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Optimization of Asafoetida gum extraction and assessments of emulsion stability

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Abstract

Response surface methodology was applied for optimization of alcoholic extraction of Asafoetida gum from *Ferula assa-foetida* L. The effects of time, temperature, ethanol to gum ratio and pH on the purity and physico-chemical properties of that gum were investigated. Extraction parameters significantly affected purity (ranged from 55.5 to 76.4 %), protein content (between 5.2 and 12.7 %), solubility (19 to 96 %), particle size (100 to 900 μm), particle size distribution (span) (0.6 to 5.4), foam capacity (20-115 %) and foam stability (13-111 %) of the gum. Effect of different concentration (5, 10, 15 and 20 % w/v) of optimum extracted gum on stability and viscosity of emulsions was studied over 28 days. The concentrations below 20%w/v showed creaming after 4 weeks. Particle size, span and microscopic analysis confirmed droplet size reduction. No significant alteration in emulsion stability and droplet size was observed over storage due to viscosity enhancement.

Key words: *Ferula assa foetida*, extraction, Asafoetida gum, arabinogalactan-protein, polysaccharides, emulsion

1. Introduction

The term oleo-gum-resin refers to products composed of a mix of essential oil, water-soluble gum and alcohol-soluble resin. These gums are obtained from some terrestrial plants as exudates after scraping the plant body. They are also generated by some natural incidences affecting the plant such as attacks by insects and animals (Nussinovitch, 2010). Myrrh and frankincense are the two well-known oleo-gum-resins usually found in African countries (Chikamai & Odera, 2002). However, literatures recently introduced other sources of natural oleo-gum-resins (Jalali et al., 2011; Sarup et al., 2015) some of them having a potential to compete with traditional gum exudates used as hydrocolloids such as Arabic gum, karaya gum and tragacanth gum (Phillips & Williams, 2000).

Ferula assa-foetida is a plant from *Apiacieae* family, native from desserts of Iran and Afghanistan. The plant root produces a fetid smell oleo-gum-resin (Anghouzeh), collected in summer by scarification. This oleo-gum-resin is composed of three fractions, including resin (40-64 %), gum (25 %) and volatile oils (3-17 %) (Fatehi et al., 2004). The main composition of resin is ferulic acid esters, coumarin, sesquiterpene coumarins and other terpenoids derivatives, soluble in ethanol and methanol. Volatile oils consist of sulphur-containing compounds, and other monoterpenes (Iranshahy & Iranshahi, 2011). The resin and volatile oil are frequently used as herbal medicine (Iranshahy & Iranshahi, 2011). The nutraceutical and functional properties of resin and/or volatile part on gut health was established recently (Vijayasteltar et al., 2017). The gum part of this oleo-gum-resin was recently characterized as a β-D-galactan with side chains composed of α-L-arabinofuranose, α-L-rhamnopyranose and D-glucuronic acid (Saeidy et al., 2018b). The average molecular weight (M_w) of this arabinogalactan identified as covalently linked to a minor proteic part (6.8 %) was 1.5×10^5 $g.mol^{-1}$. The glycoproteic nature of Asafoetida gum makes it suitable for functional applications such as emulsifier, coating agent or encapsulation of oil materials.

Rheological and emulsion properties of Asafoetida gum have been studied and compared to those of Arabic gum (Saeidy et al., 2018a). It was concluded that Asafoetida gum displays higher emulsion capacity (881.0 ±2.0 mg oil. g^{-1} gum powder against 773.0 ± 7.8 mg oil. g^{-1} gum powder) and stability (75.0 ± 0.7 % against 66.1 ± 0.4 %) than Arabic gum in O/W emulsions and can be used as a potential food ingredient. Moreover, the emulsions prepared with Asafoetida gum solution (2 %,w/w) and colza

oil (oil/gum solution ratio of 2, 3 and 4 %v/v) showed high stability over 28 days of storage time (Saeidy et al., 2019). However, no investigations were performed focusing on the effect of different Asafoetida gum on O/W emulsion properties.

In this context, the Asafoetida gum was extracted and purified using alcoholic (ethanol) extraction. The effects of time, temperature, ethanol to gum ratio (E/G) and pH on physicochemical and functional properties of the gum were studied through 30 runs of response surface methodology design. The optimum condition was selected by desirability function of numerical optimization. Then, the stability and viscosity of emulsions prepared using different concentrations of Asafoetida gum was surveyed.

2. Materials and methods

2.1. Materials

Scarification of roots of *Ferula assa-foeitida* plant was performed in Tabas (South Khorasan province, Iran) during June 2015. Oleo-gum-resin was collected, air dried and kept in freezer at -18°C until the analysis. All the chemicals were purchased from Merck (Merck, Germany).

2.2. Methods

2.2.1. Purification of Asafoetida gum

The dried yellow-brownish oleo-gum-resin was milled and suspended in several volumes of ethanol (96 % v/v) to remove resins and essential oils. Extractions were performed by heating the mixture under reflux at different temperatures (46-78°C). Ethanol to gum ratio (E/G) ranged from 5 to 45 % (w/w) and different extraction times (2-8 h) were applied. After extraction, the ethanol insoluble precipitates called ethanol extracted materials (EEM) were collected by centrifugation (10000 × g, 30 min, 20°C) and oven dried at 80°C. EEM were then extracted with five fold of distilled water at 45°C for 30 min and passed through cloth filter in order to remove water insoluble materials. The pH of filtrates (gum solutions) was adjusted at several values ranging from 3.5 to 9.5 and the gum solution was kept at room temperature during two hours. Gum solutions were centrifuged at 1500 × g (Ø30 × 115 mm tubes, rotor angel 35°C, Sigma 2-16PK, Germany) at 20°C for 15 min and the supernatant

was precipitated overnight with 3 volumes of ethanol (96 %v/v) at 4°C. Precipitates were collected and dissolved in 100 ml of distilled water before to be freeze dried. The obtained gums were passed through a 40 mesh sieve and stored at -18°C.

2.2.2. Experimental design

Response surface methodology (RSM) was used to study the effects of time (X_1), temperature (X_2), ethanol to gum ratio (E/G) (X_3) and pH (X_4). A central composite design (CCD) with 6 points was employed for designing experimental data using Design-Expert 10.0.3 trial software (Stat-Ease Inc., Minneapolis, MN, USA). **Table 1** gives independent variables and their values related to codes in RSM. Effects of each factor were studied at five levels.

