



Functional Characterization and Emulsion Stability of an Aqueous Macerate of Fenugreek (*Trigonella foenum-graecum* L.) Seeds

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Abstract: Fenugreek, a highly prized plant in traditional medicine and cuisine, for its various phytochemical with therapeutic and functional properties. The aim of this study is to evaluate the physico-chemical properties of the aqueous extract obtained by macerating fenugreek seeds and to assess its emulsifying properties. We analyzed the physico-chemical parameters of the aqueous extract of fenugreek seeds, such as dry matter, refractive index, ash content, pH, electrical conductivity, and dynamic viscosity. In the second part, we have characterized the emulsifying properties of aqueous extract by monitoring the kinetics of microscopic parameters (diameter of fat globules, interfacial surface and microscopic aspect of emulsion) and spectrometric parameters (stability and index stability). The results found that the aqueous extract of seeds fenugreek possessed desirable physical and chemical characteristics such as pH (6.32 ± 0.21), dry matter ($6.75 \pm 0.02\%$), and ash ($0.172 \pm 0.001 \text{ g L}^{-1}$). Over time, the best indicators of emulsifying properties are those observed in the emulsion prepared with extra virgin olive oil, aqueous fenugreek extract, and stabilizer (sodium caseinate) compared to the emulsion prepared without stabilizer, namely: average fat globule diameter (0.30 ± 0.01 to $0.55 \pm 0.02 \mu\text{m}$), average interfacial area (8.0 ± 0.1 to $6.0 \pm 0.1 \text{ m}^2$), average stability (100.0 ± 0.1 to $88.85 \pm 0.2\%$), and average stability index (2.5 ± 0.1 to 22.0 ± 0.3 hours). In conclusion, the macerated

aqueous extract of fenugreek seeds was characterized by significant physicochemical and emulsifying parameters that are valuable in the field of emulsions, in the formulation and development of natural products, namely in the food, cosmetics, and pharmaceutical industries.

Key words: Fenugreek, aqueous extract, physicochemical properties, emulsifying power, stability.

Leguminous plants, commonly known as legumes, represent one of the most diverse botanical families, encompassing approximately 16,400 species across 475 genera, classified into three major subfamilies (Come and Françoise, 2006). Within this family, the Fabaceae subgroup is the most significant, comprising nearly 10,000 species of considerable agronomic and nutritional importance. Legumes are widely recognized for their exceptional nutritional value, being rich sources of plant proteins, dietary fiber, essential vitamins, and minerals (Bhatty, 1999).

Among these, fenugreek (*Trigonella foenum-graecum* L.) stands out as a valuable annual herb belonging to the legume family. Its nutritional composition closely resembles that of foods from the meat, fish, and egg group, owing to its high protein content and abundance of water-soluble vitamins and minerals (Tremoullier *et al.*, 1980). Cultivated for both culinary and medicinal purposes, fenugreek seeds are notable for their richness in albumin and mucilaginous compounds (Vollak and Stodola, 1983). Traditionally, they have been used to alleviate diabetes, digestive ailments, and inflammatory conditions (Raju and Rao, 2012). Several studies have been conducted on fenugreek seeds and the effects of their bioactive compounds. According to Naidu *et al.* (2011), fenugreek is a good source of proteins (27.5%), soluble (30.6%) and insoluble (20.6%) dietary fibers, crude fats (6.71%), and minerals such as calcium, iron, and β -carotene.

Fenugreek seeds are aromatic, with a candy-like or pleasantly bitter taste, and contain galactomannans, which are polysaccharides (Sindhu *et al.*, 2012) with antidiabetic (Tavakoly *et al.*, 2018), hypocholesterolemic (Belguith-Hadriche *et al.*, 2013), antimicrobial, galactagogue, anticarcinogenic, carminative,

and anti-inflammatory properties (Pandey and Awasthi, 2015; Wani and Kumar, 2018; Dhull *et al.*, 2020a; Dhull *et al.*, 2021a; Dhull *et al.*, 2021b; Dhull *et al.*, 2023).

Fenugreek seeds, the most widely utilized part of the plant, are small, golden-yellow, and hard, with a characteristic tetrahedral shape (Altuntaş *et al.*, 2005). Once dried, they are used as spices or condiments owing to their fibrous, gummy, and adhesive texture. Botanically, the seeds are endospermic, a structural characteristic that contributes to their functional and nutritional properties (Jani *et al.*, 2009). Several bioactive compounds are predominantly present in fenugreek seeds, including pyridine alkaloids, mainly choline (0.5%), trigonelline (0.2-0.38%), carpaine, and gentianine; free amino acids, including histidine, 4-hydroxyisoleucine (0.09%), lysine, and arginine; flavonoids, mainly isovitexin, apigenin, vitexin, orientin, quercetin, and luteolin; and minerals, particularly iron and calcium (Mehrafarin *et al.*, 2010).

