min increased surface hydrophobicity, thereby reducing the average droplet size of emulsion oil, increasing the percentage of adsorbed protein, and decreasing surface load at the oil–water interface, in addition to improving the freezing–thawing stability of oil emulsions in water (Z. Cui et al., 2014).

Heat-treated soybean and pea protein showed greater surface hydrophobicity than their native forms (Z. Cui et al., 2014; Y. Zhang et al., 2020; Peng et al., 2016). The surface hydrophobicity affects the ability of the protein to adsorb at the interface and is closely related to the emulsifying properties (Damodaran et al., 2005), which can be induced by a modification of the native protein structure through heat treatment: more hydrophobic groups within the molecule are exposed as a consequence of heat-induced protein unfolding (W. Zhang et al., 2013). Moreover, heat treatment increased the surface hydrophobicity of proteins, thereby increasing their adsorption potential at the oil–air interface (Y. Zhang et al., 2018). Furthermore, heat treatment promoted interparticle interactions and induced structural rearrangements of interfacial adsorbed nanoparticles, increasing the distribution of particles on the oil drop surface, and mediating the formation of an interfacial structure with greater thickness and stability against coalescence (Sun et al., 2020).

Emulsions formed using heated pea proteins (at 95°C for 30 min) showed a higher percentage of protein adsorption and cream stability than those formed using unheated proteins (Peng et al., 2016). Shao (2014) applied heat pre-treatment (at 95°C for 15 min) and observed that whilst the emulsifying capacity of soybean protein isolate was not affected by ionic strength, it was remarkably improved by heat pre-treatment. This improvement in emulsifying capacity is consistent with the well-known fact that partial denaturation and subsequent structural unfolding of globular proteins after heating are conducive to their emulsifying properties (Damodaran & Anand, 1997).

In a study by (Peng et al., 2016b), soy protein emulsions prepared with or without heating at 95°C for 30 min showed decreased adsorption from the interface withincreasing protein concentration. Treatment of soybean proteins under more severe conditions, such as 120°C, triggered greater surface activity and faster development of intermolecular interactions in the adsorbed layer (Z. Cui et al., 2014). Typically, heat treatment above the denaturation temperature causes partial and subsequent protein aggregation (J. M. Wang et al., 2012). In soybean protein, heat treatment caused the dissociation of protein subunits, and temperature rise affected the aggregation state of proteins (J. M. Wang et al., 2012)(Rahmati et al., 2018; Zhao & Xiong, 2015).

After heat treatment at 120 °C, Wang et al. (2012) observed a more flexible protein conformation; this is due to the fact that temperature plays a crucial role in stabilising protein structure and folding, while also helping to keep the covalents within the protein structure relaxed. At higher temperatures (140°C), the protein is hydrolysed into oligopeptides due to heat energy. In a study by Wang et al. (2012), soy protein isolates were heat-treated at two temperatures (90°C and 120°C for 20 min); at 120°C, the protein presented better adsorption at the interface of oil droplets than its native form. At 90ºC showed no significant improvement (M. Kumar et al., 2021). Furthermore, heat-treated proteins reached relative adsorption equilibrium in a shorter time. These observations might be related to the adsorption behaviour of heat-induced aggregates with prolonged adsorption times (Croguennec et al., 2007). Proteins that are initially adsorbed at the oil-water interface act as a barrier to prevent the adsorption of large molecules that are anchored to the interface with only a small portion of their surface area. The more flexible conformation of soy isolates heated to 120 °C could explain the increase in surface activity after heat treatment, and this observation may be related to the flexible conformation of the heat-treated proteins (Croguennec et al., 2007; J. M. Wang et al., 2012).(Croguennec et al., 2007;J. M. Wang et al., 2012)

3.2 Ultrasound application

Ultrasound is an acoustic wave with a frequency exceeding 20 kHz and can be divided into two types: low-intensity (100 kHz to 1 MHz, power < 1 W·cm^{-2}) and high-intensity (16 to 100 kHz, power = 10–1000 W·cm^{-2}). High-intensity ultrasound can produce high shear and mechanical energy owing to the cavitation phenomenon and can be used to modify the structures and properties of globular proteins (Y. Zhang et al., 2018).

Ultrasound-assisted extraction is a rapid, efficient, and environmentally friendly technology that has been used to extract and modify plant-based proteins. At an adequate ultrasound energy density, this method can increase efficiency and shorten extraction time and temperature. In addition, ultrasound can alter the physicochemical, structural, and functional properties of proteins because of its shear and cavitation effects, as reported in soy, pea, and peanut proteins. Ultrasonic cavitation disrupts van der Waals forces, hydrogen bonds and hydrophobic interactions between molecules; in addition to reducing particle size; and induces tertiary structural changes in peanut proteins. Emulsification properties are improved as molecular bonds between proteins and other macromolecules are broken and more proteins go into solution. (Sun et al., 2020; Ochoa-Rivas et al., 2017; Sun et al., 2020; Y. Zhang et al., 2018; Z. Cui et al., 2014).

Ultrasound-assisted extraction was affected the structure and functional properties of peanut proteins; ultrasound improved water and fat absorption indices, foam activity, and emulsifying activity and increased the contact area between defatted peanut protein powder and alkaline solution, offering benefits for mass transfer and re-dictating that the emulsifying activity of peanut protein may be associated with its tertiary structure, and any change in this structure would affect its emulsifying properties (C. Li et al., 2014). Ultrasound altered the secondary and tertiary structure of pea protein, resulting in its partial unfolding and exposing the hydrophobic groups. Increased exposure of hydrophobic groups may reduce the energy barrier for adsorption at the air–water interface, thereby enhancing adsorption kinetics (Sun et al., 2020). Ultrasound-assisted alkaline extraction significantly improved functional properties, including solubility, hydrophobicity, water–oil retention capacity, foaming capacity, emulsification, stability, and gel-forming capacity (Sun et al., 2020; F. Wang et al., 2020).

There is ample evidence indicating that ultrasound pre-treatment improves protein properties. For instance, Mir et al., 2019 and Vera et al., 2019reported that high-intensity ultrasound treatment improved the functional properties of quinoa protein, particularly its gelling behaviour. In pea proteins, high-intensity ultrasound suppressed intermolecular associations, leading to the exposure of more sulfhydryl groups and hydrophobic regions to the solvent environment and increase in the sulfhydryl content and hydrophobicity(Zhao & Xiong, 2015; X. Zhang et al., 2012;Y. Wang et al., 2017; Hu et al., 2013; Xiong et al., 2018; Jiang et al., 2013). Other studies are demonstrated in Table 1 which gives a summary of publications in the area over the last few years.

4. Enzymatic modifications affecting the emulsifying properties of plant-based proteins

4.1 Enzymatic hydrolysis

Hydrolysis has been used to improve the emulsifying properties of proteins. Specifically, hydrolysis increases protein solubility, exposes hidden hydrophobic groups, improves surface hydrophobicity, and decreases protein molecular weight, allowing better adhesion at the oil-water interface. However, these improvements in protein functionality depend on the degree of hydrolysis (Zang et al., 2019; Mokni Ghribi et al., 2015), and the properties of the substrate (Jiang et al., 2013). At a high degree of hydrolysis, the protein tends to saturate in the continuous phase instead of adhering to the water-oil interface. Therefore, the use of hydrolysis to increase protein functionality is limited to a low degree of hydrolysis (Lam & Nickerson, 2013; X. Xu et al., 2016; Zhao & Xiong, 2015). The degree of hydrolysis is defined as the number of peptide bonds per gram of protein relative to the total number of peptide bonds per gram of protein in the hydrolysis process. The degree of hydrolysis is influenced by the enzyme, substrate, pH, temperature and duration; therefore, hydrolysis conditions must be controlled to a desirable degree. Alcalase, an enzyme derived from microorganisms, exhibits peak activity at pH 7-11 and has been used for protein hydrolysis to improve its bioactivity (F. Wang et al., 2020).

Enzymatic hydrolysis produces a great impact on protein emulsification capacity because of significant changes in the protein structure due to the cleavage of the peptide chain. Compared with their intact precursors, protein hydrolysates with a low degree of hydrolysis have been shown to improve emulsification capacity by increasing the conformational flexibility of large peptides. However, small peptides generated via extensive hydrolysis show a high load frequency, which may prevent their adsorption at the interface, resulting in decreased emulsification capacity and poor stability. Initially, the hydrophobicity values of hydrolysed proteins increased when at the degree of hydrolysis was below 3% (Sun et al., 2020; Zang et al., 2019), and this increase was due to partial hydrolysis, which led to protein unfolding to expose the hydrophobic groups previously buried within the structure. In contrast, when the degree of hydrolysis reached 6%, hydrophobicity decreased. This trend is consistent with the report that as hydrolysis progresses, hydrophobicity decreases gradually. This decrease can be attributed to the free hydrophobic amino acids generated by enzymatic cleavage (Zang et al., 2019b; X. Zhang et al., 2012)

In addition to modifying the emulsifying properties of proteins, enzymatic hydrolysis can induce the antioxidant activity of many proteins. As emulsions are extremely susceptible to oxidation owing to the large interfacial area created during reduction in the size of oil droplets, proteins and antioxidant peptides can be applied to inhibit the oxidation of lipids in emulsions during storage. As emulsions serve as antioxidants in food, the peptides themselves can be oxidised (Zhao et al., 2013; Chen et al., 2013; Gong et al., 2015;Zhao et al., 2013). Oxidative modification may result from the generation and accumulation of free radicals during protein preparation and storage or emulsion preparation. One of the main benefits of proteins or hydrolysed peptides used in oil dispersal products is the suppression of lipid oxidation owing to the elimination of peptide radicals and the ability to form pro-oxidative metal ions (Chen et al., 2013; Zhao & Xiong, 2015).

5. Protein conjugates

5.1 Protein-polysaccharide complex

Covalent protein-polysaccharide complexes are usually formed due to the Maillard reaction (discussed in Section 2.3). Noncovalent protein-polysaccharide complexes can be formed in aqueous solutions before homogenisation by electrostatic attraction under conditions where biopolymers have opaque charge groups (Donald, 2008), or after homogenisation by mixing a polysaccharide loaded with an emulsion containing lipid droplets coated with proteins of opposite charge (McClements, 2010).The interaction between proteins and polysaccharides has a significant impact on their properties and application in different systems (Cai et al., 2018).Proteins can interact with polysaccharides through a variety of physical and chemical mechanisms, allowing them to assemble into structures that have new functions (McClements, 2010).

Under opposing electrical loading conditions, rotaline p, which has a high net negative charge, exerts a repulsive force on the anionic polysaccharides. However, when the pH was adjusted around the pI of the protein, cationic segments in the protein interacted attractively with the anionic groups in the polysaccharide, resulting in the formation of a weak electrostatic complex (Fig 2.A). An additional lowering of the pH lead to stronger interactions between the proteins and polysaccharides, which eventually resulted in phase separation from the fully neutralised complex (Evans et al., 2013). More stable emulsions with smaller droplet sizes were observed in emulsions with a mixture of polysaccharides and proteins than in emulsions containing only proteins or polysaccharides alone. Post 24 h of storage, the emulsions containing only soy protein destabilized between pH 4-5. At pH 6 and 3, the average droplet sizes were smaller owing to the reduced repulsion of similar charges between the droplets. The stability of the emulsion was dependent on pH and concentration. Moreover, emulsion containing 0.25% of the total weight of the polysaccharides at a pH close to the protein IP exhibited a more uniform particle size and better storage stability after 28 days. In another study with zein and gum arabic, zein was found to have a pI near 6.2 and a value below the pI was considered as positive loading. When the emulsion wasmaintained at a pH of 4, a strong attraction between the gum and protein was observed, which was due to opposite charges leading to adsorption of gum arabic molecules onto the surface of colloidal zein nanoparticles. This supported the hypothesis that rubber coated the surface of the colloidal zein nanoparticles, improving the stability of the emulsion (Dai et al., 2018).

Polysaccharide and proteins can form thick interfacial films that greatly minimise contact with the droplets (Fig 2.B) (Dickinson & Galazka, 1991) .Compared to protein films, protein-polysaccharide films may have better resistance to environmental stresses such as major changes in pH, ionic strength, thermal processing, or freezing. According to Dai et al. (2018), emulsions stabilised with zein alone were unstable, resulting in the aggregation of droplets and release of oil in the upper phase of the emulsions, while the volume of the emulsified phase stabilised in the presence of a complex of gum arabic and zein increased.

In addition to their surface activity, proteins have fast interfacial adsorption kinetics, whereas polysaccharides form a thick steric layer as they protrude into the aqueous phase and provide repulsive electrostatic forces against droplet aggregation. (Yang et al., 2018). There is a wide variety of possible interactions between these types of macromolecules, including attractive non-covalent forces such as electrostatics, hydrogen bonding, hydrophobic and van der Waals forces (Semenova, 2017). Zang et al. (2019) studied xanthan gum, gum arabic, alginate, and rice bran protein. At pH 7, none of the emulsions exhibited instability, except for the emulsion with alginate, indicating that droplet aggregation occurred in the emulsion to which alginate was added, which can be attributed to the depletion mechanism (Qiu et al., 2015). Increasing the concentration of polysaccharides did not favour their adsorption, and thus enhanced the attractive osmotic forces between the oil droplets (Fig 2.C). When the critical polysaccharide concentration exceeds, presence of excess free polysaccharide in the solution promotes the coalescence of the droplets owing to the depletion mechanism (Fig 2.D). The critical concentration of each polysaccharide depends on the number and size of polymer molecules (Dai et al., 2018).

For example, due to the high degree of branching of gum arabic, its molecules are relatively small (low hydration radius). Therefore, these molecules were not present at concentrations high enough to cause depletion flocculation. However, xanthan gum contains homogeneous emulsions without cream formation, even at relatively high concentrations (Xu et al., 2016). Xanthan gum has a high charge density, stiffness, and molecular weight, resulting in stronger electrostatic and steric repulsions (Qiu et al., 2015; A. Kumar et al., 2018). Pectin and xanthan gum were able to stabilize the rice protein-coated droplets better than gum arabic and alginate. These effects are mainly attributed to the different properties of the polysaccharides, such as charge density, branching, lateral chain length, and stiffness. Emulsions stabilised with pectin or xanthan gum are tolerant to environmental changes such as pH and NaCl concentration (Zang et al., 2019).