2.2.3. Purity and protein content

Purity was reported as total sugar content (%) expressed as glucose equivalent of purified gum using phenol sulfuric method (DuBois et al., 1956). Nitrogen content was analyzed by Kjeldahl procedure after digestion and distillation using VELP system (VELP Scientific Srl, Via stazione, Italy) (AOCS Ba 4a-38). A conversion factor of 6.25 was used to convert it into to protein content. All assays were done in triplicate

2.2.4. Solubility

Solubility of Asafoetida gum was measured with an adapted method of Amid and Mirhosseini (2012). Aqueous solutions of gum at 1 % (w/v) were prepared by stirring during 30 min. Then, the solutions were centrifuged at 6000 × g (Ø17 × 120 mm tubes, rotor angel 35°C, Sigma 2-16PK, Germany) at 20°C for 30 min. Supernatant were oven-dried during 12 h at 105°C. Solubility was calculated using the equation (1):

$$\text{Solubility } (\%) = (C_1/C_2) \times 100 \quad (1)$$

where C_1 and C_2 are concentration of supernatant and initial gum solution, respectively.

2.2.5. Gum particle size

The volume weighted mean was determined by a laser particle size analyzer (Horiba, LA-930, Japan). In this experiment, 0.5 ml of 3 % (w/v) gum solution was injected into the cell of analyzer system and thoroughly diluted with distilled water. The average sizes of particles were measured by light beam scattering. Volume weighted means were reported as $D_{4,3}$ using the equation (2):

$$D_{4,3} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3 \quad (2)$$

where n_i is the number of particles with d_i diameter.

Particle size distribution or span was estimated using the equation (3):

$$\text{Span} = [D(v, 0.9) - D(v, 0.1)]/D(v, 0.5) \quad (3)$$

where D(v, 0.1), D(v, 0.5) and D(v, 0.9) are diameters at 10 %, 50 % and 90 % cumulative volumes, respectively. Results were monitored by Horiba software LA-930. Measurements were performed at least for three times.

2.2.7. Foaming properties

Foaming capacity (FC) and foaming stability (FS) were determined using an adapted method from literatures (Shahidi et al., 1995; Wang et al., 2010). Briefly, solutions of gum at 3 % (w/v) were prepared and stirred for 30 min prior to be homogenized at 15000 rpm for 2 min using an Ultra Turrax homogenizer (Ultra Turrax, model T18, Janke Kunkel, IKA Labortechnik, Germany). The whipped solutions were immediately transferred to a graduated cylinder. FC was reported as percentage of increased volume after whipping gum solution while FS was defined as a percentage of foam volume remained after 30 min. Foaming properties was calculated using equations (4) and (5).

$$FC\ (\%) = [(V_1\text{-}V_0)/V_0] \times 100 \quad (4)$$

$$FS\ (\%) = [(V_t\text{-}V_0)/V_0] \times 100$$

(5)

where V_0 is volume of gum solution before whipping and V_1 and V_t are volumes immediately and 30 min after whipping, respectively. All measurements were done in duplicate.

2.2.8. Statistical analysis and optimization

Response surface methodology (RSM) was used to determine the desirable statistical model for responses including purity, protein content, solubility, volume weighted mean, particle size distribution, FC and FS based on simultaneous optimization of responses by desirability function approach. The quadratic model (equation 6) was proposed as the best for predicting optimal point:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum_j \beta_{ij} X_i X_j + e$$

(6)

where Y is the response function, β_0 is a constant coefficient of the model and β_i, β_{ii} and β_{ij} are the linear, quadratic and interaction effects of the model, respectively. Analysis of variance (ANOVA) was performed to define significance of the statistical model, coefficient estimation of each component and interaction between them.

According to previous researches (Kuhnt & Rudak, 2013; Yuan et al., 2015), when optimizing multiple responses in a single experimental design the optimal point of each factor does not necessarily coincide. Therefore, in order to reach a compromised result, the desirability function is required for estimating the optimal point by the accepted equation of Derringer and Suich. While the desirability function for each response is expressed as $d_i(y_i)$, the overall desirability (D) represents the combination of individuals considering equation (7):

$$D = (d_1(y_1) \times d_2(y_2) \times \ldots d_m(y_m))^{1/m}$$

(7)

where m represents the number of responses. The desirability of every response may assign zero or one ($d_i(y_i)$=0 or1) if the response is totally undesirable or totally desirable, respectively. Thus,

depending on the desirability requirement, each response is maximized, minimized or assigns a target value; otherwise, it is adjusted to be in range of obtained data so that the desirability is between 0 to1. To optimize the model, responses of purity (Y_1), protein content (Y_2), solubility (Y_3), FC (Y_6) and FS (Y_7) were maximized while the volume weighted mean (Y_4) was in the range and span (Y_5) was minimized with the objective to obtain a highly pure gum powder with the most favorable functional properties.

2.2.9. Emulsion preparation

The pure Asafoetida gum was used to prepare aqueous solutions of 5, 10, 15 and 20 % (w/v) stirring 24 h at 4 °C. To prepare emulsion, sunflower oil (density= 0.92 $g.mL^{-1}$) was added gradually to the gum solution (ratio of gum solution to oil was 80:20 v/v) and homogenize using an Ultra Turrax (T18 device IKA GmbH, Germany) for 5 minutes. 50 mL of emulsion was subjected to sonication during 3 min in an ice bath using Ultrasonic processor (Ningbo Scientz-IID Biotechnology Co., Ltd.) that operated at 97.17 W (the maximum power output was 590 W) with a 10 mm probe diameter. In order to protect the emulsions from microbial spoilage, sodium azide (0.01 w/w) was added and the emulsions were stored at 4°C.

2.2.10. Creaming behavior

The glass test tubes were marked on 8 mL by stickers. Then, 8 mL of emulsions were transferred to glass tubes, capped with parafilm and creaming behavior (CI) was measured at dates of 0, 1, 3, 7, 14, 21 and 28 days using equation (8):

$$\text{Whey off} = \frac{HS}{HE} \times 100 \quad (8)$$

where HS represents the height of serum separated from emulsion and HE shows the total height of emulsion.

2.2.11. Emulsion particle size

Particle size of emulsions were analyzed as explained in section 2.2.5. Emulsion samples were diluted with 0.1 % (w/v) SDS solution at a ratio of 1:100 ratio, mixed thoroughly and injected to device cell.