Microscopically, the fenugreek seed comprises a hard, yellow embryo surrounded by a white, semi-transparent endosperm layer (Betty, 2008). Chemical analysis has revealed that the endosperm contains the highest concentrations of saponins (4.63 g/100 g) and proteins (43.8 g/100 g), whereas the husk is richer in polyphenolic compounds. Extracts from the endosperm, husk, and whole seed demonstrated notable antioxidant capacities of 72%, 64%, and 56%, respectively, as determined using a free-radical scavenging assay (Naidu *et al.*, 2011).

According to Wani and Kumar (2018), fenugreek is globally valued both as a spice and as a functional ingredient, enhancing food flavour and texture while exhibiting pharmacological effects such as antidiabetic, anticarcinogenic, hypo-cholesterolemic, antioxidant, and immunomodulatory activities. It is also used as an adhesive, emulsifier, and stabilizer in many food products. An emulsion is a mixture of two liquids that do not normally mix, such as oil and water, in which one liquid is dispersed as tiny droplets within the other (Kale and Deore, 2017). In water-in-oil (W/O) emulsions, water droplets are dispersed throughout the oil phase (Çinar, 2017). Such systems are widely used in cosmetics, pharmaceuticals, agriculture,

and food products (Ito *et al.*, 2017). They are particularly useful for encapsulating bioactive molecules, protecting enzymes, and controlling the release of hydrophilic compounds (Zhu *et al.*, 2019). A typical purpose of developing such systems is to deliver compounds with health-promoting or antimicrobial properties.

However, emulsion stability remains a major challenge because these systems are thermodynamically unstable (Goodarzi and Zendehboudi, 2019). Despite this inherent instability, the rate of phase separation can be sufficiently slow to allow an emulsion to remain metastable over time (Salager, 2000). Typically, W/O emulsions are less stable than oil-in-water (O/W) systems due to the greater mobility of water droplets, which may promote sedimentation, flocculation, or coalescence. In addition, stabilization in these systems relies mainly on steric hindrance because of the low electrical conductivity of the oil phase (Sulaimon, 2018).

Given their composition and functional properties, legumes and their components, such as proteins and starch, are increasingly being considered as health-promoting food ingredients for applications in the pharmaceutical and nutraceutical fields (Zandi *et al.*, 2015; Punia *et al.*, 2019; Dhull *et al.*, 2020b; Dhull *et al.*, 2022a; Dhull *et al.*, 2022b; Niazi *et al.*, 2023).

On the other hand, aqueous extracts of fenugreek seeds are also rich in diverse biomolecules. Benziane and collaborators (2019) concluded that aqueous extracts of fenugreek seeds are characterized by the presence of numerous phytochemical biomolecules, such as tannins, saponins, flavonoids, terpenes, nitrogen compounds, total polyphenols, and total alkaloids. In addition, aqueous extracts of fenugreek seeds have demonstrated considerable antioxidant activity (Benziane *et al.*, 2019).

In this context, the objective of the present study was to evaluate the physicochemical properties of the aqueous extract obtained by macerating fenugreek seeds and to assess its emulsifying properties.

Materials and Methods

Aqueous extract

In our experimental study, we placed significant emphasis on the careful selection

of raw materials to ensure their high quality and alignment with our research objectives. Specifically, we employed premium-grade fenugreek seeds (*Trigonella foenum-graecum* L.) as the primary plant material for the aqueous extraction process. These seeds were meticulously chosen based on their well-documented characteristics and their relevance to our study. The variety of fenugreek seeds used in this study was characterized by a hard seeds that are brownish-yellow in color, polyhedral and rhomboidal in shape.

The fenugreek seeds used in this research were collected from the Biskra region in Algeria, a representative area of the North African arid zone. This region experiences minimal precipitation. It is a typical arid climate, characterized by its high temperatures and aridity. The identification process was conducted by the Scientific Research and Technical Center on Arid Regions (CRSTRA) in Biskra to ensure that we had the appropriate seedlings (Benziane *et al.*, 2019). These individuals possess an exceptional understanding of flora that thrive in harsh, arid environments. We were able to rely on the seedlings we used in our experiments due to their expertise.

In order to maintain consistency and adhere to established protocols, we selected fenugreek seeds. By implementing these additional measures in our seed selection process, we enhance the reliability of our findings. In essence, we are aware of the precise nature of our materials, which lends credibility to our endeavor. All experiments were performed in triplicate ($n = 3$), following common practice for physicochemical and emulsifying analyses, and the results are expressed as mean $\pm$ standard deviation (SD).

First, the fenugreek seeds were cleaned and separated. Then, I mashed them together. To make the water-based extract, Benziane *et al.* (2019) and Harchane *et al.* (2012) say that you need to soak 25 grams of seeds in 100 ml of water for 24 hours at room temperature.

The chemical and physical properties of fenugreek seed

We checked a lot of things to make sure the extract was correct. We utilized an AOAC (2002) pH meter to find out whether it was acidic or basic. We used a Hoesppler BH2

ball-fall viscometer to find out how thick the liquid was. The AOAC (1990) method uses the amount of inorganic material left behind after burning a sample, or ash content, to figure out what minerals are in it. To find the density, we used a pycnometer to measure the mass-to-volume ratio.