The incorporation of anionic polysaccharides alongside proteins leads to positive or negative effects on emulsion stability, depending on protein-polysaccharide interactions. When a mixed viscoelastic protein-polysaccharide film is formed at the oil-water interface, a stable emulsion is formed (Zang et al., 2019). When evaluating different polysaccharides and lentil protein, Zang et al. (2019) found that carrageenan was the only one that exhibited better emulsion stability than lentil protein alone. In contrast, addition of carboxymethylcellulose, gum arabic, and alginate resulted in a decrease in emulsion stability compared to lentil protein alone, in descending order after 30 min. Moreover, emulsion stability can be affected by viscoelastic film coverage, film loading, biopolymer/aggregate size/conformation, and continuous phase viscosity, which prevent coalescence, leading to gravity segregation to produce a stable emulsion. To create a stable emulsion, surface-active biopolymers must migrate, adsorb, and integrate into the oil-water interface to reduce the interfacial tension at the interface, thus enabling the biopolymers to position hydrophobic amino acids towards the oily phase and hydrophilic spores towards the aqueous phase. The rate at which biopolymers migrate and the ability of biopolymers to realign at the interface greatly affect emulsion formation, droplet size and distribution, and emulsion stability (McClements, 2007; Zang et al., 2019).

Emulsion oil/water stabilised with soy protein and gum arabic at concentrations above 2% (w/w) had a larger droplet size, which may be due to competitive adsorption between soybean protein and gum. The hydrophilic sections of the carbohydrates of gum arabic oscillate, and the hydrophobic polypeptide chains are adsorbed at the oil-water interface. This competitive emulsifying activity of the gum leads to the creation of a less rigid and viscoelastic film around the oil drops, resulting in a larger drop size. The emulsions stabilised by linseed and gum had a much smaller drop size than the emulsions stabilised by soybean using the same concentration of gum. However, when the concentration of this polysaccharide was greater than 2%, stability of the emulsion diminished. This may be due to the destabilisation of emulsions, which can be attributed to flocculation and depletion (B. Wang et al., 2011).

S. Wang et al., (2020) showed a wide distribution and a substantial increase in droplet diameter for all emulsions (pH=7) after 30 days. Post 24 h of storage the emulsion prepared only with the polysaccharide extracted from the soybean hull showed the highest particle diameter compared to the emulsion that contained the polysaccharide and soybean protein (0.05–0.15% by weight of polysaccharide). This phenomenon may be related to the steric repulsion formed by electrostatic attraction between the positively charged fragments in the protein molecules and anionic groups of the polysaccharide when the concentration of this was low (Dickinson, 1999). With an increasein the concentration of polysaccharide, the particle size of the emulsion increased, and apparent aggregation occurred when the concentration of the polysaccharide exceeded 0.5% by weight. This could be caused by increased viscosity of the real medium or by the flocculation mechanism of the unabsorbed polysaccharides in the continuous phase when the concentration of the soybean polysaccharide was present in excess. When storage time increased to 30 d, van der Waals and hydrogen bonds between proteins became dominant, leading to self-assembly of (Huang et al., 2019; Xu et al., 2016) the soybean isolates, resulting in increased particle size and the amount of emulsion distribution peaks without the polysaccharide (Tran & Rousseau, 2013).

5.2 Protein- polyphenol complex

Protein–polyphenol conjugates have been widely used to improve the stability of food emulsions owing to their excellent emulsifying properties and antioxidant activities. Protein–polyphenol conjugates have been reported to exhibit various functionalities such as gelling, foaming, and emulsifying properties (F. Liu et al., 2017) Proteins and polyphenols can interact through non-covalent (hydrophobic, ionic, and hydrogen) or covalent bonds. These can participate in non-enzymatic alkaline reactions, free radical grafting, or enzymatic processes. Conjugates formed by covalent bonds have stronger and more durable interactions, in addition to high stability and are more commonly used in the food industry(F. Liu et al., 2017).

In general, protein and polyphenol interactions contribute to better emulsifying properties; however, the method used, concentration, and nature of the polyphenol directly influence the emulsifying properties of the conjugates(Pan et al., (2022) studied hydrolysed rice protein–chlorogenic acid using three methods: alkaline, free radical, and enzymatic.

Conjugated proteins showed better emulsifying and stabilising activities than proteins alone. The same behaviour was observed in the pea protein–gallic acid conjugate (M. Zhang et al., 2023) and 7S/11S–rutin protein (Jia et al., 2022). The covalent interaction causes the protein structure to unfold, resulting in the exposure of many aromatic residues, also increases the affinity of the proteins for the oil/water interface, which improves the emulsifying activity of the proteins. The emulsifying activity of the conjugate prepared by the alkaline method was higher than that of the other conjugates due to the higher content of unfolded and flexible structures in the conjugate. The conjugates prepared by the enzymatic method showed the lowest levels of free amino, thiol, and tyrosine groups among all the covalent conjugates, the lowest emulsifying activity, the lowest fluorescence intensity, and the highest β-sheet content.

Yan et al., (2022)studied and compared 3 methods for the formation of protein conjugates and phenolic compounds using soy protein and gallic acid: (a) alkali treatment, (b) free-radical induction, and (c) carbodiimide hydrochloride (EDC)/N–hydroxysuccinimide (NHS)-mediated synthesis. The results confirmed that the average diameter of the particles in the emulsions stabilised by the protein-phenol compound conjugates is significantly smaller compared to the native protein, indicating that the conjugation of the protein with the phenol compounds improves its emulsification.Particle aggregation was observed in the protein-only stabilised emulsions, possibly because of the rigid structure of soy protein. Alkaline treatment promoted the oxidation of gallic acid, favouring polyphenol-protein interactions and suppressing tryptophan fluorescence. Pham et al.,(2019) found that flaxseed protein and hydroxytyrosol (HT) conjugates decreased particle size. However, the conjugate of flaxseed protein and flaxseed polyphenol resulted in an increase in particle size that was even larger than that of the native protein.The formation of covalent sulfide bonds has been found to affect the particle size of the combinations (G. Sun et al., 2019), demonstrating that the response of the conjugates depends on the nature of the polyphenol and the protein (Yan, Yao, et al., 2022).

The concentration of the polyphenol used can also influence its behaviour in the conjugate. M. Zhang et al., (2023) observed that when the polyphenol concentration increased, the particle size of the conjugates became larger than that of the native protein. Pan et al. (2019) found an increase in the particle size of the complexes owing to the high concentrations of polyphenols in binding rice protein hydrolysates and chlorogenic acid. Undesirable changes in protein structure have also been observed in pea protein binding and high catechin (R. Li et al., 2020)Yan, Xu, et al.,(2022) studied soy protein tea saponin (TS) and hexadecyltrimethylammonium bromide (CTAB) at concentrations of 0.01–1.00%, w/v. The smallest particle sizes were observed for the protein–TS 1% protein conjugate, whereas CTAB presented better emulsions at 0.1% concentration. CTAB may have a significant positive charge, which causes electrostatic interactions to be neutralised, resulting in lower drop-to-drop repulsion (Niroula et al., 2021) et al., 2021). Above 0.1% w/v, CTAB resulted in flocculation. T. Zhao et al., (2021) observed the opposite effect of anchovy protein conjugates, Tannin and Catechin. To improve the emulsifying activity of native anchovy protein, the tannin and catechin concentrations must be 15:1; higher values have not been tested.

When the emulsions prepared with polyphenol-protein during storage were analysed, it was observed that, after 7 days, the average particle size of the emulsion stabilised by the soy protein and phenolic compound conjugates increased slightly, while that of the emulsion stabilised by protein alone was significantly larger. Polyphenols favour the slow adsorption of proteins at the oil–water interface for a prolonged period, thus increasing the physical stability of the emulsion. This may be due to the covalent binding of oxidised phenolic compounds at the interface with reactive amino acid residues, forming a stable network structure to stabilise the emulsion (Yan, Yao, et al., 2022). Yan, Yao, et al., (2022) found that after seven days of storage, the emulsion stabilised by the 1% protein– gallic acid (GA) conjugate changed less from the first day. TS molecules were adsorbed into the interfacial pores formed by proteins at low concentrations, which helped maintain emulsion stability (S. Xu et al., 2019) Excess GA molecules diffused into the interfacial pores between the protein molecules. Protein desorption at the interface interrupted bridging flocculation between droplets and separated them thus, the GA initially adsorbed on the protein surface became desorbed after prolonged storage, changing charge surface and interfacial wettability .

The protein–polyphenol interaction is an interesting modification because, in addition to improving the emulsifying properties of the protein, it can prevent lipid oxidation at the oil–water interface. Furthermore, the protein–polyphenol interaction is not only particularly affected by the structure of the polyphenol but also by the surface properties of the proteins, concentration, and method of oxidation. Thus, the same protein can present better emulsifying and surface properties when associated with certain polyphenols or can reduce their activity.

6. Conclusions

This review, it was shown how altering the structure of plant-based proteins can improve their emulsification properties, favouring the use of plant-based proteins as natural emulsification alternatives. Beneficial changes in protein structure, such as increased conformational flexibility, can affect the rate of structural reorganisation of the adsorbed protein at the interface, which in turn affects steric hindrance between droplets and alters the stability of the protein emulsion. Protein modification can result in the hydrophobic amino acids being most exposed, which lowers the energy barrier for adsorption at the air-water interface, thereby increasing adsorption kinetics at the surface, allowing better adhesion at the oil-water interface and improving protein solubility. These results suggest that vegetable proteins can be used as food ingredients not only for their nutritional function but also as emulsifiers. These proteins can partially or even completely replace artificial emulsifiers and stabilisers, thus meeting the demand of manufacturers for products targeting different consumers, including vegans, vegetarians, people with intolerances and allergies to animal and synthetic proteins. However, due to their specificity, i.e. the amino acid composition, amphiphilic nature, structure and conformation of each plant source, the desired properties of the product must be determined in advance in order to select the best protein source and modification method to achieve the ideal emulsification properties. Many studies have investigated the stability properties of the emulsion on the first day and a few days later, which requires prolonged monitoring of the emulsion. It was found that the behaviour of proteins conjugated to another molecule changes over the days of processing, the same behaviour was observed with concentration. Therefore, it is necessary to validate the results over several days or by accelerated assays to obtain a better result for a given protein. The combination of methods is also an alternative to improve the functionality of plant proteins.

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Conflicts of interests

The authors declare no conflict of interests.

Data availability

Data will be made available on request.

7. References

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Table 1. Compiled studies in which different methods typical methods of physical, chemical and enzymatic modifying plant-based proteins improved their ability to form and stabilize emulsions.

Emulsifier	Protein modification method	Emulsifier dosage	Formulation of the emulsion studied	Mechanical process applied to emulsion	Effect observed on emulsion	Reference
Soybean peptide nanoparticles	Heat treatment	0.5% (w/v)	10%(w/w) oil 90% (w/w) continuous phase	Homogenisation at 8.000 rpm for 2 min, followed by 40 MPa two times	Better inter-particle interactions Particles distributed on the surface of the droplet Greater stability against coalescence	Zhang et al. (2020)
Rice bran	Enzymatic hydrolysis	1% (w/w)	10% (w/w) corn oil 90% (w/w) hydrolysed suspensions	Homogenisation at 10.000 rpm for 2 min, followed by two times at 80 MPa	Good storage stability of the emulsion	Zang et al. (2019)
Zein	Enzymatic hydrolysis	1g/L	Soy oil in ratio of 3:1	Homogenisation at 12.000 rpm for 1 min	With the increase hydrolysis degree. the flexibility of protein enhances, and protein	A. Q. Zhang et al. (2022)

					structure unfolds. The oil-water interface tension reduces.	
Rice glutelin	Enzymatic hydrolysis-ethanol	4g/100mL	Water phase to corn oil phase was set to 9:1	Homogenisation at 10000 rpm for 5 min, sonicator operated at 450 W for 5 min in an ice bath.	Flocculation of droplets is lower in emulsions stabilized by rice proteins when hydrolysis was induced by 50 and 75 % ethanol	Yang et al. (2023)
Peanut	Microwaves and ultrasound	7 g/100 mL water	100 mL water 100 mL soybean oil	Homogenisation at 2.000 rpm for 10 min	There was no synergistic effect. Microwaves improved emulsification and foaming characteristics	Ochoa-Rivas et al. (2017)
Peanut	Spray and freeze drying	0.1% (p/v)	30 mL solution (water and protein) 10 mL oil	Homogenisation at 10.000 rpm for 3 min	Emulsions produced using lyophilised proteins were more stable.	Gong et al. (2015)

Quinoa	Spray, freeze, and vacuum drying	1.75 g/50 mL	25 mL water 25 mL soybean oil	Homogenisation at 10.000 rpm for 3s (water and protein), following another round for a further 3s after oil addition	Lyophilisation was better than the three techniques and improved technological properties of the protein.	Shen et al. (2021)
Pea	Drying and spray drying	3% (w/w)	Distilled water 15% (w/p) soybean oil	Homogenisation at 1.000 rpm for 1 min, followed for 3.000 psi	Better solubility and emulsifying capacity	Burger et al. (2022)
Soy	Cold plasma	0.5% (w/v)	10 mL canola oil	Homogenisation at 13.000 rpm for 5 min	Treatment with up to 18 kV reduced interfacial tension. However, stability of the emulsion decreased.	Sharafodin & Soltanizadeh, (2022)
Pea	Cold plasma	1.0% (w/v)	25 mL solution (10 mM phosphate buffer and protein)	Homogenisation at 17.000 rpm for 3 min	Stability of emulsions stabilised with protein treated at 18.6 kVpp for 60 s was higher.	Mahdavian, Mehr & Koocheki, (2020)

			5 mL corn oil			
Soy	Electrolysis	1.0% (w/v)	90 mL solution (10 mM phosphate buffer and protein) 10 mL corn oil	Homogenisation at 8.000 rpm for 5 min, followed by 80 MPa	The method altered protein structure and improved the ability to retain oil	Yu et al. (2018)
Avocado	Alkaline extraction	0.5, 1.0, 1.5, 2.0% (w/w)	10 mL phosphate buffer 20 mmol /L (pH 7.0) 1 mL soybean oil	Homogenisation at 6.000 rpm for 5 min, followed by 500 bar	Improved stability of the oil emulsion system in water.	J. S. Wang et al. (2019)
Mushroom	Extraction with pH change	5, 10, 15, 20 mg/mL	20 mL suspension 0.5% (w/w) corn oil 20 mL suspension [0.1–0.8% (w/w) corn oil]	Homogenisation at 10.000 rpm for 1 min	Improved protein solubility after extraction at alkaline pH. Stable emulsions for 30 days	Fan et al. (2021)
Fava	High-pressure homogenisation	10 mg/mL	2 mL canola oil 6 mL protein solution (10 mg/mL, pH 7)	Homogenisation at 25.000 rpm for 2 min	Emulsifying capacity and emulsion stability decreased with increasing	Yang et al. (2018)

					homogenisation pressure	
Cyperus esculentus L.	High-pressure homogenisation	2%(w/v)	10% soybean oil	11.000 rpm for 3 min, followed by 0 60, 80, 100, 120 and 140 MPa	The untreated protein had the lowest emulsion stability and then gradually increased with homogenization pressure at 100 MPa.	A. Zhang et al. (2022)
Millet	Ultrasound	1% (w/w)	10 mL vegetable oil	Homogenisation speed 5 of a rotor–stator homogenizer for 2.5 min	Increased tendency of protein adsorption at the oil–water interface after treatment at 73.95 W·cm^{-2} for 12.5 min	Nazari et al. (2018)
Rice	Ultrasound-assisted alkali	1% (w/v)	6 mL of protein solution (pH 7.5) 2 mL olive oil	Homogenisation at 10.000 rpm for 1 min	The emulsifying activity of proteins increased with increasing alkaline concentration	Qu et al. (2018)