Oil droplet diameters and particle size distribution were expressed as volume weighted mean ($D_{4,3}$). Measurements were performed in triplicate at dates of 0, 1, 3, 7, 14, 21 and 28 days.

2.2.12. Microscopic analysis

Emulsion microstructure was observed using a light microscope devise (Olympus, Japan) coupled with a digital camera. The samples were magnified by ×1000.

2.2.13. Viscosity measurements

Emulsion viscosity was determined using DV-II Brookfield viscometer (Brookfield Eng. Labs Inc., USA) equipped with a small sample adaptor regulated at 25 °C and at a rotation speed of 200 rpm.

2.2.14. Statistical analysis of emulsion assessments

Statistical analysis of emulsion data was performed using fully factorial design. All the experiments were performed in triplicates and analysis of variance (ANOVA) was used for data processing (SPSS, 20). Least significant difference (LSD) test was applied to compare the data at 5 % significant level.

3. Results and discussion

In the current research, the effects of independent variables including extraction time (X_1), extraction temperature (X_2), ethanol to gum ratio (X_3) and pH (X_4) on the quality and functional properties of the Asafoetida gum were studied. Multiple regression analysis using RSM was applied. It was found that the effects of all variables on responses followed the second-order. Analysis of variance (ANOVA) was carried out to obtain p-value, F-value and lack of fit of the independent variables (**Table 2**).

It was shown that all the response variables were significant (p-value less than 0.05) following quadratic model with regression coefficients (R^2) between 0.90 and 0.97. It represents that the extraction variables had significant effect on physicochemical and functional properties of the gum. The current models showed no significant lack of fit (**Table 2**).

Table 2 also shows the linear, quadratic and interaction effects of extraction variables on physicochemical and functional properties of Asafoetida gum. It can be concluded from p-values that extraction temperature and E/G ratio had higher ($p < 0.05$) effects on the properties of Asafoetida gum contrary to pH. Among all the responses volume weighted mean was affected by the majority of extraction variables. On the contrary, gum purity and foam capacity were affected by the least variables of the extraction. E/G ratio was the most effective factor on the physicochemical and functional properties of gum, as it possessed the highest F-value among variables.

3.1. Gum purity

Purity of the Asafoetida gum was significantly ($p < 0.05$) influenced by temperature (X_2), E/G ratio (X_3) and pH (X_4) of extraction. Time of extraction did not have a significant effect on purity of the gum. However, the interaction terms of time and temperature (X_1X_2) influenced the gum purity significantly ($p < 0.05$). In other words, when planning an extraction process it is important to consider both the effect of each single variable and the interaction terms. Based on F-value the effects of extraction variables on gum purity were in descending order: E/G > temperature > pH (**Table 2**). Gum purity ranged from 55.54 to 76.35 %.

Effect of independent variables on extraction yield (the amounts of pure gum recovered from impure gum, %) were also studied. The yield ranged from 32.15 to 52.68 %. However, no significant ($p < 0.05$) effect of model terms including time, temperature and pH on extraction yield were observed (data not shown). Taking into account that ethanol-soluble materials of Asafoetida have a putrid odor that can be used separately as herbal medicine (Iranshahy & Iranshahi, 2011) and that the gum can be used as a by-product, an ideal extraction would achieve by the appropriate purity content not by higher yield of gum extraction. Therefore, the purity was considered as a better parameter to explain the impact of extraction variables on quality of Asafoetida gum.

The increasing of extraction temperatures influenced significantly and positively the purity of the Asafoetida gum (**Table 2**) as the resins and volatile compounds became more soluble in ethanol contrary to water-soluble materials including gum and ash. Jones and Thomas (1961) applied hot methanolic extraction to separate Asafoetida resins from water-soluble residue. Oleo-gum-resins of

Ferula gummosa was also purified using high temperature ethanol (Jalali et al., 2011; Mohammadzadeh Milani et al., 2007).

However, when considering high temperature during long time of extraction, the purity reduced (**Fig 1** and **2 (a)**). It means that the interaction of extraction time and temperature had a significant ($p < 0.05$) decreasing effect on gum purity. It can be due to the fact that at high temperatures over long times the gum residue starts chemical reaction such as degradation, Millard reaction and/or pyrolysis. Moreover, in these conditions low soluble pollutants can be released from the raw materials. This result is in accordance with the data reported by Wu et al. (2007) that found the interaction of time and temperature as a negative factor reducing the purity of polysaccharide from Sterculia seeds.

The E/G (w/w) ratio had a high significant ($p < 0.05$) effect on gum purity (**Table 2**). Using much amounts of alcohol for purification led to remove resin and volatile compounds (**Fig 1** and **2 (b)**).

Higher gum purity achieved at lower levels of pH (**Table 2**). The natural pH of aqueous solution of Asafoetida gum was from 5.3 to 5.8 depending on gum purity. The low purity of gum obtained for extraction with higher pH could be explained by a co-extraction of proteins. Protein was more soluble at higher pH values of extraction which led to loss of proteins linked to polysaccharide domains.

3.2. Protein content

Protein content of the gum was influenced significantly ($p < 0.05$) by time and temperature of extraction and E/G ratio; meanwhile, quadratic terms of temperature (X_{11}) and pH (X_{44}) and interaction terms of time and temperature (X_1X_2) and temperature and pH (X_2X_4) had also significant effects on protein content. The effect of extraction variables were as following descending order: E/G > temperature > time (**Table 2**). Protein nature and content of the gum is abundantly described as responsible for some of its functional properties such as emulsion stability, foaming ability, gelling properties and solubility (Williams & Phillips, 2014).

Protein content of various extracted gums ranged from 5.20 to 12.70 %. These results were higher in comparison with protein contents reported for Arabic gum (0.88-4.06 %) (Islam et al., 1997; Sanchez et al., 2018), gum from *Lepidium Perfoliatum* seed (1.27-4.87 %) (Koocheki et al., 2009b) and ghatti gum (4.34 %) (Kang et al., 2011), but lower than those of flax seed gum (14.4 %) (Wang et al., 2010).

Fig 1 and **2 (c)** represents the interaction effect of time and temperature on protein content by means of contour plot and 3D surface plots, respectively. Increasing both time and temperature led to an increase of protein content, followed by a decrease at higher temperatures during long times of extraction. This decreasing phenomenon was explained by the denaturation and then the insolubilization of protein. These results were similar to those obtained by Jouki et al. (2014) in the case of quince seeds mucilage extraction. Generally, the interaction term of time and temperature had a significant ($p < 0.05$) decreasing influence on the amount of protein extract (**Table 2**).