We also utilized the AOAC (1990) method for my electrical conductivity test. This test shows the ionic makeup of the extract by seeing how well it carries an electric current.

Finally, we utilized a refractometer to check the dry matter content and refractive index according to the AOAC (2000) rules. Thanks to all these tests, we now have a better understanding of what the extract really is.

Emulsifying properties

The emulsion system was formulated using extra virgin olive oil as the dispersed phase and an aqueous fenugreek extract as the continuous (dispersing) phase. The emulsions were prepared either with or without sodium caseinate, which acted as a stabilizing agent. The components were mixed in a volume-to-volume ratio (V/V) of 0.05263%, and each formulation was homogenized at 20°C using an Ultra-Turrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik) operated at 8000 rpm for 30 minutes.

The mean droplet diameter of the fat globules was determined under a light microscope (PHYWE, Hund Wetzlar, Germany) equipped with a micrometer scale (0-10 μm , with 0.1 μm divisions). Based on these observations, the interfacial surface area was subsequently calculated according to the equations described by Acem and Choukri (2012) and Arditty *et al* (2005).

To assess the physical stability of the emulsions, emulsion stability and stability index were quantified using spectrometric analysis, following the methodology outlined by Acem and Choukri (2012) and Pearce and Kinsella (1978). In general, emulsion stability refers to its ability to produce an emulsion that remains stable over time in the face of creaming, which is related to the coalescence of droplets.

Emulsion stability can generally be characterized by the emulsion stability index,

which indicates the number of hours required for total destabilization of the emulsion, i.e., to find $At \approx 0$ (absorbance in time t).

The stability (S) and stability index (SI) of the emulsion are determined by measuring the absorbance (A) of a diluted emulsion at a specific wavelength (500 nm) using a UV-visible spectrophotometer.

Statistical analysis

Statistical analysis of the experimental data was performed using Excel statistical software (Kumar, 2023). At a confidence level of 95%; the results were presented using statistical parameters corresponding to the mean of the replicates, i.e., three for each parameter, with the standard deviation and standard error.

Results and Discussion

Physicochemical Parameters

The Figure 1 showed average physicochemical parameters of the aqueous extract of seeds fenugreek.

The aqueous extract obtained by maceration of fenugreek seeds was characterized by remarkable physicochemical parameters, namely: ash ($0.172 \pm 0.001 \text{ g L}^{-1}$), dynamic viscosity ($1.19 \pm 0.04 \text{ cP}$), dry matter ($6.75 \pm 0.02\%$), refractive index (1.345 ± 0.001), density (0.964 ± 0.014), and pH (6.32 ± 0.21).

The physicochemical parameters analyzed of the aqueous extract obtained by maceration of fenugreek seeds were related to the extraction method, the ratio of seeds/solvent, the variety of seeds, the physico-chemistry of the potable water, the quality and the quantity of water-soluble metabolites. These findings are consistent with previous studies (Adrian *et al.*, 1981; Amellal, 2008; Messaid, 2008). The results of the study of Buba *et al.* (2015) on physicochemical properties of fenugreek seeds powder suggested that the aqueous extract of fenugreek contains bioactive constituents that may be beneficial as a spice in food and management of diseases.

This could be due to the effect of physicochemical properties of potable water and maceration conditions on the solubility of molecules, minerals, and dissolved gases in the aqueous phase. According to Acem and Choukri (2012), the density and the viscosity

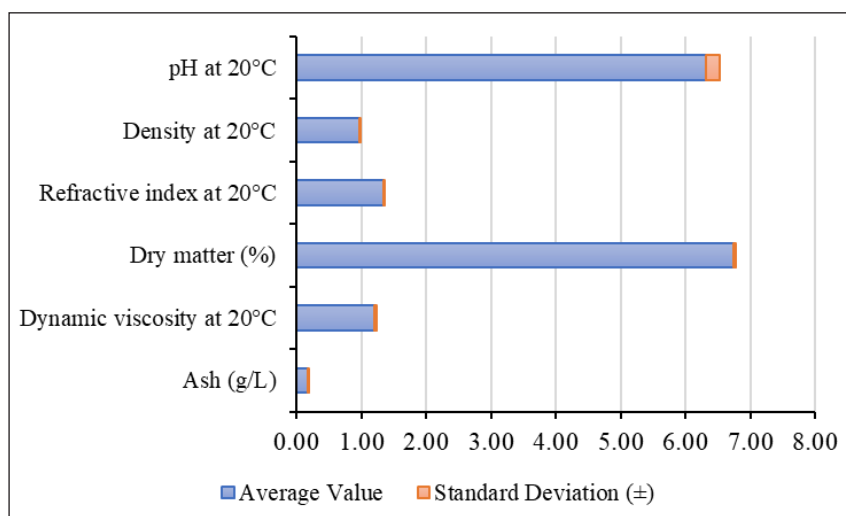


Fig. 1. Averages values of physicochemical parameters of aqueous extract of seeds fenugreek.

of product depend on its dry matter and fat content as well as temperature.