Rice	Oxidation	1% (w/v)	Aqueous protein solution, volumetric oil fraction (φ) from 0.25	Homogenisation at 6 MPa	Moderate oxidation favoured the increase in emulsifying capacity and emulsion stability	Wu et al. (2020)
	Oxidation					
Wheat germ	Electron beam irradiation	1% (w/v)	30 mL solution (pH 7.0) 10 mL soybean oil	Homogenisation at 10.000 rpm for 1 minute	Protein stability and emulsifying activity increased up to 10 kGy; larger doses produced the opposite effect.	Li Wang et al. (2019)
Black gram by-product	Ultrasonication and micronisation	1% (w/v)	5 mL sunflower oil 15 mL	Homogenisation at 14.000 rpm for 1 min	Ultrasound treatment for 5 min improved emulsifying activity and emulsion stability. Micronisation at 110°C improved emulsifying activity	Kamani et al. (2021)

Rice bran	Radio frequency	2.0% (p/v)	15 mL solution (protein and water) 15 mL soybean oil	Homogenisation at 10.000 rpm for 2 min	Treatment at 100°C increased emulsion stability and emulsifying capacity	Ling et al. (2019)
Lentil	high-pressure	2,5, 5,0 e 10 g/100 g	5g/100g soybean oil	Homogenisation at 10.000 rpm for 5 min followed by 50, 100, 200 and 300 MPa were applied with 1, 2 and 3 passes	Best nanoemulsion stability was achieved with 1:1 protein:oil ratio and 300 MPa.	Munizaga et al. (2019)
Coconut	non-enzymatic deamidation using temperature and HCl concentration	0,5 g/100 mL	10 mL palm oil 30 mL suspension	Homogenisation at 22.000 rpm for 1 min	The optimal conditions to obtain Emulsifying properties were a temperature of 75 °C and HCl concentration of 0.3 mol/L.	Meenmanee et al. (2022)
Soy	Preheating and	5 mg/mL	corn oil at a 4:1	homogenization at	The Emulsifying	Y. Jiang et al.

	enzymatic deamidation		ratio	13.500 rpm for 2 min	activity and emulsifying stability improved with increased heating intensity and deamidation except for preheating at 121 °C.	(2022)
Soy, Mung bean, Pea	Xanthan; Gellan complex	1% (w/v) protein 0.1% (w/v) polysaccharide	10% (v/v) soybean oil	Homogenization at 10.000 rpm for 2 minutes followed by homogenization 45 Mpa for 5 minutes	Xanthan gum conjugates and mung beans / gellangum and soybean showed more stable emulsions.	Zhang & Li, (2022)
Hemp	Ultrasound and alkali treatment (Gallic acid; Catechin)	10 mg/mL	10 mL corn oil, 30 mL sample solution	Homogenization at 18.000 rpm for 5 min followed by ultrasonicated for 10 min (500 W; 20 kHz)	Conjugation with polyphenol significantly improved solubility and emulsifying properties. Protein-gallic acid	M. Zhang et al. (2023)

					conjugates produced through ultrasound had better functional properties.	
Soy	Covalent-alkaline (Galato de (-)-epigalocatequina; Quitosana)	1.4 % (w/v) proteins 0, 0.25, 0.5, 1, 2% (w/v) polyphenol	10% (w/v) soybean oil	Homogenization at 13.600 rpm for 5 min	the interaction between polyphenol and protein improved the properties at a concentration of 0.15%. The addition of chitosan enhanced the emulsifying effect of the conjugate	Yan et al. (2021)

Figure captions

Fig. 1. Oil emulsion formation scheme in water, with and without the addition of proteins as emulsifier, and emulsion stability after rest (storage).

Fig. 2. Schematic representation of protein-polysaccharide application in an oil/water emulsion: protein-polysaccharide interaction (A), protein-polysaccharide film stabilized emulsion (B), protein-polysaccharide interaction with excess polysaccharide (C); stabilized emulsion with excess protein and polysaccharide (D)

Figure 1.

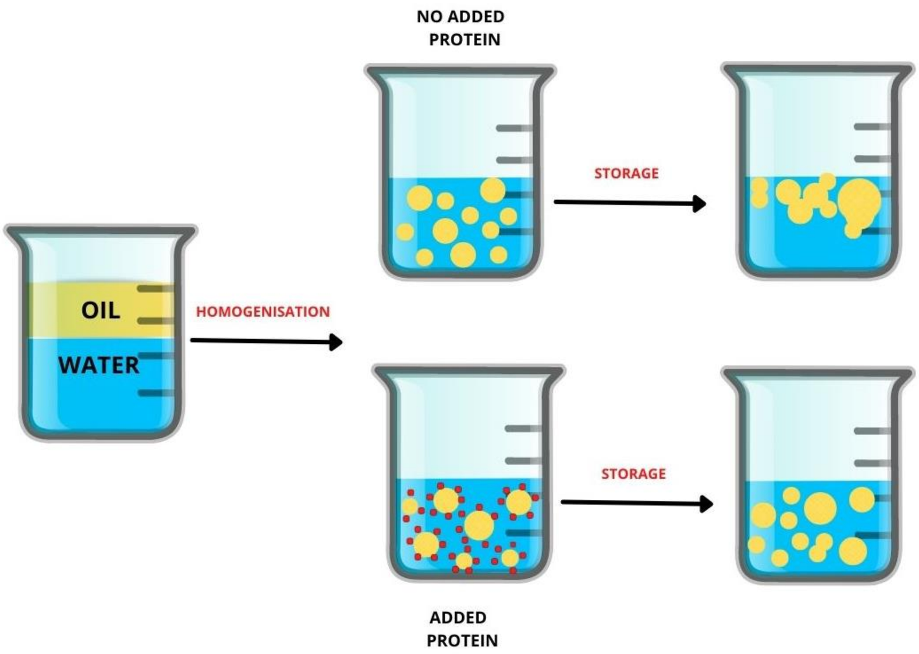
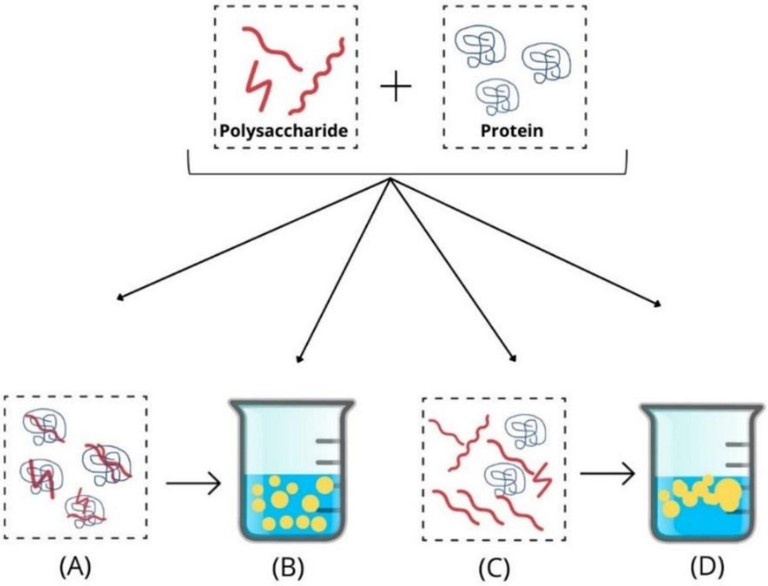


Figure 2.



3. CHAPTER 2

Synergism between different plant-based proteins, their effect on emulsifying properties applied to oil/water type model emulsions

Abstract

This study investigated the isolated and synergistic effects of three protein concentrates from legumes (pea, lentil and lima bean) as emulsifiers and stabilisers of oil-in-water emulsions using simplex- centroid mixture design. The aim was to check whether proteins combined in different proportions have better emulsifying properties than isolated proteins. During the study, each protein concentrate was characterised by different evaluated parameters. After statistical analysis of desirability, it was found that the best formulation for stabilising an oil-in-water emulsion under the conditions tested (2% total protein; 3% sunflower oil) was the protein blend containing 21.21% pea, 32.78% lentil and 46.01% fava bean. The blend exhibited better emulsification properties compared to the individual proteins. Synergism was found to be a way to improve the emulsification properties of plant proteins and these results can serve as a basis for further research into new natural emulsifiers.

Keywords: Protein mixtures, pea protein, lentil protein, fava bean protein, response surface

1. Introduction

The growing interest in protein-based products, such as beverages, has stimulated research and development activities to explore different plant protein sources with emulsifier function. The use of plant proteins as ingredients in product formulations is extremely attractive due to their functionality, ecology, nutritional value and sustainability (Jeske et al., 2017; McClements & Grossmann, 2021).

Soy protein products (concentrates or isolates) are commonly used for plant protein-based emulsifiers, but due to allergen concerns, the industry is looking for other alternatives. (Huang et al., 2019; Ray & Rousseau, 2013; Tran & Rousseau, 2013; L. Wang et al., 2017).Emerging protein ingredients include those derived from peas, lentils and fava beans. The use of legume proteins is quite justified as the food industry is very interested in replacing synthetic proteins in foods to produce healthier products. Several studies have investigated the use of fava bean, lentil and pea proteins as emulsifiers and demonstrated their potential to stabilise oil-in-water emulsions (Ghumman et al., 2016; Ladjal-Ettoumi et al., 2016a; C. Li et al., 2014; Sharan et al., 2021).

Pulses contain large amounts of protein, usually between 20 and 40%, and are a rich source of essential amino acids such as leucine and lysine (Jiang et al., 2014; Stone et al., 2015; Tang & Shen, 2013). The major proteins in lentils are globulins (~50 %) and albumins (~16.8 %), both of which are considered globular proteins . Globulins consist of vicillin-like trimeric proteins (175-180 kDa) and legumin proteins (300-370 kDa) with sedimentation coefficients of 7S and 11S, respectively. In contrast, proteins from the albumin fraction have a lower molecular weight (5-80 kDa) and are considered compact globular proteins (Boye et al., 2010; Shewry et al., 1984) .Similar to other protein-based emulsifiers, these proteins act by diffusion at the interface, realign with the interface for better binding, and then form a viscoelastic film to stabilise the oil droplets by charge repulsion at pH values away from the isoelectric point (pI) of the protein or by steric stabilisation (Wanasundara, 2011). However, since plant proteins have low solubility compared to animal proteins, mechanisms to modify the protein structure and improve its technofunctional properties are being investigated (Alves & Tavares, 2019).

Studies show that combining vegetable protein with other macromolecules such as gum, pectin, polyphenols or combining vegetable protein with animal protein enhances the emulsifying properties of vegetable protein. The association of pea protein and whey showed that there were profound differences in the interfacial properties between the proteins alone and their mixtures, and that these were dependent on the order of adsorption (Grasberger et al., 2022). Another study using milk proteins and pea protein found that mixing pea proteins with milk proteins increased emulsion stability (Hinderink et al., 2019); pea proteins also increased the elasticity of casein-stabilised interfaces (Hinderink et al., 2020);Whey-plant and plant-plant protein blends behaved synergistically leading to enhanced emulsion stability (Ho et al., 2018). However, little is known about the synergistic effect of different plant sources on the stability of oil-in-water emulsions.

The aim of the present study was to investigate the isolated and synergistic effect of protein concentrates from legumes (peas, lentils and broad beans) on the formation and stabilisation of oil-in-water emulsions. Our hypothesis is that combined proteins have better emulsifying properties than separate proteins. The proportions of the proteins tested were composed according to a simplex centroid mixture design and the desirability function was used to optimise the formulations (Hayati et al., 2016; Zhang et al., 2022).

2. Materials and methods

2.1. Materials

Pea protein concentrate 52% (w/w), lentil 53% (w/w) and fava bean 56% (w/w) were purchased from Ingredion®. Sunflower oil was purchased from a local supermarket (Juiz de Fora, Brazil). All chemicals were of analytical grade.

2.2 Simplex-centroid design

The experiment was designed according to a simplex-centroid delineation and consisted of three of individual protein and seven groups of protein blends.The component proportion of blends is illustrated in Table 1.The experimental domain of this research consisted of different proportions of components of X1- Pea protein concentrate (PPC), X2- lentil protein concentrate(LPC), and X3 - Fava bean protein concentrate(FPC) between zero and one ($0 \leq Xi \leq 1$; $\sum Xi = 1$). All emulsions with respective blends were prepared in two independent replications in order to allow error estimation (Hayati et al., 2016).

Table 1: Sample codes and experimental design.

Emulsion code	Component proportion		
	Pea (%)	Lentil (%)	Fava bean (%)
T1	100	0	0
T2	0	100	0
T3	0	0	100
T4	50	50	0
T5	50	0	50
T6	0	50	50
T7	50	25	25
T8	25	50	25
T9	25	25	50
T10	33.33	33.33	33.33

2.3 Emulsion preparation

To prepare O/W emulsions, protein concentrates (2.0 %, w/w) were dissolved in a solution of filtered water at 45 ℃ and sunflower oil (3.0 %, w/w) under 8.000 rpm for 2 minutes in a homogeniser (Silverson L4RT) followed by homogenisation (APV homogeniser) at 20 MPa (15/5 first and second stage). For all emulsions, the pH was adjusted to 7 with 0.1 M NaOH.

2.4 Size and ζ-potential

ζ-potential was measured according to the method of (H. Li et al., 2021), by using Zetasizer Nano ZS90, (Malvern Instruments, city, Country). Before measurement, the emulsion was diluted 500 times with 10 mmol/L phosphate buffer (pH 7.0). Each record was scanned twelve times, and three repeated measurements were taken for each sample.