When considering the interaction effect of temperature and pH, there was a primary enhancement in protein content of the gum that ended with a decrease at upper levels of temperature and pH (**Fig 1** and **2 (d)**). The results of this study are similar to those reported by other researchers (Cui et al., 1994; Koocheki et al., 2009b). The third structure of protein began to unfold at high temperature and therefore might expose more readily to structural changes related to enhanced levels of pH. Thus, the history of heat treatment significantly ($p < 0.05$) changed the amount of protein at different values of pH.

The most significant ($p < 0.05$) influential variable on surveyed responses was E/G ratio (**Table 2**). Much amounts of ethanol facilitated the separation of impurities, which led to a more efficient extraction with higher purity and protein content.

3.3. Solubility

Fully dissolution of hydrocolloids plays an important role in their techno-functionality in many products such as foods (Laaman, 2010). Arabic gum, as an arabinogalactan-protein (AGP), is readily dispersible in cold and hot water even in high concentrations and yields solutions with low viscosity due to compact branched structure. This characteristic makes Arabic gum favorable to various applications as emulsifying agent (Verbeken et al., 2003).

Solubility of Asafoetida gum was significantly ($p < 0.05$) affected by extraction variables (**Table 2**). Time of extraction, temperature, E/G ratio and pH had increasing impact on solubility as following descending order: temperature > pH > E/G > time (**Table 2**). Solubility ranged from 19 % for the sample treated at 54 ºC for 3.5 h with E/G ratio of 15 and pH of 5 (X_1= -1, X_2= -1, X_3= -1, X_4= -1) to

96 % for sample extracted at 62ºC, 5 h with E/G ratio 25 and pH of 9.5 (X_1= 0, X_2= 0, X_3= 0, X_4= 1). Forty percent of the runs had the solubility higher than 80 %. The solubility of Arabic gum (80-90 %), flax seed mucilage (70-90 %), guar gum (50-60 %) and locust bean gum (20-25 %) were reported previously (Mazza & Biliaderis, 1989).

Temperature and pH were the dominant factors determining the solubility of gum during extraction procedure. Higher pH increases the overall negative charge on the structure of gum including peptide residue. This phenomenon strengthened repulsive forces between molecules leading to a superior dispersity. However, at high levels of pH the solubility decreased for samples that underwent high temperature of extraction. It means that the interaction of temperature and pH expressed a reducing effect (**Fig 1** and **2 (e)**). This result was in accordance with protein result so that the solubility of gum decreased by decreasing protein content at high temperature and pH.

E/G ratio had an increasing ($p < 0.05$) effect on solubility of the gum. In order to have a readily soluble gum, it is required to perform a complete purification that might achieve by using high amounts of solvent. It was reported by Koocheki et al. (2009b) that solubility of crude *Lepidium perfoliatum* seed gum did not exceed 25 % probably due to presence of impurities and insoluble matters in it. Low solubility of crude durian seed gum (7-19.21 %) was also attributed to its impurities (Amid & Mirhosseini, 2012).

3.4. Gum particle size

It was investigated that volume weighted mean ($D_{4,3}$) was significantly ($p < 0.05$) influenced by all the extraction factors as well as the interaction terms of time and temperature (X_1X_2), time and E/G ratio (X_1X_3) and temperature and pH (X_2X_4) (**Table 2**). The effects of parameters were in the following descending order: E/G> pH> temperature> time. In the current research, particle sizes of Asafoetida gum were ranged from 100 to 900 μm.

The effects of time and temperature are illustrated in **Fig 1** and **2 (f)**. These two parameters as well as their interaction had a significant increasing influence on $D_{4,3}$ ($p <0.05$). Raising temperature during longer periods of extraction yielded larger particle sizes. This may be due to the chemical interactions of gum molecules under long time heat treatment. Asafoetida gum, as an AGP macromolecule could

unfold over long time of heat treatment reinforcing the formation of polysaccharide-protein complexes with larger dimensions (Jones et al., 2010; Saeidy et al., 2018b).

The interaction of time and E/G ratio have significantly ($p < 0.05$) decreased the particle sizes (**Table 2**). It points out that higher amounts of ethanol during high extraction time removed the particles with larger sizes and led to a reduction of average size of molecules (**Fig 1** and **2 (g)**).

On the other hand, the interactions of increasing temperature and pH increased the particle size to a certain level. However, it followed by a decrease when the temperature and pH got higher degrees (**Fig 1 (h)**). The pH increment led the structure of gums treated with high temperatures to unfold due to intramolecular electrostatic repulsions resulting in higher light scattering (Weinbreck et al., 2003). However, particle size decreased due to the breakage that might occur in the gum residue at high pH.

Span was also influenced significantly ($p < 0.05$) by time, temperature and E/G ratio and the quadratic effect of all factors (**Table 2**). Higher time, temperature and E/G ratio yielded a more complete extraction process and more uniform particle dimensions. Therefore, the distribution range got narrower significantly. In the present study, span of samples varied from 0.6 to 5.4. Higher values of span imply more diversity of compounds present in a suspension obtained from the extraction of gum including insoluble materials and impurities. The most impressive factor was E/G ratio, which reduced the distribution range to a significantly ($p < 0.05$) lower level (**Table 2**). Many impurities such as volatile components and resins are soluble in ethanol and were eliminate while consuming much amounts of ethanol.

3.5. Foaming properties

Foams are colloidal systems containing gas bubbles randomly dispersed in aqueous phase. Destabilization of foams occurs when the bubbles tend to join and form large bubbles. This coalescence phenomenon is accelerated by drainage of water between the bubbles. Surface-active agents such as proteins can slow down the motion of bubbles and water drainage so that the tendency of bubbles to unify would reduce (Kalsbeek & Prins, 1999).

FC and FS of the Asafoetida gum were effected significantly ($p < 0.05$) by time, temperature and E/G ratio of extraction and the quadratic terms of temperature and pH (**Table 2**). It was determined that

the interactions of factors had no significant effects on foam properties of samples were ranged from 20 to 115 %. FC of flaxseed gum from 10 to 40 % depends on drying procedures (Wang et al., 2010). FC of 1% *Acanthophyllum bracteatum* gum was reported to be 44% and FS was 73% after 60 min (Jahanbin et al., 2012). Solutions of 0.1 – 0.3% basil seed gum and its fractions changed foaming capacity of albumin in the rage of 11% to 31% (Naji-Tabasi & Razavi, 2016).