These findings suggest that the refractive index depends on the chemical composition of the substance being studied, as reported by Adrian *et al.* (1981). The pH values of the aqueous extracts were found to be slightly neutral. The variability of the pH of a solution is strongly linked to the concentration of H⁺ ions.

According to the findings reported by Benziane *et al.* (2019), the analytical investigation of aqueous extracts from fenugreek seeds revealed that this species possesses a distinctive metabolic profile enabling it to synthesize various bioactive compounds, particularly flavonoids, which play a crucial protective role against both biotic and abiotic stresses affecting the plant. The same study further indicated that phytochemical screening and quantitative analysis of the aqueous extract obtained through maceration of fenugreek seeds demonstrated a remarkable diversity of secondary metabolites, including tannins, saponins, terpenes, nitrogenous compounds, polyphenols, alkaloids, sterols, and polyterpenes, among others.

Emulsifying properties

Diameter of fat globules: The Fig. 2 presented the kinetics of the average diameter of fat globules of emulsions. Generally, the evolution of the average diameters of fat globules in emulsions tends to increase over time, especially in emulsions prepared with

stabilizers. In line with this, studies have shown that stabilizers play a crucial role in controlling the size and stability of fat globules in emulsions (Desrumaux and Marcand, 2002). These stabilizers help to inhibit coalescence and flocculation, thus preventing the aggregation of fat globules and leading to smaller average diameters (Singh and Ye, 2013).

In the case of emulsions formed from aqueous extract without stabilizers, an increasing rate of fat globule diameter over time has been observed. In the study, the diameter of fat globules in the analyzed emulsions ranged from 0.43 to 1.2 μm . This increase in size can be attributed to the absence of stabilizers, which are known to provide a protective layer around the fat droplets, preventing their aggregation and subsequent growth (Singh and Ye, 2013).

On the other hand, when the aqueous extract of fenugreek seeds is combined with a stabilizer such as sodium caseinate, the emulsion demonstrates a growing and slightly stable rate in terms of fat globule diameter. Research has shown that fenugreek seeds possess natural emulsifying properties, which can contribute to the stability of the emulsion (Garti and Leser, 2001). The addition of sodium caseinate as a stabilizer further enhances the emulsion's stability by forming a protective barrier around the fat globules, limiting their growth. For instance, in the study, the diameter of fat globules in such emulsions evolved from 0.3 to 0.55 μm over time.

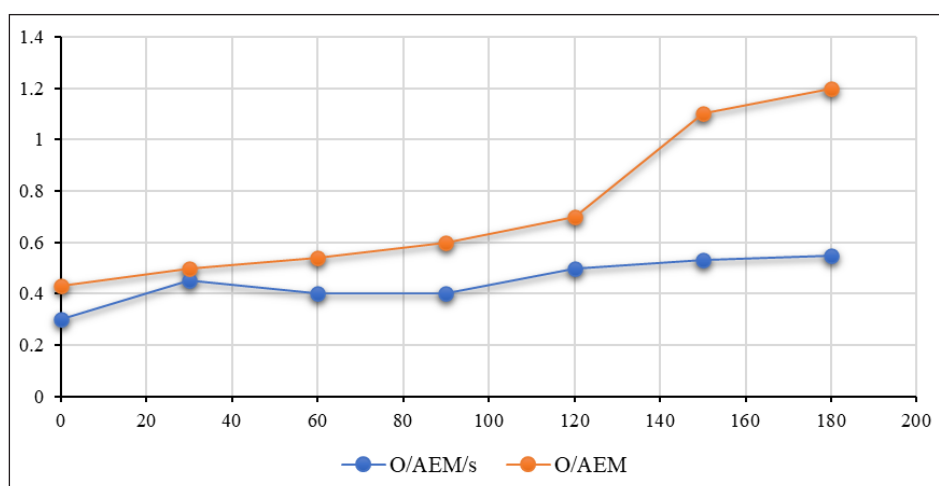


Fig. 2. Kinetics of the average diameter of fat globules of emulsions at base on aqueous extracts of fenugreek seeds over time. (O/AEM/s: Emulsion at base virgin olive oil and aqueous extract macerated with sodium caseinate as stabilizer; O/AEM: Emulsion at base virgin olive oil and aqueous extract macerated without sodium caseinate as stabilizer).

Interfacial surface: The Fig. 3 shows the kinetics of the interfacial surface of emulsions as a function of time.

On the other hand, the evolution of the interfacial surface of emulsions tends to decrease over time, particularly in emulsions prepared with stabilizers. Stabilizers play a crucial role in reducing the interfacial area between the oil and water phases, resulting in a lower interfacial surface (Kowalczyk and Baraniak, 2014). These stabilizers form a protective layer around the oil droplets, inhibiting coalescence and promoting a more compact interface.