The emulsion particle size measurement method was based on H. Li et al., (2021) with some modifications. The particle polydispersity index (PDI) and size of the emulsion droplets dispersed in deionized water and 10 mg/mL sodium dodecyl sulfate (SDS) after storing for 4 h and 24 h were determined using a static polyangular dispersion particle size analyser (Zetasizer Nano ZS90, Malvern Instruments) The refractive index of soybean oil was 1.474 and the imaginary part of the refractive index (due to absorption) was fixed at 0.001.

2.5 Emulsion flocculation index measurement

With reference to the method of H. Li et al., (2021) the d4,3 values were acquired by using deionized water and SDS (10 mg/mL) as the dispersant were expressed as d4, 3-SDS and d4, 3+SDS, respectively. During the research, all emulsion samples were diluted with deionized water or SDS (10 mg/mL) solution to avoid the influence of multiple scattering. The flocculation index (FI) of the emulsion was evaluated after 0 or 24 h of static storage. The FI was calculated by the following formula:

(1) $FI\ (\%) = [(d_{4,3\text{-}SDS}) / (d_{4,\ 3+SDS}) - 1.0] \times 100\%$

2.6 Flocculation and coalescence measurement

The calculation method of the stability of coalescence and flocculation ((C + F) %), coalescence (C%) and flocculation (F%) of the emulsion during storage for 4–24 h was based on the method of Tang et al. (2013). Using the following formula:

(2) $(C + F)\ \% = [(d_{4,\ 3\text{-}SDS,\ (24\ h)} - d_{4,\ 3+SDS,\ (0h))} / d_{4,\ 3+SDS,\ (0\ h)]} \times 100\%$

(3) $C\% = [(d_{4,\ 3+SDS,\ (24\ h)} - d_{4,\ 3+SDS(0h)}) / d_{4,\ 3+SDS,\ (0h)]} \times 100\%$

(4) $F\% = (C + F\%) - C\%$

2.7 Emulsion Activity Index (EAI) and emulsion stability Index (ESI)

Protein emulsification activity was determined according to the method by Du et al., (2018) with some modifications. The protein sample (30 ml with a concentration of 2% (w/v)) was dispersed in 10 ml of soybean oil. The mixture was homogenized (Ultra-Turrax IKA

T25) at 15.000 rpm for 2 min. Then, 200 µL of the emulsion sample was immediately mixed with 25 mL of 0.1% SDS and the absorbance (Shimadzu 1800 UV-Vis Spectrometer) of the solution was read at 500 nm. Emulsifying activity index (EAI, expressed in m2/g) and emulsion stability (expressed in min) were calculated using Eqs. (5), (6), respectively.

(5) EAI (m2/g) = [2x 2.303 x A_0/ 0.25 x protein concentration]

(6) ESI (min)= [A_0 x Δ_t/ Δ_A]

where, A_0 = absorbance at 0 min, A_{10} = absorbance at 10 min, $\triangle A = A_0 - A_{10}$ and $\triangle t$ = 10 min.

2.8 Determination of centrifugal stability constant

The measurement of the emulsion centrifugal stability constant (KE) was based on the method of H. Li et al. (2021) with appropriate modifications. The emulsion was centrifuged for 10 min (3000 g), and 50 µL lower layer of the emulsion before and after centrifugation was taken and then diluted with distilled water in a 25 mL volumetric flask. Distilled water was used as a blank and the absorbance at 500 nm was measured, and A_0 and A were recorded. The following formula was used to evaluate the centrifugal stability of the emulsion:

(7) KE (%) =(A_0-A)/A × 100

2.9 Emulsion stability high temperatures

Emulsion stability (ES) to high temperatures was determined following the methodology of Rahmati et al., (2018) with some modifications. Emulsions were heated to 80 °C for 30 min and centrifuged at 1000 g for 10 min. ES was calculated using formula:

(8) ES= (F_{eh}/I_{eh}) x 100

where F_{eh} is the final emulsion height and I_{eh} is initial emulsion height.

2.10 Determination of heat coagulation time (HCT)

Heat stability was determined according to the oil bath method proposed by Dumpler & Kulozik, (2015) with some modifications. 10 ml of the samples were placed in a 50 ml glass tube with screw cap (Schott). The samples were immersed in an oil bath at a constant temperature of 140 °C so that the sample level was equal to the oil bath. The sample was shaken continuously and the time in minutes taken for the sample to coagulate was recorded.

2.11 Optimization design

Mixed regression analysis was applied to the data to determine the estimated coefficients and significance of the model terms, the F-test and the coefficient of determination (R^2). The results were first fitted to all available mixture regression models of increasing complexity, from linear to special cubic models. Model significance, lack of fit significance and adjusted R^2 value were used to assess the adequacy of model fit. The adjusted R^2 value describes the proportion of variation in responses explained by the model, and the value was adjusted for the number of terms in the model. The models were then reduced (if any), leaving only significant terms. Finally, it was found that the responses for the analysis were best fitted using a quadratic model or the special cubic model (Cornell, 2002):

$Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_2+\beta_{23}X_2X_3$ (Quadratic)

OU

$Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_2+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3$ (Special Cubic)

Where Y is the predicted response of the analysis, β_1, β_2 and β_3 are the estimated coefficients of the individual linear effect terms, β_{12}, β_{13} and β_{23} are the binary interaction effect terms, and β_{123} is the ternary interaction effect term. Once the estimated model equation for each response was obtained, contour and surface plots were generated.

The simultaneous optimisation of the response variables was performed using the desirability function approach, which is the most popular method for solving optimisation problems with multiple quality features (Derringer & Suich, 1980) This technique consists of converting each response variable into an individual desirability function that varies in the range 0-1. A value of 0.0 was assigned to the predicted values of the response variable outside the region of interest, and a desirability value of 1.0 corresponded to the response variable when it reached its target. The most influential response variables for emulsion stability were selected and thus the desirability function was applied. After applying the desirability function, a new ternary mixture was used to produce new emulsions (Three repetitions were performed. n = 3). Finally, the model was validated by comparing the average responses of these emulsions with the respective value predicted by the model equations.

2.12 Statistical analysis

All tests were performed in triplicate. A one-way analysis of variance (ANOVA) with Tukey's Multiple Comparisons (family error rate) was applied to the data to determine a significant difference ($p < 0.05$) between emulsions. the experimental design, data analysis, contour and surface plots and simultaneous optimization of the response variables was performed were developed using a Minitab ™ 19(Minitab Inc., EUA) statistical software package. The level of confidence used was at $\alpha = 0.05$. (Estevão et al., 2020; Hayati et al., 2016).

3. Results and discussions

3.1 Surface characteristics

The surface charges (ζ-potential) of all protein mixtures at pH 7.00 are presented in Table 2. In all cases, the proteins carried a net negative charge at neutral pH. The measured surface charge values were comparable to those reported in the literature for pea protein -20 to -30 mV (L. Cui et al., 2020); -20 to -22mV (Stone et al., 2015); -25mV (Wei et al., 2020). Lentil protein -25 to -30 mV (Lee et al., 2021); -30 mV (Tabilo-Munizaga et al., 2019); -30 mV(Galvão et al., 2022). Fava bean protein -22 mV (Felix et al., 2018); -20 mV (Choe et al., 2022); -28.3 mV (Liu et al., 2019).

The surface charge values determined in this work are in agreement with the literature for legume proteins. The T9 (-32.15 ± 1.27 mV) showed higher surface charge values than the samples of the individual proteins found in this work and also in the literature. It can be observed that the association between the proteins improves the surface charge of the mixtures, as the mixed proteins have higher negative charges than the protein alone. For example, pure lentil protein (T2=-23.77 ± 2.33) in association with pea or fava protein showed higher surface charge (T4=-26.75 ± 2.34 and T6=-28.05 ± 1.20).

Primozic et al., (2017) relates ζ-potential to solubility and hydrophobicity, i.e. the more negative the charges, the greater the solubility of the protein and the lower the hydrophobicity of the surface. Chang et al., (2015) found that protein solubility is directly related to charge, suggesting that proteins with higher charge are more soluble. Karaca 2011 also studied proteins from peas, lentils and broad beans and found a correlation between surface charge and solubility, but inversely to hydrophobicity. The solubility of proteins is important because proteins, when well dispersed and in solution, generally have good emulsifying properties and can easily migrate to the oil-water interface.

3.2 Emulsifying properties

The emulsion activity index (EAI) and emulsion stability are two key parameters for the emulsification properties of proteins. EAI is a measure of the ability to form the emulsion produced by the protein, while emulsion stability is a measure of the ability of the protein to form a stable emulsion within a given time period (Boye et al., 2010; Z. Cui et al., 2014). The emulsion activity and emulsion stability of the different protein mixtures are shown in Fig. 1. Treatment T2 with 100 % lentils showed the highest emulsifying activity, followed by T4 and T8, which have a higher proportion of lentils in the formulation. The emulsion stability was affected by fava bean protein. The treatments T3, containing 100% fava bean protein, and T9, containing a higher percentage of fava beans in its formulation, showed the best results.

The emulsifying properties of plant-based protein are determined by its amphiphilic nature. Balanced hydrophobicity and hydrophilicity of proteins are required for good adsorption at the interface between the aqueous and oil phases (Shao & Tang, 2014) .Protein adsorption at the interface generally occurs in two stages. First, the proteins migrate and bind at the oil-water interface (McCarthy et al., 2016). Once the protein molecules are transported and bound to the interface, their hydrophobic part promotes adsorption. Lentil protein has a higher hidrophobicity (Karaca et al., 2011) than pea and fava bean protein, which could explain its greater activity at the surface to form the emulsion, however its stabilising effect is low Chang et al., (2015) observed that pea protein was able to stabilise the emulsion longer than lentil protein, it was hypothesised that this was due to the high negative charge on the surface of the protein coating the oil droplets, in addition to the vicillin protein in the globulin fraction of pea shows better emulsion stability than albumins due to the high surface hydrophobicity of the vicillin fraction (Ghumman et al., 2016).

Previous studies with pea, lentil and bean proteins at pH 7 gave EAI and ESI values for fava beans 8 m^2/g; 14 (Choe et al., 2022), 28 m^2/g; 25 min(Yang et al., 2018). Lentil protein 18 to 25 m^2/g; 12 to 18 min (Lee et al., 2021). Pea protein 13.1 to 14.1 m^2/g ; 53 to 95 min (Shevkani et al., 2015), 19.3 ± 0.2 m^2/g; 61.6 ± 0.2min (F. Wang et al., 2020). The values found in this work are consistent with those found in the literature. Blends can improve both emulsifying activity and emulsion stability. Lentil protein in combination with pea protein showed better ESI average values, while T5, a combination of pea and fava protein in equal amounts, showed lower average values than pea and fava protein alone.

The T10 treatment (Figure 1) showed better results in both the emulsifying and stabilising effects of the final emulsion. The combination in equal proportions favoured both EAI and ESI. Lentil proteins are believed to be responsible for the rapid adsorption at the interface. These proteins contain a considerable amount of polar and hydrophilic amino acid residues such as glutamine/glutamic acid, asparagine/aspartic acid, lysine and arginine (Lee et al., 2021), which contribute to favourable hydrophilic interactions between protein and water molecules such as hydrogen bonds and electrostatic interactions (Jarpa-Parra et al., 2014). Since pea and bean proteins are more soluble, they can stabilise the emulsion (Karaca et al., 2011; Liang & Tang, 2013). The higher solubility of the proteins allows more solubilised proteins to adsorb rapidly at the oil-water interface and facilitate the formation of densely packed films around the oil droplets, resulting in the emulsion (Deng et al., 2019).

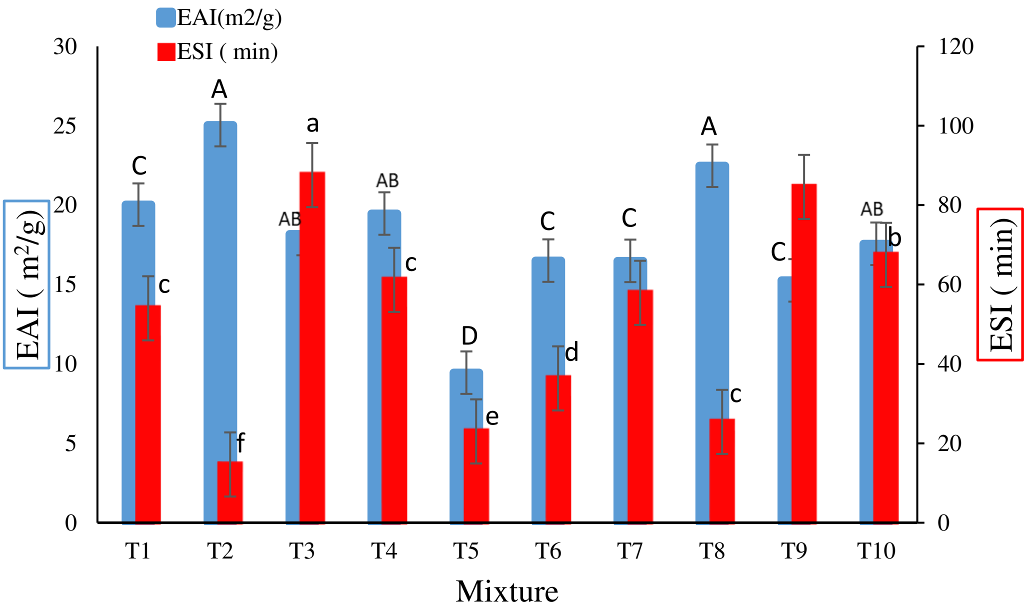


Figure 1: Emulsion activity (EAI, m2/g) and stability indices (ESI, min) for 10 treatments of protein mixtures from peas, lentils and fava beans. Data represent the mean ± one standard deviation (n=3). Means followed by the same capital or small letters, do not differ from each other.

3.3 Stability to flocculation and Coalescence

Flocculation is considered an important destabilisation mechanism in the stabilisation of emulsions (Delahaije et al., 2013; Tcholakova et al., 2006) Flocculation can be explained by interactions between emulsion droplets (Delahaije et al., 2015; Tcholakova et al., 2006) , which may be caused by a reduction in electrostatic repulsion or poor adsorption of proteins at the interface. Emulsions stabilised with lentil showed a lower flocculation index than emulsions stabilised with pea and fava, as also observed by (Ladjal-Ettoumi et al., 2016), which could be due to the lower content of SH and SS bonds, as SH groups can form bridges and increase the flocculation of droplets (Ladjal-Ettoumi et al., 2016). The flocculated state of the droplets showed an inverse trend in emulsions stabilised with bean proteins, with FI % increasing with the increase of bean content in the formulation. When flocculation reaches a certain level, coalescence usually occurs. Severe coalescence leads to creaming. This occurs when the size of the oil droplets is large enough to increase the oil density, which favours phase separation (Bouyer et al., 2013) shown in Table 2, coalescence was lower in all treatments with low flocculation. Treatment T4 showed higher flocculation index, lower flocculation stability and coalescencand larger particle size after 24 hours (Figure 2).