FC of the Asafoetida gum ranged from 13 to 111 %. The most stable foam was assigned to the run treated 6.5 h at 70 ºC with E/G ratio of 35 and pH of 5 (X_1= 1, X_2= 1, X_3= 1, X_4= -1). In this case, 97 % of the initial foam remained after 30 min. It was higher than the values reported for guar and xanthan (0.1-0.5 % w/v) which were 45 and 91.3 %, respectively (Sciarini et al., 2009). The lower FC and FS were obtained at low time (3.5 h), temperature (54 ºC) and E/G ratio (15) and the high value of pH (8) i.e. X_1= -1, X_2= -1, X_3= -1, X_4= 1.

The results of foam properties were in accordance with protein content, which demonstrated the significant increasing effects of time, temperature and E/G ratio. Higher levels of these factors improved the extraction quality, which in turn resulted in much protein content. Foam properties of gums are usually attributes to their peptide residue as proteins can reduce the surface tension between air and water by 15 to 20 $mN.m^{-1}$. Proteins contain both hydrophobic and hydrophilic parts; however, in a foam system only the hydrophobic regions are exposed to air surface and cover the bubbles. The hydrophilic section is mostly oriented to aqueous medium. This phenomenon makes a foam stable (Damodaran, 2005). Polysaccharides usually cannot stabilize a foam system because of their hydrophilic natures. Nonetheless, they are able to enhance FC and FS of proteins by playing the role of thickening and/or gelling agents (Dickinson, 2010).

Literature indicate that extraction conditions influence the amount of protein in gums which itself may change the foaming properties. Structural changes resulting from various pH, ionic strength and temperature of extraction may alter the quality of adsorption, conformation and arrangement of film lamella at the air-water interface (Fidantsi & Doxastakis, 2001; Tsaliki et al., 2002). Makri and Doxastakis (2006) studied the foaming properties of proteins extracted from two beans specious (*Phaseolus vulgaris* and *coccineus*) at different pH. The pH of 5.5 showed the highest foam ability and stability because it was close to the isoelectric point of proteins. As a reason, the protein layer was

more compact, rigid and seems to increase the viscosity of interfacial layer. Therefore, high foaming ability of Asafoetida gum was related to its protein content as well as the structure of the polysaccharide-protein chain.

3.6. Optimization using desirability function

The numerical optimization of extraction was applied in order to determine the most desirable extraction conditions (**Table 3**). When selecting the goal of optimization in software, following options are represented: maximize, minimize, target to and in range. In this case, the four independent variables including time, temperature, E/G ratio and pH were selected in the range of measurement. About the purity, the goal of optimization was to reach the maximum value. Moreover, in order to obtain the most functional properties of a gum, it is favorable to approach the maximum protein content; therefore, this factor along with functional properties including solubility, FC and FS were maximized. There was no special objective for optimum value of volume weighted mean ($D_{4,3}$) so it was chosen to be in range. Low particle size distribution shows more uniformity of extracted materials, consequently, the goal for optimization was minimized. The optimum points obtained from RSM design were as following: time = 6.50 h, temperature = 70°C, E/G ratio = 35 and pH = 5.42 that yielded purity = 73.54 %, protein content = 11.82, solubility = 94.74 %, $D_{3,4}$ = 900 μm, span = 0.60, FC = 105.23, FS = 101.75. The overall desirability of responses was 0.92. . Design validation represented almost a good agreement of theoretical and experimental data ($R^2 \approx 1$). These results indicated that response surface method could be an appropriate design for finding the optimal extraction responses by using desirability functions.

3.7. Emulsion stability

The results of creaming are represented in **Fig. 2** in the form of whey off percentage. Creaming index increased for emulsions prepared by 5 to 15 % (w/v) Asafoetida gum solution over the storage time. The creaming indexes were obtained after 4 weeks at respectively 45.4 ± 3.2 %, 17.8 ± 0.7 % and 12.0 ± 2.3 % for the emulsions prepared with solutions of 5, 10 and 15 % (w/v) Asafoetida gum. Emulsions formulated with a solution of 20 % (w/v) of gum did not display any phase separation showing that high amounts of gums decreased the creaming index significantly ($p<0.05$). There was a slight increase in creaming during the storage time. This slow phase separation may be due to increase

in viscosity of aqueous phase in emulsions containing high amounts of gum, limiting the movement of oil droplets to rise upward the surface. The same result was given by Koocheki et al. (Koocheki et al., 2009a). Their investigations showed that increasing concentration of *Alyssum homolocarpum* seed gum to 0.75-1 % resulted in higher viscosity and stable emulsions during storage period.

3.8. Emulsion particle size

Volume weighted means ($D_{4,3}$) of emulsions decreased proportionally with the increase of gum solution (**Fig. 3**). It can be inferred that increasing gum concentration resulted in more coated oil droplets. In other words, high concentrations of Asafoetida gum did not limit the movement of gum to the interface of oil droplets during the emulsion preparation because Asafoetida gum produces low viscosity solutions even in high concentrations resembling Arabic gum rheological properties (Saeidy et al., 2018a). On the contrary, increasing the concentration of highly viscous gum solutions like xanthan and Alyssum homolocarpum gum solutions led to enhancement of average oil particle sizes (Chivero et al., 2015; Koocheki et al., 2009a). Moreover, applying high rotational speed of homogenizer and the subsequent ultrasonication caused the oil droplets to break into fine particles and led the gum molecules to overcome the viscosity in the emulsion medium. In fact, high surface activity and low viscosity of the asafoetida gum solutions resulted in formation of fine oil droplet size up to 2 μm in O/W emulsions. $D_{4,3}$ increase during storage time was insignificant indicating slow coalescence and/or flocculation of droplets.

Particle distribution of emulsions is illustrated in **Fig 5**. Distribution curves shifted to the left, got narrower width and less peak area for the emulsions prepared by higher gum concentrations. It obviously was indicant of reduction in $D_{4,3}$ and span with increasing gum content. Span was found to be in the rage of 1.4-1.6, 1.2-1.4, 1.1-1.3 and 1.0-1.3, respectively for emulsions made by 5, 10, 15 and 20 % w/v gum solutions during four weeks. Effect of storage time on span was negligible but it was observed that distribution curves tailed to higher values and got much extended peak areas after 28 days. Moreover the appearance of curves change to bimodal which represented the presence of oil droplets with heterogeneous dimensions. Similar conclusions were reported for Arabic and Angum gums (Huang et al., 2001; Jafari et al., 2013).