In the case of emulsions formed from an aqueous extract without stabilizers, a decreasing rate of interfacial surface over time has been observed. For instance, a study reported a

reduction in the interfacial surface area from 6 to 2 m² in such emulsions. The absence of stabilizers allows for increased interfacial mobility and facilitates the coalescence of oil droplets, leading to a larger interfacial surface (Kokal, 2005).

Conversely, when the aqueous extract of fenugreek seeds is combined with a stabilizer, the emulsion demonstrates a gradual and relatively stable rate of interfacial surface. Fenugreek seeds possess inherent emulsifying properties that contribute to the stability of the emulsion (Kasran *et al.*, 2013). The addition of sodium caseinate further enhances stability by reducing the interfacial surface area. In our study, the interfacial surface of such emulsions evolved from 8 to 6 m² over time.

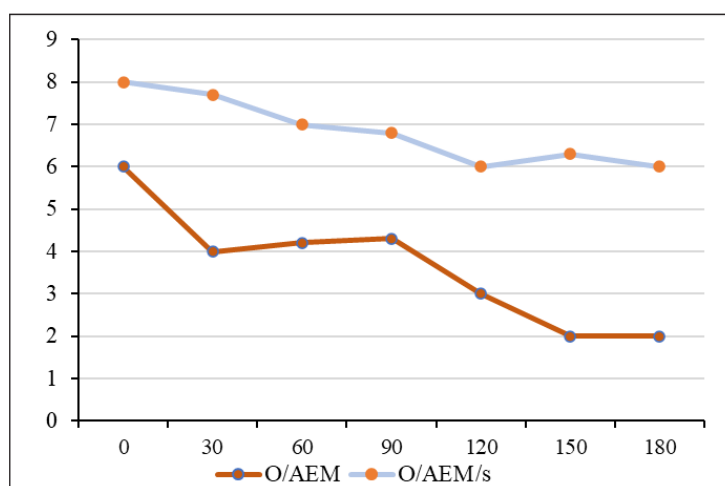


Fig. 3. Kinetics of interfacial surface of emulsions at base on aqueous extracts of fenugreek seeds over time.

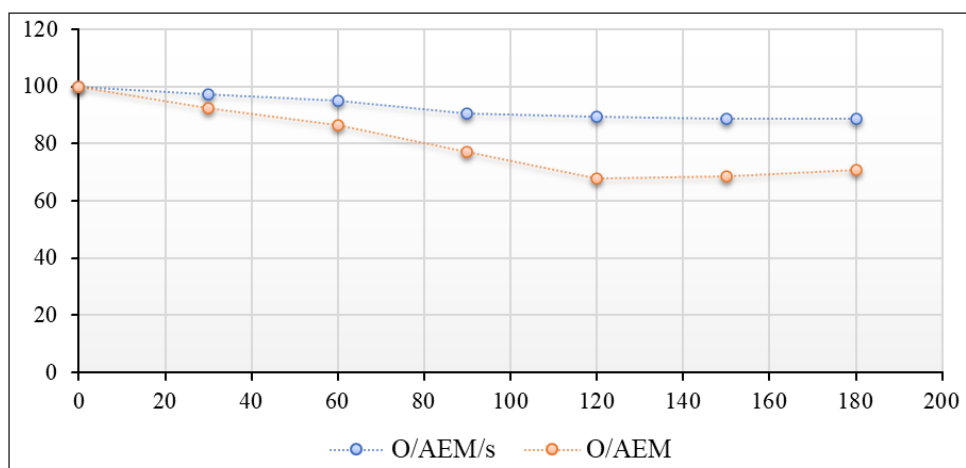


Fig. 4. Kinetics of the stability of emulsions at base on aqueous extracts of fenugreek seeds over time.

Stability

The Fig. 4 shows the kinetics of stability of emulsions over time.

The same observation was recorded for emulsion stability. The evolution of the stability of the emulsions tends to decrease over time with two different rates and the higher values were observed for the emulsions prepared with stabilizer.

The emulsion formed from aqueous extract and without stabilizer showed a decreasing rate over time with a stability which varies from 100 to 67.9%; and the emulsion prepared with the aqueous extract of fenugreek seeds and the stabilizer (sodium caseinate) showed a low decreasing and a high stability, the stability of which varied from 100 to 88.85%.

Stability index: The figure 5 shows the kinetics of the stability index of different emulsions over time.

For the stability index of emulsions, the estimated number of hours until complete destabilization of the emulsion varies depending on the formulation. One study reported that the emulsion formed from the aqueous extract of fenugreek seeds and the stabilizer (sodium caseinate) exhibited a stability index of approximately 22 hours. The presence of stabilizers, such as sodium caseinate, contributes to the formation of a more robust interfacial film, resulting in improved emulsion stability and a longer duration before destabilization occurs.