In all treatments, droplet size increased after 24 hours, except for T7, where it remained the same, and treatments T8 and T10, which showed a decrease in droplet size.It is noted that pea protein is present in the formulation in all 3 treatments. From the contour plot (Figure 3 -A; D) it can be seen that pea protein is most responsible for reducing both coalescence and flocculation. It is believed that the combination of proteins in the proportions tested at T8 and T10 reduces the diffusion rate of proteins at the interface, allowing for better emulsion formation. Treatment T10 formulated with the combination of the three proteins in the same proportions showed less flocculation and coalescence, indicating a more stable emulsion than the formulation with single proteins.

This divergence in emulsification behaviour of the proteins could be due to different physicochemical properties such as molecular weight, hydrophobicity, free SH and SS bonds, as previously noted (Ladjal Ettoumi et al., 2016; Ladjal-Ettoumi et al., 2016).

3.4 Centrifugation stability

The coalescence of the emulsion droplets can be accelerated by mechanical stirring or by centrifugal force, as the frequency of collision of the emulsion droplets is increased. Centrifugation is a simple and practical method for determining the effects of formulation on the coalescence stability of emulsions by forcing the droplets to collide with each other through centrifugal force (H. Li et al., 2021).

The stability of the emulsion is weakened by the centrifugal force, forcing phase separation. Table 3 lists the stability constants (KE). T3 with 100% bean protein was the most stable emulsion and resisted gravity better. The protein can stabilise the emulsion

Table 2: Flocculation and coalescence stability, average flocculation index (FI), centrifugal stability, HCT (heat coagulation time) stability constant (KE) and ζ-potential of emulsions of emulsion prepared with different mixtures of pea, lentil and fava bean protein.

Treatament	Coalescence stability(%)	Flocculation Stability(%)	flocculation index (%)	HCT (minutes)	KE (%)	ζ-potential (mV)
T1	22.18 ± 0.03^{c}	4.13 ± 0.014^{j}	5.7 2± 0.01^{e}	18.34 ±3.99^{ab}	24.46 ± 0.03^{d}	30.28 ± 2.62^{b}
T2	6.92 ± 0.016^{e}	4.77 ± 0.014^{h}	3.93 ± 0.09^{f}	13.53 ± 2.11^{c}	65.58 ± 3.84^{b}	-23.77 ± 2.33^{a}
T3	49.41 ± 0.23^{b}	27.18 ± 0.13^{a}	25.80 ± 0.12^{a}	20.3 ±1.80^{ab}	3.45 ± 2.05^{h}	-26.25 ± 2.33^{a}
T4	78.39 ± 0.38^{a}	26.69 ± 0.13^{b}	26.02 ± 0.12^{a}	19.7 ± 0.83^{ab}	40.53 ± 0.05^{c}	-26.75 ± 2.34^{a}
T5	5. 25 ± 0.02^{f}	15.15 ± 0.06^{d}	13.13 ± 0.05^{b}	18.4 ± 4.14^{ab}	10.91 ± 2.65^{f}	-26.15 ± 2.19^{ab}
T6	11.58 ± 0.04^{d}	4.49 ± 0.07^{h}	12.79 ± 0.04^{c}	18.96 ± 0.56^{ab}	16.11 ± 0.11^{e}	-28.05 ± 1.20^{b}
T7	4.93 ± 0.07^{f}	1.31 ± 0.07^{k}	3.21 ± 0.07^{g}	25.78 ± 0.67^{a}	16.39 ± 0.09^{e}	-28.10 ± 2.12^{b}
T8	11.67 ± 0.02^{d}	18.56 ± 0.03^{c}	0.53 ± 0.07^{i}	16.85 ± 0.50$_{b}$	74.01 ± 0.07^{a}	-29.90 ± 2.69^{b}
T9	7.07 ± 0.01^{e}	4.24 ± 0.01^{j}	5.76 ± 0.06^{e}	25.00 ± 0.79^{a}	17.41 ± 0.49^{e}	-32.15 ± 1.27^{c}
T10	5.19 ± 0.01^{f}	6.82 ± 0.02^{g}	3.38 ± 0.04^{g}	21.63 ± 0.60ab	6.87 ± 0.02^{g}	-30.40 ± 1.27^{b}

Different letters in the same column indicate significant differences at $P < 0.05$.

by adsorbing at the interface and forming an interfacial film, and the steric hindrance formed can inhibit the movement of small droplets (Hu et al., 2016). These results are in agreement with the discovery made by Gumus et al., (2017) that the fava bean protein adsorbs much better at the interface than the pea protein, followed by the lentil protein. It was observed that all the emulsions that contained higher percentage of fava bean in their formulation showed higher stability after centrifugation.

According to Gumus et al., (2017), the adsorption rate of fava bean protein when added to an oil-water emulsion is much higher than that of pea > lentil protein at the same concentrations, which could explain the lower contribution of lentil proteins to emulsion stability. Indeed, both protein solubility and surface charges are important for the stability of emulsion systems, as emulsions are stabilised by a combination of electrostatic and steric repulsions (Petursson et al., 2004).

Gravity separation often causes droplets to remain in contact for prolonged periods of time, which can lead to increased flocculation or coalescence. The highest coalescence was observed in T2 (100 % lentils) after centrifugation, with a thicker oil layer forming. Samples with a higher proportion of lentils in the formulation had a thick oil layer above the bottom layer. The bean protein, on the other hand, was able to inhibit the movement of the oil droplets. In sample T3, the presence of pure broad beans in the emulsion improved the resistance of the oil droplets to high gravity, resulting in greater homogeneity with a creaminess threshold of less than 5%.

Analysis of the contour plot (Figure 3-C) shows untested areas responsible for better emulsion stability. Higher concentrations of fava beans may reduce coalescence of the samples during centrifugation, resulting in more stable emulsions, but mixtures with lentils and peas may also exhibit the same property. Lentil protein in combination with pea protein did not lead to higher stability at the concentrations tested.

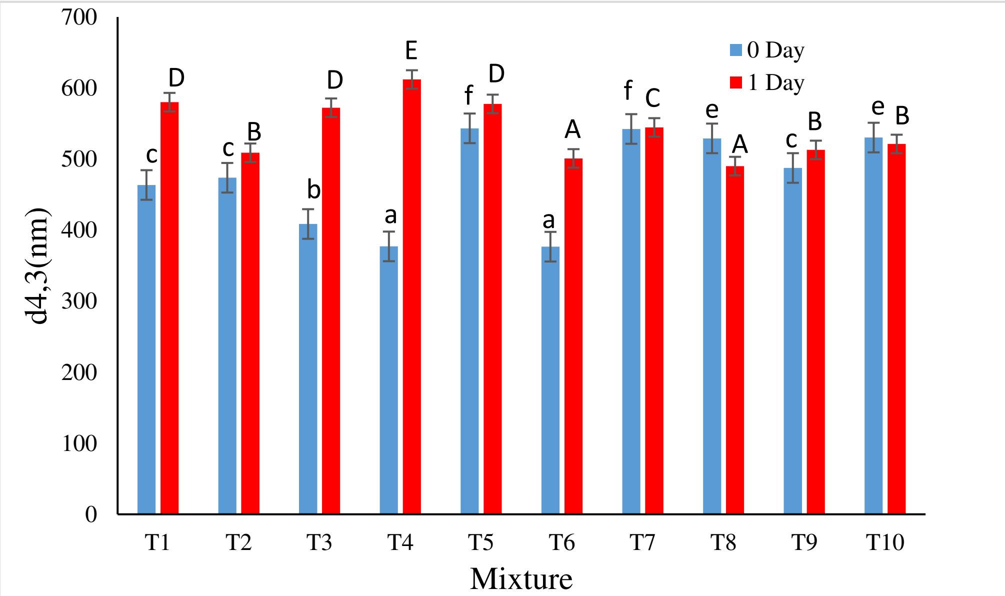


Figure 2: $D_{4.3}$ value of emulsions with protein mixtures at time zero and after 24 hours.

Heat treatment can lead to greater movement of the oil droplets, their flocculation and a loosening of the protein structure, resulting in the formation of interactions between the protein molecules (hydrophobic or disulphide bonds) (McClements, 2004).These phenomena reduce the thermal stability of the emulsion(Rahmati et al., 2018.) When the samples were heated to 80 ºC for 30 minutes and then centrifuged, only treatments T2 (100 % lentil protein) and T4 (50 % pea, 50 % lentil) showed extreme phase separation, which completely changed the appearance of the samples. All other emulsions remained stable, proving that they could withstand heat treatment at 80 °C. The fava bean protein was able to thermally stabilise the emulsion with lentils and also the emulsion with peas and lentils. Joshi et al., (2011) found a peak of denaturation of the lentil protein between 118 and 123 ºC, temperatures well above those tested. However, the lentil protein were not able to withstand such stress, so the solid part settled at the bottom of the falcon tube. This sedimentation was also observed by (Jeske et al., 2019) in samples lentil protein that were not treated with high pressure when subjected to the accelerated stability test. A wide range of commercial plant-based milk substitutes analysed in another study showed high deposition rates for almost all products due to insoluble plant material by flocculation and precipitation(Jeske et al., 2017).

In HCT (Time of Thermal Coagulation) analysis, coagulation is induced by applying heat in order to measure the thermal stability of the sample. Methods of this type can be useful to some extent to simulate batch sterilisation in an autoclave, where heating of the sample is as slow as in laboratory-scale thermal stability tests.

The mixed protein suspensions had a thermal clotting time (HCT) of 13.53 ± 2.11 to 25.78 ± 0.67 min in an oil bath at 140 °C (Table 2). The treatment T2 (100% lentil) had the shortest thermal clotting time. The longest time was observed in the mixtures with the highest concentration of pea and bean protein. The combination of lentil protein with pea our bean resulted in a prolongation of the clotting time. In a study with lentil protein at 3.3 % (w/w), a value of 8.28 minutes was determined for thermal stability(Jeske et al., 2019). In general, it can be stated thatthe proteins were stabilised to a certain degree and protected from heat-induced coagulation. As long as the proteins are at the interface with the oil, their hydrophobic groups are aligned with the oil phase, so that no interactions are forced between the exposed hydrophobic sites of the protein molecules. However, as the degree of denaturation increases, the proteins may lose their ability to stabilise the interface and eventually the system becomes unstable, leading to exposure of the hydrophobic groups and aggregation.

Alonso-Miravalles et al., (2020) observed that HCT increased from 2.8 to 6.7 minutes when pH was increased from 6.3 to 7.2. This heat-induced coagulation is determined by a balance between attractive and repulsive forces, as an increase in the latter has been shown to increase HCT. The charge distribution between amino acid side chains is altered by pH, and for lentil proteins, negative charges increase with increasing pH, resulting in greater repulsion between lentil proteins. The results of this work are consistent with those obtained by (Alonso-Miravalles et al., 2020). The surface charge of the emulsions, with the lentil protein having the highest charge (-23.77 ± 2.33), showed a shorter coagulation stability time, while the other emulsions, which had longer HCT times, showed lower surface charges. This inverse correlation was observed in the correlation test, although it did not reach significant values ($r = -3.64$; $p < 0.05$), indicating a weak correlation.

3.5 Surface response graphs- response optimization variable

To make the effect of the independent variables on the reaction of interest clear, contour diagrams for the variables flocculation, coalescence stability, emulsification activity, centrifugal stability, surface charge (ζ-potential) and HCT were shown in Figure 3.

On the basis of the contour graph, the influence of the individual proteins or their combination on the most important reactions can be understood. It can be seen that the pea protein has the strongest influence on the coalescence and flocculation stability and the thermal stability (HCT). The bean protein was responsible for the accelerated stability when the sample was subjected to mechanical stress and the pea protein influenced the emulsifying activity.

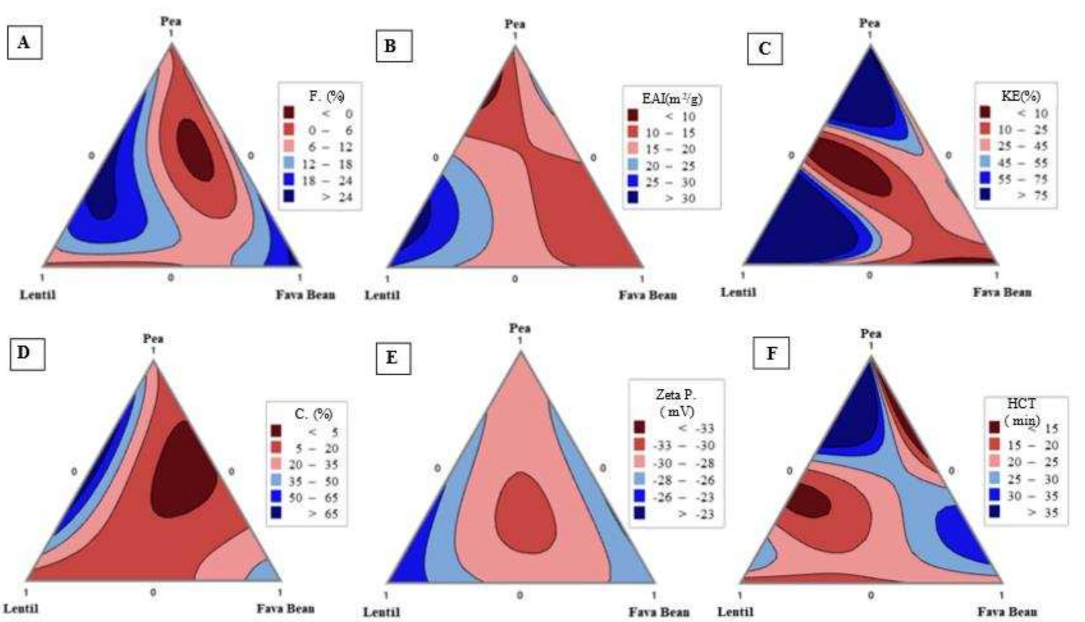


Figure 3. Contour plots showing the effects of pea protein, lentil protein and fava bean protein proportion on: A- Flocculation stability (%); B- EAI(m^2/g) C-Centrifugal stability constant -KE(%); D-Coalescence stability -C (%); E- ζ-potential (mV); F-HCT(min).