3.9. Light microscopy

Optical microscopic images of emulsion samples were in accordance with the results of particle size measurements. Oil droplets were larger for the emulsions with less amount of Asafoetida gum. However, no remarkable size increment was observed comparing the emulsions evaluated immediately after preparation and emulsions after 28 days. Samples that were pictured directly after production, showed randomized motions due to the effect of ultrasonication. In another publication, it was indicated that ultrasonication reduced the size of oil droplets in an emulsion prepared with different concentrations (0.2-2 %w/v) of coconut milk protein and sunflower oil. They showed that the protein concentrations beyond 1.2 % did not make so significant reduce in the oil droplet size (Lad & Murthy, 2012). In an ultrasonic assisted production of O/W emulsion using Arabic gum as the emulsifier, the droplet size decreased by increasing emulsifier concentration from 6 to 10 % w/w (Gharibzahedi et al., 2013).

3.10. Emulsion viscosity

Table 4 shows viscosity of emulsions prepared with 5-20 %w/v gum solutions over the storage time. Obviously, emulsion viscosity increased with increasing Asafoetida gum concentration. Enhancing the viscosity of emulsions containing higher concentrations of biomacromolecules is a fact that has been reported in several researches (Bouyer et al., 2012; Jafari et al., 2012). Viscosity of emulsion samples decreased 24 h after preparation, increased slightly during the first week followed with sharp enhancement up to 28th day of storage. Higher values of viscosity obtained for the samples assessed immediately after emulsion preparation might be due to more mobility of oil droplets treated with high speed homogenizer and ultrasonic process. The increasing trend observed for the emulsions during the storage time was attributed to flocculation effect rather than coalescence. In this phenomenon, oil droplets physically attached together without complete joining so that the emulsion viscosity increases with developing creaming rate. Note that in the absence of flocculation, emulsion viscosity decreases with creaming during storage time as a reason of coalescence (Tadros, 2004). However, in the current research, the slow creaming rate and droplet size increment indicated a negligible coalescence.

4. Conclusion

The present study showed that extraction condition significantly influenced purity, protein content, solubility, volume weighted mean, particle size distribution, foaming properties of Asafoetida gum. The optimum condition was determined as extraction time 6.50 h, temperature 70°C, E/G ratio 35 and pH 5.42. The solutions of optimum purified gum (5-20 %w/v) were used as emulsifying agent in O/W emulsions. Only the emulsions prepared with 20 %w/v of gum solution remained stable over storage time. The maximum oil droplet size was 2.2 µm using ultrasonication. Preservation time did not show significant effect on creaming behavior and particle size of emulsions due to viscosity enhancement.

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Figure captions

Fig 1. Contour plots showing the effects of time, temperature, ethanol to gum ratio (E/G) and pH on depended variables of Asafoetida gum extraction.

Fig 2. 3D response surface showing the effects of time, temperature, ethanol to gum ratio (E/G) and pH on depended variables of Asafoetida gum extraction.

Fig 3. Effect of Asafoeitida gum concentration (5-20 %w/v) on emulsion whey off over 28 days of storage.

Fig 4. Volume weighted mean of emulsion prepared with 5-20 % (w/v) Asafoetida gum solution over 28 days of storage.

Fig 5. Particle distribution in emulsions prepared with 5-20 % (w/v) solutions of Asafoetida gum: (A) immediately after production and (B) after 28 days.

Table 1. Independent variables and value of levels.

Independent variables	Coded factor levels				
	-2	-1	0	1	2
X_1: Extraction time (h)	2	3.5	5	6.5	8
X_2: Extraction temperature (°C)	46	54	62	70	78
X_3: Ethanol to solid gum ratio (E/G)	5	15	25	35	45
X_4: pH	3.5	5	6.5	8	9.5

Table 2. R^2, p-value, F-value and lack of fit of the independent variables and F-value and p-value of extraction variables obtained using ANOVA.

Variable	Purity (%,Y_1)		Protein content (%,Y_2)		Solubility (%,Y_3)		$D_{4,3}$ (µm,Y_4)		Span (µm,Y_5)		FC (%,Y_6)		FS(%,Y_7)	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Linear														
X_1	0.94	0.35	5.11	0.04*	13.08	0.0025*	5.25	0.04*	45.80	<0.0001*	12.33	0.0031*	21.67	0.0003*
X_2	34.19	<0.0001*	27.70	<0.0001*	67.36	<0.0001*	27.52	<0.0001*	41.71	<0.0001*	39.66	<0.0001*	59.89	<0.0001*
X_3	85.56	<0.0001*	163.59	<0.0001*	23.62	0.0002*	46.17	<0.0001*	135.20	<0.0001*	52.28	<0.0001*	69.80	<0.0001*
X_4	9.70	0.01*	0.19	0.67	29.23	<0.0001*	45.46	<0.0001*	0.71	0.41	0.06	0.82	0.03	0.86
Quadratic														
X_{11}	0.31	0.58	1.55	0.23	3.31	0.09	2.42	0.14	14.42	0.0018*	3.11	0.10	5.55	0.03*
X_{22}	11.01	0.0047*	38.28	<0.0001*	18.43	0.0006*	16.30	0.0011*	188.73	<0.0001*	11.23	0.0044*	15.48	0.0013*
X_{33}	1.19	0.29	3.56	0.08	33.88	<0.0001*	21.69	0.0003*	48.74	<0.0001*	2.44	0.14	10.53	0.0054*
X_{44}	0.74	0.40	11.85	0.0036*	1.58	0.23	20.76	0.0004*	8.78	0.0097*	20.89	0.0004*	10.85	0.0049*
Interaction														

X_1X_2	12.28	0.0032*	6.07	0.03*	3.06	0.10	30.34	<0.0001*	0.03	0.87	1.38	0.26	1.84	0.20
X_1X_3	3.06	0.10	0.78	0.39	1.75	0.21	7.89	0.01*	3.58	0.08	0.12	0.74	0.09	0.78
X_1X_4	0.70	0.42	3.59	0.08	0.36	0.56	0.33	0.58	1.22	0.29	0.67	0.43	1.09	0.31
X_2X_3	2.35	0.15	0.02	0.89	1.00	0.33	0.95	0.34	3.13	0.10	0.41	0.53	1.13	0.30
X_2X_4	0.16	0.90	7.59	0.02*	5.25	0.04*	15.25	0.0014*	3.87	0.07	1.13	0.30	0.45	0.51
X_3X_4	0.19	0.67	0.45	0.51	0.33	0.57	0.59	0.45	0.10	0.76	0.76	0.40	3.28	0.09
R^2	0.92		0.95		0.93		0.94		0.97		0.90		0.93	
p-value of model	<0.0001*		<0.0001*		<0.0001*		<0.0001*		<0.0001*		<0.0001*		<0.0001*	
F-value of model	11.56		18.64		13.68		16.11		32.24		9.86		13.54	
Lack of fit of model	0.33		0.06		0.10		0.08		0.06		0.05		0.06	

*p-values less than 0.05 indicate model terms are significant.