In contrast, the emulsion prepared without a stabilizer, based on the aqueous extract alone, recorded a lower stability index of approximately 8 hours. The absence of

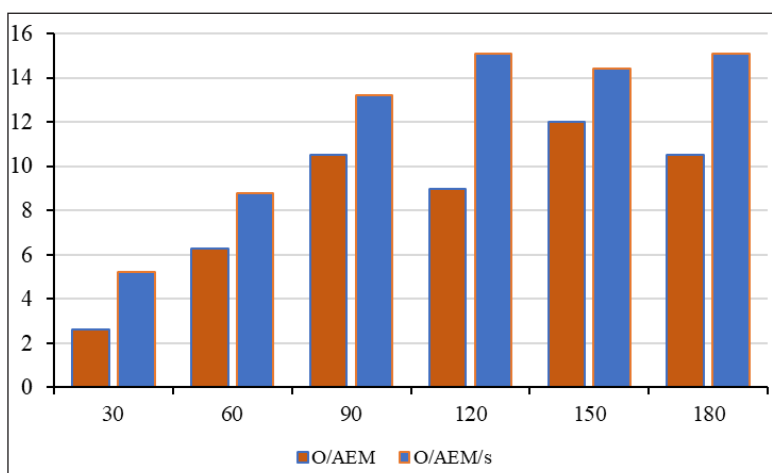


Fig. 5. Kinetics of stability index of emulsions at base aqueous extracts of fenugreek seeds over time.

stabilizers in this formulation leads to weaker interfacial interactions and a higher propensity for droplet coalescence, resulting in a shorter stability duration.

Microscopic aspect: The Fig. 6 showed the microscopic aspect of emulsions (magnification $\times 100$).

In the emulsion made with virgin olive oil and aqueous extract without sodium caseinate as a stabilizer (O/AEM) (Figure 6(a)) there are these big, red, spherical fat globules. They don't really blend into the blue-colored aqueous extract; instead, they clump together because they've coalesced, and they're larger than the ones shown in Fig. 6(b). Take away the sodium caseinate, and you end up with poor interfacial protection between the oil and water. That lets the fat droplets stick together more easily, so you get these bigger clusters and a pretty uneven emulsion. Overall, it just doesn't hold up well physically.

Now, with the version that includes sodium caseinate as a stabilizer (O/AEM/s) (Figure 6(b)), it's a different story. Here, the red fat globules stay nicely separated and float evenly throughout the blue aqueous extract. You

don't see them agglomerating no coalescence happening here and they're noticeably smaller than those in Figure 6(a). Adding sodium caseinate really helps, since it forms a protective layer around each droplet, keeping them from glomming together. This leads to smaller, evenly spread-out fat globules, which shows just how much sodium caseinate boosts the emulsion's microstructural stability.

According to Kinsella (1979), the mechanism of fat or oil absorption is primarily governed by the physical entrapment of oil within the matrix. This property is a key determinant of flavor retention, whereas fat emulsion capacity and stability represent essential characteristics for ingredients used as emulsion stabilizers (Ziani *et al.*, 2011; Chau and Cheung, 1997) said that both the surface area and the hydrophobicity of food items have a large effect on how effectively they soak up oil. Proteins from fenugreek seeds dissolve better in acidic and alkaline circumstances than they do in neutral situations. They also operate better than other beans when it comes to emulsifying and foaming, which makes them great for salad dressings, meat products, ice cream, cake mixes, and mayonnaise (El Nasri and El Tinay, 2007).