It is possible to identify several experimentally untested regions that can assume optimal values for each tested variable. For coalescence and flocculation stability, the formulations can vary between pea and bean protein, while the lowest values for surface charge are in the central region of the diagram, that is, when the three proteins are combined. The experimental values proved that for the ζ-potential, T10 (combination of the three proteins in equal proportions) was the best formulation, so Table 3 shows experimental values of possible optimal formulations for the main tested variables.

Table 3: Experimental optimization of the response variable

Evaluated parameters	Pea (%)	Lentil (%)	Fava Bean (%)	Experimental value	Adjusted R^2
ζ-potential	33.33	33.33	33.33	-30.43	0.99
Coalescence	34.87	27.76	37.37	2.99	0.98
Flocculation Index	36.73	21.85	41.41	1.0	0.99
Centrifugal stability (%)	41.41	30.60	27.99	0.5	0.98
HCT(min)	56.73	43.26	0	27.00	0.99
EAI (m2/g)	19.19	80.81	0	32.85	0.95

3.6 Optimization of multiple parameters

For each parameter assessed, a regression analysis was performed to determine the model that best explained the responses. In this study, it was possible to predict significant effects on several parameters such as coalescence stability, flocculation stability, particle size, HCT, emulsifying activity and ζ-potential. The quadratic and special cubic models allowed the evaluation of the influence of the variables on the responses. In addition, the analysis of variance (ANOVA) confirmed the significance of the influence of the model on these responses (Table 4).

To select the parameters for the optimization of the emulsion, a correlation analysis was performed between the analyzes carried out to determine which variables are related to each other and, thus, select the relevant variables. Table 5 shows the significant correlation values between the variables.

Table 4: Analysis of variance of experimental responses to pea, lentil and fava bean protein concentrate mixture.

Dependent variable response	SS	MS	R^2	*P-Value*
Flocculation stabilit (%)	1706.33	213.29	0.9800	0.000*
Coalescence stability (%)	11442.4	1430.29	0.9955	0.000*
Flocculation index (%)	1591.08	235.181	0.9469	0.000*
Centrifugal stability (%)	12086.6	1342.95	0.9958	0.000*
Particle size ($D_{4,3}$)	11426.4	1269.60	0.9404	0.000*

EAI(m_2/g)	351.21	39.023	0.8494	0.000*
ESI (min)	8822.9	1470.5	0.5626	0.016
HCT (min)	209.66	23.21	0.6436	0.020
ζ-potential (mV)	12.975	14.44	0.6781	0.023
IPD	0.0079	0.0013	0.3007	0.345

SS sum of squares, MS mean square. Statistical significance: *p ≤ 0.05, nsp > 0.05.

Table 5: Pearson correlation analysis between the tested variables that were significant.

Sample 1	Sample 2	Correlation	*p- Value*
EAI (m^2/g)	Centrifugal stability (%)	0.759	0.024
EAI (m^2/g)	IPD	0.695	0.002
HCT(min)	Centrifugal stability (%)	0.725	0.008
Coalescence stability (%)	Flocculation stabilit (%)	0.765	0.000
Coalescence stability (%)	Flocculation index (%)	0.845	0.000
Flocculation stabilit (%)	Flocculation index (%)	0.725	0.000
Flocculation stabilit (%)	$D_{4,3}$(nm)	0.758	0.008
ζ-potential (mV)	ESI (min)	0.657	0.005

Through the correlation analysis it was possible to correlate the results of the analysis of the emulsifying activity with the results of the centrifugation stability and the polydispersity index. The ζ-potential correlated with the emulsion stability. Coalescence stability correlated with flocculation stability, which in turn correlated with particle size. Based on these correlations, the following parameters were selected for optimisation of the mixture: EAI, HCT, ζ-potential, particle size and coalescence stability.

For each analysis the parameters of interest, was defined. The variables EAI and HCT were maximised, while the variables ζ-potential coalescence stability and IPD were to be minimised (Table 6). The lower and upper limits for each dependent variable were set to values that guarantee the integrity and intrinsic properties of the product, based on the maximum or minimum values obtained for each response (Table 3). The optimisation results provide information about the influence of the mixture on the stability of the emulsion.

After setting the optimisation parameters (Table 6), the desirability function was used to calculate a new optimal formulation that satisfied the previously set values with greater accuracy. The optimal experimental formulation 21.21% pea protein, 32.78% lentil and 46.01% broad bean (R2 Adj, 0.9057).

Table 6: Predicted and experimental values for optimization parameters of the protein mixture.

Dependent variable response	Goal	Lower limit	Upper limit	Predicted value	Experimental value	Relative Error (%)
EAI(m^2/g)	Maximize	16.00	32.00	16.09	23.75 ± 1.18	41.0
ζ-potential (mV)	Minimize	-28.00	-31.00	-30.9	-29.16 ± 1.46	0.90
Coalescence stability (%)	Minimize	4.0	7.0	3.56	2.53 ± 0.69	0.91
$D_{4.3}$(nm)	Minimize	400.00	420.00	421.28	436.16 ± 16.06	3.56
HCT(min)	Maximize	18.00	26.00	24.69	22.40 ± 2.54	6.73

3.7 Verification of predictive models

After calculating the new formulation for the mixture of the three proteins, the predicted concentration (21.21% of body weight; 32.78% lentils, 46.01% fava beans) was tested in the laboratory and the analyses were repeated for comparison with the values predicted by the programme (n=3). The (experimental) values obtained in the laboratory are shown in Table 6.

It can be noted that the values obtained are close to those predicted by the mathematical optimisation model, with the exception of EAI, and therefore the fitted model is considered valid for the formulation. Through the desirability function, it is possible to optimise each parameter independently, as shown in Table 3, to modulate, maximise or minimise each response variable, in addition to optimising multiple responses to obtain the ideal formulation.

The predicted value and the experimental value are close in most analyses, with a relative error of less than 5%. It can be concluded that the mathematical optimisation model, with the exception of EAI, is considered valid for the formulation. The equation predicted formulation showed better results for the tested dependent variables than the mixture formulations and even better than the isolated protein.

4. Conclusion

This study showed that mixture design is an efficient statistical tool to determine the best combination of different proteins when used to stabilise oil-in-water emulsions. The single lentil protein concentrate was efficient in emulsifying activity but with lower values of ζ-potential. The pea showed better thermal properties but was only able to achieve good coalescence and flocculation stability when combined with the broad bean. In general, it was observed that the combined proteins showed greater functionality than the individual isolated protein sources. The synergism between the proteins favoured their emulsifying properties and improved most of the tested parameters. With a desirability analysis, it was possible to optimise the reaction variables and select parameters of grater relevance to find concentrations of the individual protein econcentrates that are ideal to obtain a more stable emulsion.

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4. CHAPTER 3

Nutritional and technological aspects of vegetable oils that stand out for the prevalence of medium-chain triacylglycerides: A review

Abstract

The nature of the fatty acid presented in each type of vegetable oil will determine the oil's characteristic. The purpose of this review is to describe the medium chain fatty acids present in vegetable oils absorption mechanism and their benefits for human body, combined with technological advantages in the preparation of food formulations. Highlighting how the source of the raw material and the extraction method interfere with the concentration of fatty acids present in the oils. It is a review of narrative literature, which used as a theoretical framework recent scientific articles in the English language. Oils derived from coconut, palm kernel, and babassu, for example, have a solid or semi-solid characteristic at room temperature. This happens due to the presence of saturated fatty acids with medium-length carbon chains, that is from 6 to 12 carbons. Although medium-chain fatty acids are saturated, they present characteristics that are different from other long-chain saturated fatty acids. They are quickly digested by the body, which favors the absorption and the using of these nutrients, leading an impact on diseases' control, such as obesity, diabetes and hypertension. In addition, due to their chemical structure, medium chain fatty acids can be used in the food industry as a substitute for animal fat, combining their nutritional benefits with technological advantage, being used in food preparations to impact texture, flavor, stabilize emulsions, and improve the rheological characteristics of the product.

Keywords: obesity, coconut oil, Kernel palm oil, babassu oil, medium-chain fatty acid.

1. Introduction

Vegetable oils can be extracted from vegetable raw materials such as grains, seeds, cereals, fruits and other food types (Costa et al., 2019).The main component of oils are lipids that account for different functions in the body, such as providing energy, contributing to hormone formation and producing satiety (Lichtenstein,2021) ; in addition, they are of great importance for the food industry since they can change the flavour and texture of processed food. Lipids found in oils are mostly composed of both triacylglycerols and small amounts of mono and diacylglycerols (Lieb et al., 2019; Kumar & Krishna, 2015).

The physical, chemical and nutritional properties of oils depend on the nature of fatty acids found in them, which, in their turn, are influenced by the source of oil's raw material (Table 1). The raw material for oils can be influenced by the variety and maturation stage of the fruits, climate where it was cultivated other factors capable of interfering with the features of the vegetable raw material (Santos et al., 2013; Srivastava et al., 2016; Costa et al., 2019)

Figure 1- Overview on the technological and nutritional features of medium-chain fatty acids extracted from vegetable oils.

Each fatty acid has a different feature, i.e., number of carbons in the chain, degree of saturation, which determines the chemical behaviour of this oil and its stability in the face of changes in temperature, storage and other factors(Bach & Babayan, 1982; Oliveira et al., 2019; Lieb, 2019; Jeyarani et al., 2009; (Dhamodharan & Bakthavatsalam, 2020). Among saturated fatty acids, we can highlight those of medium-chain, which are found at high concentration in the composition of some vegetable oils such as coconut, babassu and kernel palm oil (Ferrari & Soler 2015; Kiliç & özer, 2019; Costa et al. 2019; Zicker et al., 2019). The presence of medium chain fatty acids has straight impact on the absorption of these oils by the body, a fact that means several benefits to consumers' health (figure 1) (Lee et al., 2019;

Roopashree et al., 2021). In addition to the nutritional aspects, the chemical structure of these fatty acids presents technological features such as low melting point, which cause these oils to solidify at temperatures below 25 °C and to return to the liquid state when the room temperature increases – this process does not make these oils lose any technological or nutritional property (Bach & Babayan, 1982; (Kumar & Krishna, 2015) (figure 1).This phenomenon results from the prevalence of medium-chain fatty acids(MCFA) in the composition of these oils(Jeyarani et al.,2009).The aim of the present review is to address vegetable oils that have high concentrations of medium-chain fatty acids (MCAF) in their composition, the features of these fatty acids and how they influence the physical-chemical properties of oils, as well as their nutritional impact on and health benefits to consumers.

2. Methodology

A narrative review was carried out (Sant'Anna Ramos Vosgerau & Paulin Romanowski, 2014), through bibliographic research in different indexing bases, on topics related to the nutritional and technological properties of oils with predominance of medium chain fatty acids. In the search for scientific articles, the keywords "vegetable oils", "metabolic syndrome", "fatty acid nutrition and metabolism", "functional fatty acids", "medium chain fatty acid rheology" and "technological properties" were used of medium chain fatty acids".

In order to obtain reliable information on the topic, only scientific articles were searched in electronic databases such as: Scopus, Scielo, Science Direct, Web of Science and Wiley Online Library. The criteria adopted for the selection of articles included publications in the English language, preferably between the years 2015 to 2021, relevant and recent research in the nutritional and technological area on the use of medium-chain fatty acids.

3. Results and Discussion

3.1 General aspects

Vegetable oils consist of triacylglycerides extracted from plant that present glycerol molecules linked to three fatty acid molecules (figure 2) (Lee et al, 2022). Some oils, such as coconut, kernel palm and babassu oil, are mainly composed of medium chain triglycerides such as caproic acid (C: 6) caprylic acid (C: 8), capric acid (C:10) and lauric acid (C: 12), which are saturated fatty acids presenting carbon chains ranging from six to twelve carbons

(Table 1) (Jeyarani et al., 2009; Santos et al., 2013; kumar & krishna 2015; Srivastava et al., 2016).

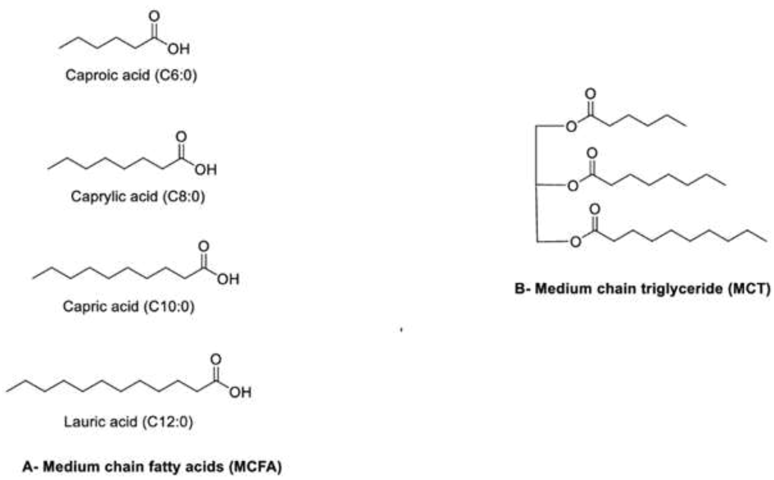

Figure 2: Chemical structure of medium-chain fatty acids (A). Chemical structure of a medium-chain triacylglycerol (B).

Medium-chain fatty acids have low water solubility, which is a factor related to the presence of a hydrocarbon chain, increase in acyl chain and to less double bonds. It is also associated with the presence of the carboxylic acid group, which is polar and ionized at neutral pH (Jeyarani et al., 2009; Lee et al, 2022).The melting point is also influenced by the length and degree of hydrocarbon chain unsaturation. Saturated fatty acids presenting from 12: 0 to 24: 0 carbons have wax consistency at room temperature (25 °C), whereas unsaturated fatty acids (at the same length) are oily liquids. Coconut oil has melting point close to 22.3 °C (Dhamodharan & Bakthavatsalam, 2020), Kernel palm oil presents melting point at approximately 28.2 °C and babassu oil has melting point ranging from 24 °C to 26 ° C. Soybean oil, which is composed of long chain unsaturated fatty acids, has melting point of -20 ° C. This difference in melting points results from different fatty acid-molecule packaging degrees (Norizzah et al., 2018).

Carbon chain packaging is possible due to chain flexibility. Completely saturated compounds account for great hydrocarbon chain flexibility as rotation of the hydrocarbons is free around each carbon-carbon bond. The linear conformation is the most stable one, and it minimizes the steric impedance of neighbouring atoms. These molecules can group into almost crystalline arrangements - atoms along their entire length are at Van der Waals interaction with atoms of neighbouring molecules Jeyarani et al., 2009.The double cis bond forces the hydrocarbon chain to fold in unsaturated fatty acids Fatty acids with one or more of these folds cannot clump as tightly as fully saturated fatty acids, fact that makes interactions between them weaker. Since less thermal energy is needed to clutter these poorly ordered arrangements of unsaturated fatty acids, they have considerably lower melting points than saturated fatty acids presenting the same chain length (Heil et al., 2019).