X_i, X_{ii} and X_iX_j represent linear, quadratic and interaction effects of variables, respectively. X_1: Extraction time; X_2: Extraction temperature; X_3: Ethanol to gum ratio (E/G); X_4: pH.

Table 3. Numerical optimization using desirable function.

	Goal	Lower limit	Upper limit	Optimum theoritical point	Optimum experimental data
Time (h)	is in range	3.5	6.5	6.50	
Temperature (ºC)	is in range	54	70	70	
E/G ratio	is in range	15	35	35	
pH	is in range	5	8	5.42	
Purity (%)	maximize	55.54	76.35	73.56	
Protein content (%)	maximize	5.20	12.70	11.82	12.30
Solubility (%)	maximize	19	96	94.74	92.51
$D_{3,4}$ (μm)	is in range	100.20	900	900	818.20
Span	minimized	0.60	5.39	0.60	0.57
FC (%)	maximize	20.21	115.38	105.23	111.20
FS (%)	maximize	12.73	111.54	101.75	110.83
Desirability				0.92	
R^2					1

Table 4. Viscosity of emulsions prepared by different concentrations of gum solution during 28 days.

Gum concentration (%,w/v)	Time (day)						
	0	1	3	7	14	21	28
5	12.70 ± 0.35	9.85 ± 0.50	9.85 ± 0.50	10.50 ± 0.70	18.00 ± 0.70	21.75 ± 0.35	22.55 ± 0.35
10	22.50 ± 0.70	18.75 ± 1.80	19.00 ± 0.70	20.00 ± 0.35	34.00 ± 0	36.75 ± 0.35	37.70 ± 0.40
15	34.00 ± 2.80	26.75 ± 1.80	27.50 ± 0.70	29.25 ± 1.80	46.25 ± 1.80	57.00 ± 1.40	61.60 ± 0.85
20	57.25 ± 1.80	43.90 ± 1.60	45.50 ± 2.80	45.35 ± 1.60	85.00 ± 1.40	89.00 ± 2.80	92.80 ± 1.00