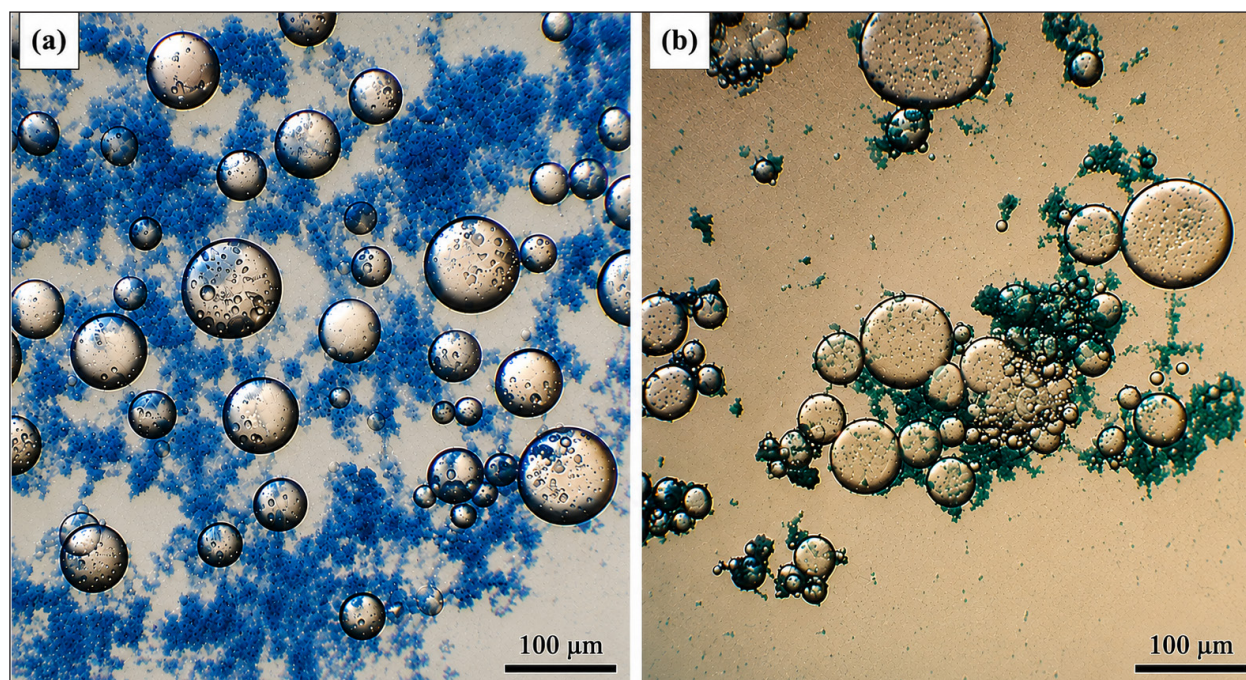


Fig. 6. The microscopic aspect of emulsions after three hours of their stability. (Images were observed using an optical microscope at $\times 100$ magnification with a scale bar of $100\ \mu\text{m}$. (a) Emulsion based on virgin olive oil and aqueous fenugreek extract obtained by maceration, stabilized with sodium caseinate (O/AEM/s), and (b) emulsion based on virgin olive oil and aqueous fenugreek extract obtained by maceration, without sodium caseinate as a stabilizer (O/AEM)).

Kiosseoglou and his colleagues (1999) discovered that the way you handle seed proteins and what they're comprised of influences how well they mix together. Other studies have shown that emulsion capacity is significantly influenced by the hydrophilic-lipophilic balance (HLB), which varies with variations in pH. El Nasri and El Tinay (2007) noticed that fenugreek protein concentrate mixes best at pH 10 about 69.2%. When the pH drops to 7, its mixing ability falls to 65.35%, and at pH 4.5, it drops even further to 41.3%. Basically, fenugreek proteins still break down in both acidic and basic settings. According to Kinsella (1979), proteins that blend well usually perform better in other ways too, like emulsifying and foaming. That's especially useful in baking, like when making cookies.

A lot of research shows that emulsifying ability doesn't rely on just one thing. Net surface charge, pH, interfacial tension, protein structure, and how sodium chloride interacts with the protein (the "salting-in" effect) all matter (Hung and Zayas, 1991; Lawal *et al.*, 2004). Similarly, Góral and Wojciechowski (2020) observed that saponin-rich extracts differ markedly in their ability to lower surface tension, form viscoelastic interfacial layers, and stabilize foams.

Proteins, by nature, are amphiphilic molecules composed of both hydrophilic and hydrophobic regions, allowing them to act as effective natural surfactants that readily adsorb at oil-water interfaces, even at low concentrations in aqueous solutions (Murphy *et al.*, 2010). According to Mathieu (1998), emulsion destabilization occurs when the equilibrium of intermolecular forces is disturbed by external parameters such as pH, ionic strength, or temperature. Likewise, Xalabarder (1994) reported that the stability index of an emulsion depends on both the type of oil and the stabilizing medium employed. The system will be stable as long as the particles in a dispersed system don't come together (Mathieu, 1998; Cheftel *et al.*, 1985). Murphy and colleagues (2010) say that smaller droplets make the overall surface area bigger, which frequently makes the emulsion more stable (Jensen *et al.*, 2000).

Plant and dairy proteins are often used in culinary practices due to their capacity to

facilitate emulsification. Amine *et al.* (2014) discovered that protein type, dose, and water pH substantially influenced their effectiveness. Amine *et al.* (2014) shown by the pendant drop method that augmenting the concentrations of sodium caseinate, pea, and potato proteins diminishes interfacial tension and decreases the average droplet size.

The pH of the aqueous phase also plays a crucial role in emulsification efficiency. At higher pH values, protein molecules tend to unfold, enhancing emulsifying properties through increased electrostatic repulsion. However, as Amine *et al.* (2014) noted, while interfacial elasticity contributes to stability, it alone is not sufficient; other physicochemical factors must be considered. Moreover, an excessively low interfacial tension may disrupt the measurement of interfacial elasticity. To produce smaller droplets, it is necessary to reduce interfacial tension by increasing the protein concentration; however, beyond a certain point, excess protein can lead to a reduction in interfacial elasticity, ultimately decreasing emulsion stability (Amine *et al.*, 2014).

The average droplet size in an emulsion system is strongly influenced by the interfacial tension between the dispersed and continuous phases (Schroën *et al.*, 2020; Ravera *et al.*, 2021). Surfactant molecules form a stabilizing interfacial film around oil droplets, providing mechanical strength to the interface and minimizing the attractive forces responsible for droplet coalescence and aggregation (Zhao *et al.*, 2020; Kovalchuk *et al.*, 2018).