3.2 Extraction method

Vegetable oil is extracted through different ways, the main extractions are artisanal, mechanical and made with solvent. Based on the artisanal extraction method, the fruit pulp is subjected to intensive cooking in container filled with water. After boiling the mixture, the supernatant oil is separated from the aqueous part. Then, the oil is left to dry over low heat until it loses opacity, the oil is filtered. The artisanal method has some drawbacks, such as low yield, product with opaque aspect and high humidity. Artisanal coconut oil must be centrifuged to allow better oil phase separation and waste extraction in order to avoid the oxidation process within few days (De Pinho & Souza, 2018).

Mechanical extraction can be carried out by using the pressing technique, without solvents; thus, the obtained product maintains its natural properties. However, as the result from its lower efficiency in removing oil from the fruit or seed, this extraction method can be combined to solvent extraction in order to increase yield. Press extraction does not generate oil and cake heating, since it only requires a press and an extractor cylinder (Bhatnagar et al., 2009; De Pinho & Souza, 2018).

Solvent extraction consists of putting an organic solvent in direct contact with the plant matrix. The oil derives from the evaporation of the solvent used in the liquid phase. This methodology is recommended for very unstable oils that do not handle temperature increase. Hexane, benzene, methanol, ethanol, propanol, acetone, pentane and several chlorinated solvents are the organic solvents mostly used in this process for oil extraction purposes. The difficulty in removing all residual solvent and non-volatile compounds that remain in the oil after extraction is one of the negative effects of using organic solvents (Nik Norulaini et al., 2009; Santos et al., 2013).

Virgin coconut oil, which is extracted from fresh coconut pulp. Coconut oil extraction process does not use heat treatment or food preservatives. It can also be called cold extraction, as the oil does not reach temperatures higher than 40 ºC. Conut milk is left to stand for 20–24 h, under favourable conditions (35–40 °C, 75% relative humidity). Autochthonous lactic acid bacteria, which have the ability to break protein bonds, act in the coconut milk mix by causing the separation of virgin coconut oil. The separated oil is filtered through sterile paper filter placed in a large funnel. Refined and deodorized coconut oil derives from the extraction of dehydrated copra oil – this process is followed by alkali refining, bleaching and deodorization(Kumar & Krishna, 2015a).

Extraction can still be done by using the supercritical fluid method. Carbon dioxide is the most used solvent, mainly by the food industry, as it is not toxic, not combustible, available at high purity rates and at low cost, has low critical temperature (31ºC) and critical pressure (74 bars) 'which are advantages when it comes to the required energy and to the conservation of thermolabile substances' has low boiling point, does not leave solvent waste in the extracted material and accounts for good extraction yield. When this method is used to purify edible oils, it presents high solvation power and does not have its properties changed (Nik Norulaini et al., 2009).

Table 1 shows that the fatty acids concentrations in the oils do not present sudden variations when they are subjected to different extraction methods; however, the adopted method can influence the phytochemical compounds found in plants, which are transferred to the oil and can be lost after extraction (Srivastava et al., 2016). These compounds can also influence oil solubility depending on the applied temperature and pressure (Soares et al., 2007).

Table 1: Composition of fatty acids from different food sources

Food Source	Extraction method	C6:0 (%)	C8:0 (%)	C10:0 (%)	C12:0 (%)	C14:0 (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	Reference
Coconut	solvent	0.35	7.87	6.07	49.55	17.03	8.02	2.71	7.01	1.39	Srivastava et al., (2016)
Coconut	Virgin	48.03	6.27	7.64	0.64	16.23	8.40	3.46	5.80	0.90	Marina et al.,(2009)
		48.08	6.48	6.01	-	17.72	7.79	6.94	0.85	1.61	Zicker et al., (2019)
		50	5.55	7.10	0.45	18.1	7.05	2.42	7.26	1.66	Srivastava et al.,(2016)
Coconut	Refined	47.42	6.53	8.24	0.63	18.26	9.33	2.68	5.25	1.57	Marina et al., (2009)
		-	7.24	5.25	50.90	21.38	9.22	0.38	4.81	0.81	Kumar & Krishna, (2015)
Coconut	Supercritical fluid	42-47	8-10	10-12	-	21-23	7-9	1-2	3-5	0.5-1.5	Norulaine et al., (2009)
Babassu	Cold Press	42.79	5.21	4.77	0.27	14.11	8.85	3.57	17.48	2.88	Ferrari & Soler, (2015)
Babassu	Solvent extraction	48.93	7.99	4.54	-	15.64	7.46	1.65	10.55	3.24	Santos et al., (2013)
		47.42	6.46	8.06	-	15.47	7.60	2.81	10.28	1.90	D'Anton et al.,(2011)
		-	-	4.1	36.1	19.4	12.3	5.8	19.4	2.8	De Oliveira et al., (2019)

Kernel palm	Solvent extraction	0.32	3.24	3.6	47.73	15.91	8.25	2.45	15.18	3.22	Kılıç & Özer, (2019)
		0.20	3.13	3.18	45.51	15.64	9.48	2.55	16.90	2.64	Rodrigues, (2010)
		-	4.45	-	49.1	17.00	10.0	2.30	11.00	1.60	Halim et al., (2019)
		-	3.01	3.06	47.85	17.25	10.31	2.63	14.43	1.19	Costa et al., (2019)
Kernel palm oil	Supercritical fluid	-	2.33-4.34	2.56-4.37	39.4-45.5	15.9-17.25	9.44-10.31	1.14-3.35	17.9-24.3	0.9-4.36	Costa et al., (2019)
Kernel palm oil	Inter esterified	0.17	7.06	7.97	39.97	12.46	7.52	1.31	13.84	8.17	Kiliç & özer, 2019
Cow milk	-	0.92	0.62	1.47	1.71	6.44	29.13	9.13	20.89	1.78	Rennó et al., (2013)
Human milk	-	-	0.17-0.22	1.46- 2.00	4.24- 5.86	4.91- 6.80	18.60-22.59	5.77- 6.25	-	-	Yuhas et al., (2006)
Ghee Buffalo	-	1.02	0.47	0.97	1.55	9.64	33.13	13.07	22.49	0.67	Ali et al., (2020)
Ghee Bovine	-	1.01	0.61	1.37	1.79	7.89	29.76	13.18	28.76	0.62	Ali et al., (2020)
Anhydrous fat milk	-	1.76	1.28	2.91	4.89	13.00	35.20	10.24	-	1.51	Neves et al., (2020)

Wherein: C6: 0- caproic acid, C8: 0- caprylic acid, C10: 0- capric acid, C12: 0- lauric acid, C14: 0- myristic acid, C16: 0 –palmitic acid, C18: 0- stearic acid, C18: 1-oleic acid, C18: 2-linoleic acid.

In addition to the extraction method, the variety of the raw material and the place of origin can influence the characteristics of these oils. Ferreira et al. (2019) and Kumar & Krishna (2015) evaluated the lipid profile of different varieties of coconut (Cocos nucifera L.) And observed that the composition of fatty acids in the triacylglycerol chains was not the same for all varieties. These variations in the concentration of fatty acids can also be seen in Table 1.

The composition of triacylglycerols has an impact on their molar mass, density, melting point, solubility, a fact that has a direct influence on the stability of oils against oxidative rancidity. This stability allows the use of these oils in confectionery products due to the increased shelf life of products without trans fatty acids (Jeyarani et al., 2009). According to (Azeez 2007), the fatty acid profile of coconut oil can change depending on the season and the maturation of the nut, as well as the differences between the cultivars. Fatty acid rates can increase or decrease from small to medium cadence due to the maturity of the nut and the concentration of unsaturated fatty acids (Ferreira et al., 2019).

3.3 Nutritional Aspects

Closer attention has been paid to the role of fats and oils in modern diet in recent decades due to their high caloric contribution to the diet itself and to health issues associated with the growing concern about obesity. However, studies show that some specific saturated fatty acids account for a wide spectrum of critical functions in physiological activities, depending on the unsaturation length and degree of the hydrocarbon chains. Fatty acids are not only essential nutrients, but they can also modulate many human diseases and conditions (Legrand & Rioux, 2015; H. Lu et al., 2020).

3.3.1 Absorption of medium-chain triglycerides

Lipid catabolism coordinates lipolysis, which is the hydrolysis of triacylglycerides into free fatty acids, which account for the peroxisomal and mitochondrial β-oxidation of fatty acids to produce chemical energy (ATP) and heat (Rial et al., 2020). The digestive bioavailability of medium-chain triacylglycerides(MCT) is greater than that of lon-chain triacylglycerides(LCT). The comparison between MCT and LCT has shown that MCT hydrolysis, which starts in the stomach, is faster and more complete than that of LCT, as well as its gastrointestinal transit speed is faster and its absorption occurs in the proximal portion of the small intestine, a fact that makes it faster and more efficient (Bach & Babayan, 1982;(Papamandjaris et al., 1998); Ferreira et al., 2003 Lee et al., 2022).

Fatty acids undergo different metabolic pathways depending on their saturation degree and on chain length. Medium-chain saturated fatty acids (carbon chains presenting up to 12 carbons) can be transported right to the mitochondria without the aid of membrane transporters. They are promptly absorbed by the enterocytes and transported directly to the liver by the blood in order to produce energy. On the other hand, long-chain fatty acids travel through the lymphatic system via chylomicrons to extrahepatic tissues where they can be stored in the form of triglycerides (Papamandjaris et al. 1999; Heil et al., 2019; Lee et al., 2022). Thetransport of lipids in the body is overall described in the exogenous and endogenous metabolic pathways. The exogenous pathway represents the transport of dietary lipids from the intestine to the liver. The endogenous pathway describes the transport of lipoproteins synthesized in hepatocytes from the liver to peripheral tissues. Medium-chain fatty acids are easily transported in the plasma since they bind to albumin during the post-absorptive phase; they also take the portal vein and quickly reach the liver (triglycerides (Papamandjaris et al. 1999; Montgomery et al., 2019; Heil et al., 2019; Lee et al., 2022).

Approximately 80% to 100% of medium-chain fatty acid (MCFA) found in the entire portal flow are captured by the liver and the remaining portion of them flows through the bloodstream and becomes available to peripheral tissues (Ronis et al., 2013)(X. Lu, Chen, et al., 2019). MCFAs are partially converted into ketone bodies and released into the blood due to fast metabolism of MCT in the liver. Approximately 10% of ingested MCFAs are detected as chylomicrons in postprandial venous blood (Ronis et al., 2013; Wang et al., 2016) and it turns medium-chain fatty acids into a fast source of energy (Yoshihara et al., 2020).The high intake of medium-chain fatty acids rich in triglycerides leads to the significant intracellular incorporation of them into chylomicrons (in the resynthesized triacylglycerol form). This incorporation likely depends on the consumed amount and distribution of medium-chain fatty acids in the ingested triacylglycerides (Bach & Babayan, 1982; Heil et al., 2019; Lee et al., 2022).

3.3.2 Medium-chain fatty acid in lipid oxidation

Mitochondria are important sites for lipid oxidation. Several mitochondrial metabolism markers are used to determine whether intracellular lipid levels can be related to differences in fatty acids size of the chain. Diets high in fat and MCFAs increase mitochondrial biogenesis in skeletal muscle(Ishizawa et al., 2015; Montgomery et al., 2019).In addition to the postprandial absorption pathway, MCFA effects on muscle mitochondrial biogenesis are affected by metabolic degradation of the liver(Thevenet et al., 2016). MCFAs induce much greater increase in the activity of the muscle oxidative enzyme (Montgomery et al., 2019) (Thevenet et al., 2016). A study carried out with sedentary individuals in the age group 40 and 60 years who received a diet rich in medium-chain fatty acids showed that exercising increases and improves the aerobic capacity in terms of oxygen transport and cardiac output capacity (Nosaka et al., 2020).

High fat-based MCFA diet increases the activity of the Citrate Synthase (CS) mitochondrial oxidative enzyme in both fast and slow skeletal muscle contractions (Ishizawa et al. 2015). However, based on the current results, MCFA-based diets increased the activity of the mitochondrial oxidative enzyme in slow muscle contraction. The ability of the muscle to increase mitochondrial concentration, density and oxidative enzymatic activity (e.g., CS activity) is an important muscle adaptation to resistance exercises. Oxidative enzyme activity, in its turn, is associated with significant mitochondrial biogenesis simulation (Ströher et al., 2020).

3.3.3 Medium-chain fatty acid in obesity control

Some studies have suggested that medium chain fatty acids are potential obesity control factors (Assunção et al., 2009; Zhou et al., 2017). Studies with humans and rodents have shown that MCFA induces higher energy expenditure than LCFA, but such an outcome has also been associated with lower adipose tissue accumulation (Ishizawa et al., 2015).

A study carried out with rats fed on diet rich in MCFA has evidenced that rats' body weight was lower when they fed on diet rich in MCFA than that of rats who did not. Rats who fed on long-chain fatty acids (LCFA), even at equal energy value, have shown that diets rich in MCFA can reduce fat accumulation. Plasma glucose concentrations were significantly higher in the LCFA group than in the MCFA group (Ishizawa et al., 2015; Turner et al., 2009).

Such features are indicative that MCFA can be recommended as dietary supplement to prevent obesity and high fat diet-related diseases.

3.3.4 Medium-chain fatty acid for diabetes control

Resistance to insulin is defined as the reduced ability of insulin to regulate the metabolism of carbohydrates and lipids in target tissues. It is one of the main metabolic obesity and type 2 diabetes issues (Bach & Babayan, 1982; Roopashree et al., 2021).Diets rich in MCFA reduce plasma glucose levels and improve insulin sensitivity in comparison to diets rich in LCFA (Ferreira et al., 2019; Turner et al., 2009). A study based on coconut oil addition to the diet showed increased fatty acids and triglycerides availability, and it may have changed glucose using in oxidative mitochondrial metabolism (Yoshihara et al., 2020), since metabolic changes related to glucose metabolism and resistance to insulin are closely associated with increased dietary fat intake.

Progressive resistance to insulin and the subsequent hyperglycaemia caused by high-fructose intake diet leads to symptoms similar to those of hepatic steatosis, in rats(De Moura et al., 2008). Coconut oil supplementation reversed the hepatic steatosis condition in addition to having better resistance to insulin, lower glucose levels, higher HDL levels and lower levels of total cholesterol and triacylglycerols. These outcomes are attributed to improvement in the hepatic steatosis-associated dyslipidemic condition (Narayanankutty et al., 2018).

Other studies have reported that diets rich in coconut oil have hypolipidemic activity mediated by its effect on lipid metabolism; they also have antidiabetic activity in diabetic models due to high fructose and fast MCFA absorption and metabolism. Narayanankutty et al. (2018) observed that diets rich in coconut oil have shown that MCFA improves fat's beta oxidation and protects individuals from liver disease caused by excess of fat (Ronis et al.,2013). MCFA markedly decreased the percentage of apoptotic and late cells necrosis in comparison to the long-chain fatty acid. Moreover, it did not induce apoptosis, oxidative stress and chronic inflammatory responses in liver cells of individuals with steatosis. (Wang et al.,2016).

3.3.5 Medium-chain fatty acid to control cardiovascular diseases

Postprandial triglycerides (TG) are among factors accounting for the development of cardiovascular diseases (Jackson et al.,2012). TG levels reflect the presence of remaining lipoproteins that can cause atherosclerosis (Austin et al., 2020).

MCFA reduced postprandial TG levels since it was transported through portal circulation and was little incorporated to chylomicrons. MCAF fast beta oxidation in the liver results in decreased TG synthesis (Jackson et al., 2012). There was significant reduction in serum TG levels due to MCFA diet intake in comparison to the LCFA diet.

The MCFA diet did not affect the total levels of free fatty acids. Postprandial hypertriglyceridemia appeared to be related to fasting atherogenic dyslipidaemia, increased stress oxidation and impaired haemostasis. All together, these factors can contribute to impair the vascular function and to the development of atherosclerotic cardiovascular disease. Thus, MCFA oil consumption can limit the entry of circulating chylomicron-TG and, consequently, reduce the residence time of remaining lipoproteins rich in atherogenic TG (Lee et al., 2019). However, the study by Austin et al. (2020) did not demonstrate any beneficial effect of MCFA-rich diets on postprandial TG reduction. These effects were only observed when MCFA was combined to fish oil.

The table 2 shows clinical studies in vivo and in vitro on the use of triglycerides in the diet. Featuring type of used oil, dosage administered and observed benefits.

Table 2: Physiological effects of dietary MCFA / MCT observed in clinical studies

Assessed Component	Declared Composition	Dosage/ period	Assessed Individual	Observed Effect	Reference
MCFA	C8:0- C18:3	45% to 60% calories of diet / 21 days	Mice	Adiposity reduction and glucose intolerance improvement.	Turner et al., (2009)
MCFA	MCFA-rich oil	60% of calories of diet/ 4 weeks	5-week-old male Wistar rats	Increases the activity of the mitochondrial oxidative enzyme	Ishizawa et al., (2015)
MCFA	C8:0- C18:3	45% of calories of diet/ 8 weeks	Mice (absence of Gpr84 protein)	Mitochondrial regulation, insulin response	(Montgomery et al., 2019)
MCT	Nestle Nutrition (82%MCT /18% Bovine tallow)	20%, 35%, 50%, 65% for 21 days	Male rats	Metabolic syndrome, hepatic steatosis	Ronis et al., (2013)
MCT	FA C8:0	12 months	Mice	Treatment for Acil -CoA dehydrogenase deficiency	Tucci et al., (2015)
MCT	FA C8:0, C10:0	10%, 20%, 30% p/p	Mice	Obesity control, body weight reduction	Zhou et al., (2017)
MCT	40:60 ratio of MCT C8:10	5% of diet	Wistar rats	Social behaviour	Hollis et al., (2018)

MCT	C8:0, C10:0 – Nestlé Switzerland	30g/ 2.000kcal	18-65 year old individuals BMI ≤5.0 kg / m^2	Glucose and insulin metabolism	Thomas et al., (2019)
MCT	FA C8:0, C10:00	6g/ day	40-60 year old sedentary individuals	Increased aerobic capacity, oxygen transport and cardiac output capacity	Nosaka et al., 2020)
MCT	Dermol M-5 (ALZO international)	45kcal (40%MCT - 60%C:8 and C:10/ 5% TCL-soy)	Mice	Hepatic thermogenesis	Rial et al., (2020)
Coconut oil	Virgin commercialisation	5% and 15% of basal diet-10 days	Wistar rats	Antioxidant and hepatoprotective	Famurewa et al., (2017)
Coconut oil	Santa Cruz Biotechnology, TX, USA	4.5g/Kg- 180 days	Wistar rats	Hypertrophy	Quitete et al., (2019)
Coconut oil	Organic commercialisation	1.3 and 9 g/kg- 12 weeks	Mice	Inflammatory and metabolic dysfunction	Zicker et al., (2019)
Coconut oil	Virgin	2ml/kg -30 days	Rats	Metabolic changes due to lipid accumulation in the liver	Ströher et al., (2020)

Wherein: MCFA: medium chain fatty acid, MCT: medium chain triacylglycerol, FA: fatty acid

3.4 Technological Aspects

The concern of consumers to have a healthier life shows the search for products with less fats, mainly of animal origin (Kiliç & Özer 2019). In order to meet consumer demands, there is a growing interest in studying vegetable oils to partially or totally replace animal fat in order to improve the textural quality, appearance and oxidation stability of products(Goibier et al., 2020; Nguyen et al., 2021).

3.4.1 Application of the MCFA in food formulations

Bhatnagar et al., (2009) tested blends of vegetable oils such as flaxseed, rice, sunflower and soy added with coconut oil. They evaluated the oxidative stability of mixes and observed that blends with coconut oil showed greater oxidative stability because it is less likely to form peroxide due to the presence of medium chains.

Coconut oil was also used by Szafrańska (2020) in cheese sauce formulations to replace anhydrous milk fat. Visually the sauces with milk fat and the sauce with coconut oil showed differences, however the technological characteristics such as stability, viscosity, water activity of the coconut oil made it a good alternative for the production of analogues to the cheese sauce (Szafrańska et al., 2020).

Ali (2020) showed used coconut oil in vegetable meat formulations as ingredient added to unsaturated oil in order to add texture and sensory aspect similar to that of beef. Saturated and unsaturated oils are mixed to form white dots in hamburgers and vegetable sausages, and to simulate animal fat(Chatterjee et al., 2020; Guimarães et al., 2007).

Kiliç et al. (2019) assessed animal fat replacement by kernel palm oil in sausages, which showed excellent sensory results and no significant differences between treatments focused on colour, odour, flavour, juiciness, texture and general acceptance. Kiliç et al. (2017) also assessed animal fat replacement by kernel palm oil in sausage and found that bovine fat replacement by kernel palm oil can be a strategy to improve nutritional quality without having negative effect on quality features. In addition, meat fat replacement by kernel palm oil has not significantly contributed to lipid oxidation development (Kiliç & Özer 2017, 2019).

Goibier (2020) used triacylglycerides that crystallize at 20 ºC, such as coconut oil, as a stabilizer for a double (W1 / O / W2) emulsion. emulsions are complex liquid dispersions of oil globules containing small aqueous droplets. These systems have great potential to arrest and control the release of aroma and flavor in the field of food production, to protect and arrest probiotics, to mask flavor and to reduce the fat content in foods. Initially obtained emulsions were relatively fluid, but after a few hours at 20°C, they presented considerable yield, strength and firmness. As crystallization proceeded, solid bridges formed between aggregated crystals that further strengthened the network. The W / O emulsion obtained was stabilized only by triglycerides; it did not demand the addition of surfactants, resisted osmotic shocks and was not subjected to coalescence under storage conditions, at room temperature (Goibier et al., 2020).

3.4.2 Stability of oil-in-water emulsion systems

Most industrialized foods are oil-in-water emulsions, such as beverages, sauces and ice cream, and are also used in the pharmaceutical, cosmetic and oil industries. Naturally, this system does not remain stable, making them dependent on the use of emulsifiers (H. Lu et al., 2020).

Many emulsions based on coconut oil and water as vegetable drinks are being introduced to the market, it is necessary that this emulsion remains stable.Emulsification is characterized by the dispersion of immiscible liquid in other liquids based on the use of high shear energy. Requires the use of an emulsifying agent to avoid separation of the subsequent phase(Ramisetty et al., 2015). in addition to the type of emulsifier, the mixing method, pressure of homogenization influences the stability of the emulsion, as shown in table 3.

In general, the mixing process is one of the most important unit operations that influence the size of the drops and the stability of the emulsion. Studies have already shown the effect of different homogenization pressures and their response to the stability of the emulsion (Cheng et al., 2020; Matsuura et al., 2015). Other types of processing have been used to prepare ultrasonic emulsions that have shown excellent results in reducing particle size and excellent morphologies: particles of uniform size that favor emulsions and, consequently, avoid coalescence (Gulão et al., 2018; X. Lu, Su, et al., 2019; Ramisetty et al., 2015; Saricaoglu et al., 2018). The final average size of the drops and their distribution depend on the dissipation of energy, which, in turn, depends on the intensity of the cavitation (M. Lu et al., 2020).

Emulsifying agents are responsible for the development of bonds between two immiscible liquids, acting as a surface-acting agent. The hydrophilic head of the emulsifying agent has a good connection with the aqueous phase and the lipophilic tail connects with the oil phase to bring these two liquids together. The lipophilic tips of the tail move towards the center of the oil drop and the hydrophilic layer goes to the emulsifying agent around the surface of the spherical oil droplets which prevent the oil droplets from coalescing and stabilize the emulsion. (Ramisetty et al.,2015). Therefore, using sufficient amount of emulsifying agent allows the emulsion to be made more stable for long periods of time (Table 3)

Table 3: Coconut oil-in-water-type emulsions stabilized with different types of emulsifiers

Emulsifying agent	Source of emulsifying agent	Emulsifier dosage	Formulation of the assessed emulsion	Mechanical process applied to emulsion	Effect observed in the emulsion	Reference
Lactoferrin	Bovine milk	3% p/v	10% coconut oil 90% water	Ultrasound 30KHz/3min-25º	Emulsion stability during 7-day storage.	Gulão et al., (2018)
Albumin and Globulin	Coconut pulp	1% p/p	1:9 coconut milk/water	Homogenizer 3000psi- 2x	The globulin fraction is more effective in stabilizing the emulsion.	Patil & Benjakul, (2017)
Pre-gelatinized Rice Starch	Rice	5% to 7%	10% coconut oil	Ultra-Turrax at 8.000, 11.000, and 15.000 rpm/ Calorimetry 30°c and 50°C	It showed better results in both heating and stirring conditions than the other oil.	Yulianingsih & Gohtani, (2020)
Egg yolk	Egg	6%w/w	Coconut oil + Palm oil diaglycerol (0%, 10%, 20% ,30%)	Agitator 12.500rpm/6min	Samples with coconut oil alone were more stable for 30 days.	Ng et al., (2014)
Tween 80 and Span 80	-	0.02, 0.04, 0.05, 0.07, 0.09, 0.11 and 0.12w/w	0.1%, 0.2%, 0.3% and 0.4 % of coconut oil.	Ultrasound Processor -20 KHz and 750W -20 KHz 1500W -20 KHz 1000W	The greater the power of the processor, the smaller the size of fat droplets, the better the stability of the emulsion.	Ramisetty et al., (2015)
WPC 80%	Whey	2%, 3%, 4%, 5%, 6%, 7%, 8 %	10% Anhydrous milk fat 10% milk and coconut oil	Agitator 10.000rpm / 2min 80°C	Both are efficient in producing cheese sauce.	Szafrańska et al., (2020)

Sucrose esters (SE)	Sucrose	0.25% in oil weight	5% oil in coconut milk	Agitator 11.200rpm / 3min	Emulsions with sucrose esters were thermally more stable than emulsions with Tween 60.	Ariyaprakai et al., (2013)
Glyceryl monostearate (GME)	Fat	0-0.40%	10% coconut oil 4%protein 14%sucrose 0.25% carboxymethylcellulose	Pasteurization 65 ° Homogenizer of 2 pistons 20 Mpa in the first stage and 4 Mpa in the second stage.	GME reduced fat cell deformation and inhibited partial coalescence	Cheng et al., (2020)
Corn grain and starch with different amylose levels	Corn	0.3 to 0.5g/L	6% Coconut cream 30.8% fat 3.9 protein	Mixer 13,500rpm / 3 minutes - sterilization after filling 115 ° C / 20 min	The higher the amylose content, the higher the viscosity and the better the thermal stability.	M. Lu et al., (2020)
Glyceryl monostearate (GME) Waxy Starch	Fat and corn	0.3 to 0.5g/L	6% Coconut cream 30.8% fat 3.9 protein	20KHz high intensity ultrasound	Ultrasonic treatment resulted in particle size reduction to monomodal size distribution.	X. Lu, Chen, et al., (2019)
Dextrose (DE) and Maltodextrose (MC)	-	27.5% p/p- (2,10,25 DE)	20% (w/w) coconut oil	High pressure homogenizer - ¨60Mpa in the 1st stage and 40Mpa in the 2nd stage	The best results were obtained by using 10% dextrose associated with sugar stearic acid esters	Matsuura et al., (2015)

Wherein: w / v- weight / volume ratio, w / w: weight / weight rati

4 Conclusion

This review explored the technological and nutritional functions of vegetable oils that have medium-chain fatty acids in their composition. Fatty acids can be free or observed in their triacylglycerol form. They are intrinsically connected to the control of some diet-related diseases and to their technological applications in the food industry. Experimental studies have shown that dietary MCFA suppresses fat deposition through thermogenesis and fat oxidation both in animals and humans. In addition, several reports have suggested that MCFA has the therapeutic advantage of preserving insulin sensitivity in type 2 diabetes' animal and human models. MCTs enable technological benefits for the food industry when they are used as emulsion stabilizers, since they provide texture and flavour to products.

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5. GENERAL CONCLUSIONS

This paper deals with oil-in-water emulsions stabilised by plant proteins. Proteins can be extracted from different sources, each with different properties. The functionality of protein as an emulsifier has been discussed and associated proteins have been found to have better technofunctional properties than the native protein. Studies have demonstrated the efficacy of protein association with polyphenols, polysaccharides, animal proteins and papper 2 concluded that plant based proteins from pea, lentil and fava, when associated, gave better results than alone. These results suggest the potential applicability of plant proteins as food ingredients in formulated foods that could be beneficial to industrial manufacturers. The work also addressed nutritional and technological aspects of medium-chain vegetable oils such as como oil and babassu oil. Studies have shown that the protein associated polyphenol can not only improve emulsification properties but also prevent lipid oxidation of the emulsion. It is believed that emulsions formulated with FAMC and vegetable proteins can be attractive to both consumers and industry as they combine nutritional and technological factors.