During emulsion destabilization, the droplets located at the bottom of the sample gradually migrate upward. As they rise, they tend to aggregate and merge into larger droplets, which continue to ascend and eventually coalesce into the upper oil layer, resulting in progressive oil-water phase separation (Dekker *et al.*, 2021; Abedi *et al.*, 2019).

Sodium caseinate (NaCas) has proven to be a highly effective emulsifying protein for the formation of stable oil-in-water (O/W) emulsions. Owing to its high surface activity, it promotes the formation of small, stable droplets by inducing electrostatic repulsion among oil droplets, while maintaining a moderate interfacial tension (Fang and Dalgleish, 1993).

The minimum protein load required for caseinate-stabilized emulsions is approximately 1 mg/m²; below this threshold, droplets are prone to aggregation and coalescence. Nonetheless, a caseinate concentration as low as 0.3% (w/w) has been found sufficient to yield a stable emulsion (droplet diameter < 1 µm) containing 20% (w/w) oil (Fang and Dalgleish, 1993).

As reported by Walstra *et al.* (2005), NaCas product derived from the neutralization of acid-precipitated casein consists of α s1-, α s2-, β -, and κ -casein in a ratio of 4:1:4:1, respectively. Among these, α s1- and β -casein display the highest surface activity, enabling rapid adsorption at the oil-water interface, thereby enhancing emulsion stability (Dickinson, 1994).

The findings of Ma and Chatterton (2021) demonstrated that high-energy processing methods such as high-pressure homogenization (HPH), ultrasonication (US), and microfluidization (MF) effectively reduce droplet size and enhance emulsion stability. Another approach involves incorporating biopolymers that either form complexes with NaCas or interact independently. In complex-forming systems, biopolymers associate with NaCas, increasing the thickness of interfacial layers and reinforcing electrostatic and steric repulsion. Conversely, in non-complexing systems, biopolymers enhance emulsion stability by increasing the viscosity of the aqueous phase and forming a weak structural network that limits droplet mobility and coalescence (Ma and Chatterton, 2021).

The protein-to-oil ratio really shapes how stable NaCas-stabilized emulsions turn out. If there's not enough protein compared to oil, you run into bridging flocculation basically, there's not enough NaCas to cover the droplets, so the protein molecules end up connecting neighboring droplets like little bridges, and that clumps everything together. On the flip side, when there's way too much protein, you get depletion flocculation. Here, the extra NaCas floating around in the continuous phase creates forces that pull droplets together (Czemierska *et al.*, 2015; Liang *et al.*, 2014). If you see instability at high NaCas concentrations, bumping up the oil content helps. At around 30% oil (by weight) and NaCas between 5 and 10%, emulsions get a lot more stable. What's happening is that

a three-dimensional network forms in the continuous phase, and the viscosity goes up this slows down droplet movement and keeps them from clumping or rising to the top (Liang *et al.*, 2014). Still, if there's not enough protein actually sitting at the droplet interface, droplets can merge and the emulsion can break down (Liang *et al.*, 2014).

Consequently, flocculation and coalescence are induced either by pH adjustment toward the pI or by calcium ion addition. This destabilization is associated with both a loss of electrostatic repulsion, due to reduced surface charge, and a collapse of steric barriers, caused by the retraction of charged molecular tails (Ma and Chatterton, 2021).

The primary function of emulsifiers is to facilitate the formation of fine droplets during homogenization by rapidly adsorbing to newly created interfaces, lowering interfacial tension, and providing a protective layer that prevents droplet aggregation (Walstra, 2003). Furthermore, advances in physical, chemical, and membrane-assisted technologies have been shown to improve the techno-functional performance of surfactants. For instance, ultrasound treatment significantly enhances the emulsifying and foaming properties of proteins, increasing their potential for industrial applications (Cheftel *et al.*, 1985; Taha *et al.*, 2020).

Conclusions

The study of the physicochemical and emulsifying properties of the aqueous extract of fenugreek seeds (*Trigonella foenum-gracum* L.) revealed interesting results in terms of both physicochemistry and functionality. The physicochemical parameters analyzed in the aqueous extract obtained by maceration of fenugreek seeds were related to the extraction method, the seed/solvent ratio, the seed variety, the physicochemistry of the potable water, and the quality and quantity of water-soluble metabolites. Compared to the emulsion (O/AEM), the best emulsifying behavior of the aqueous extract of fenugreek seeds was observed in the emulsion based on extra virgin olive oil dispersed in the macerated aqueous extract and in the presence of sodium caseinate as a stabilizing agent (O/AEM/s). The emulsifying properties of the aqueous extract obtained by maceration are closely related to

the quality and composition of the emulsion, the speed, time, and emulsification technique. In conclusion, this study will open up new approaches to improving the aqueous extract of fenugreek seeds obtained by maceration in order to enhance its value in the food and non-food sectors.

Conflicts of Interest

The authors declare no conflict of interest.

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