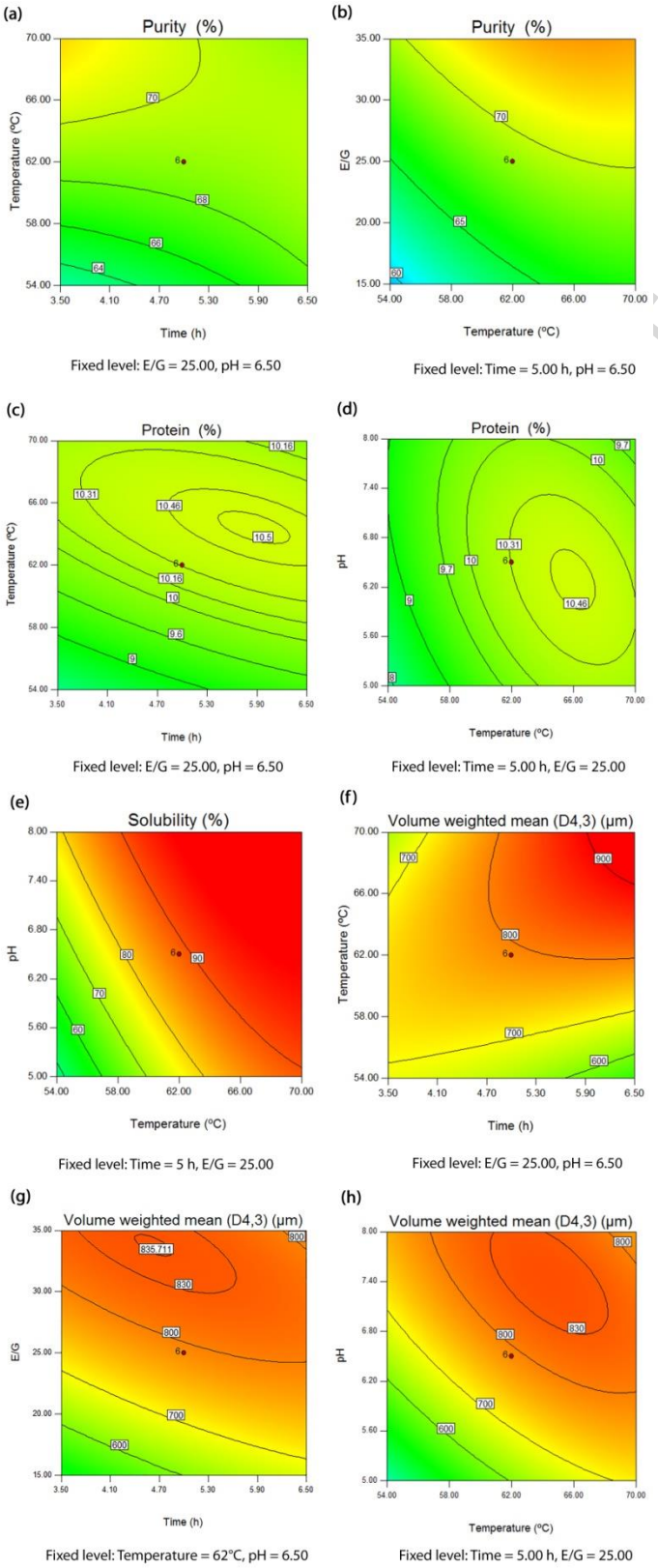


Fig. 1

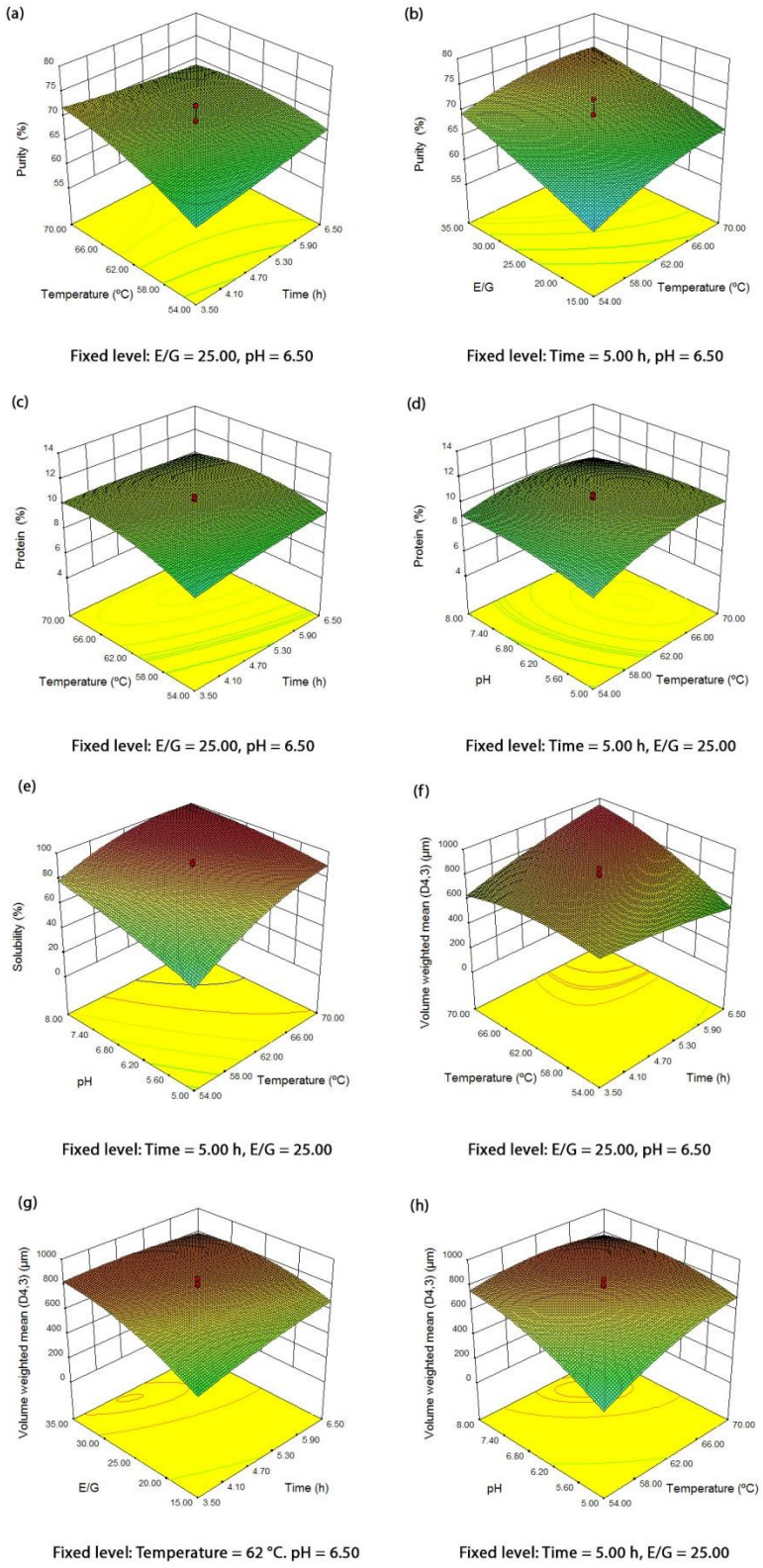


Fig. 2

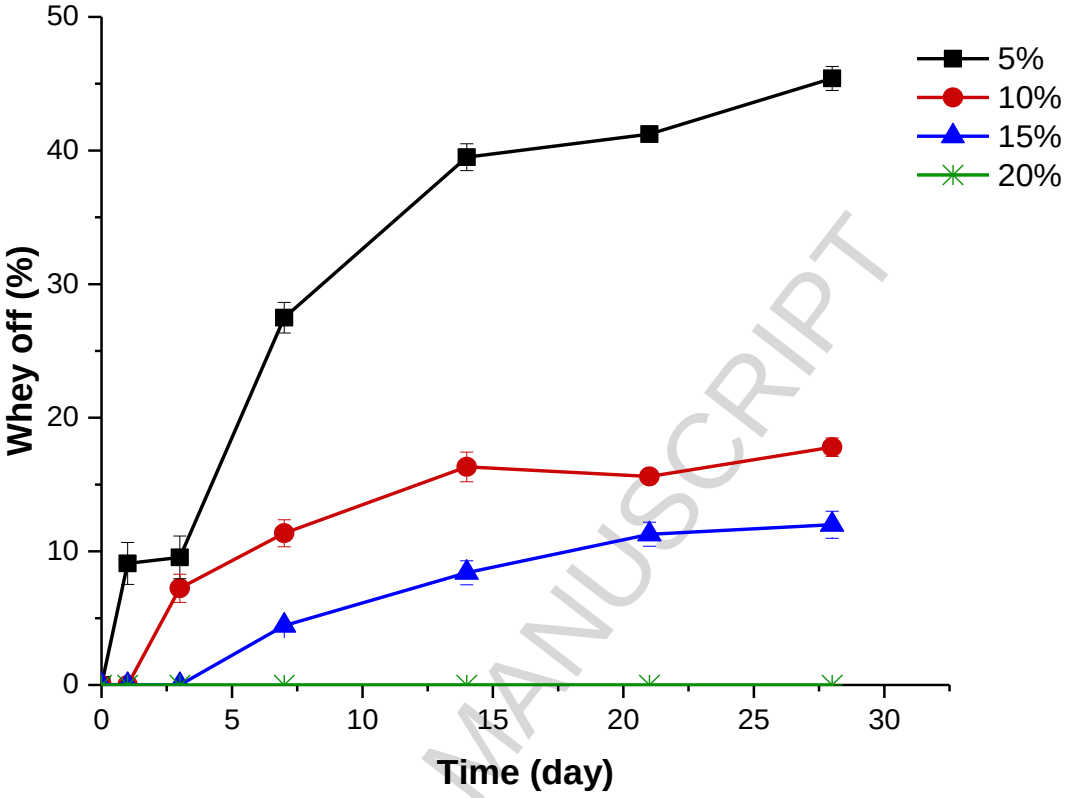


Fig 3

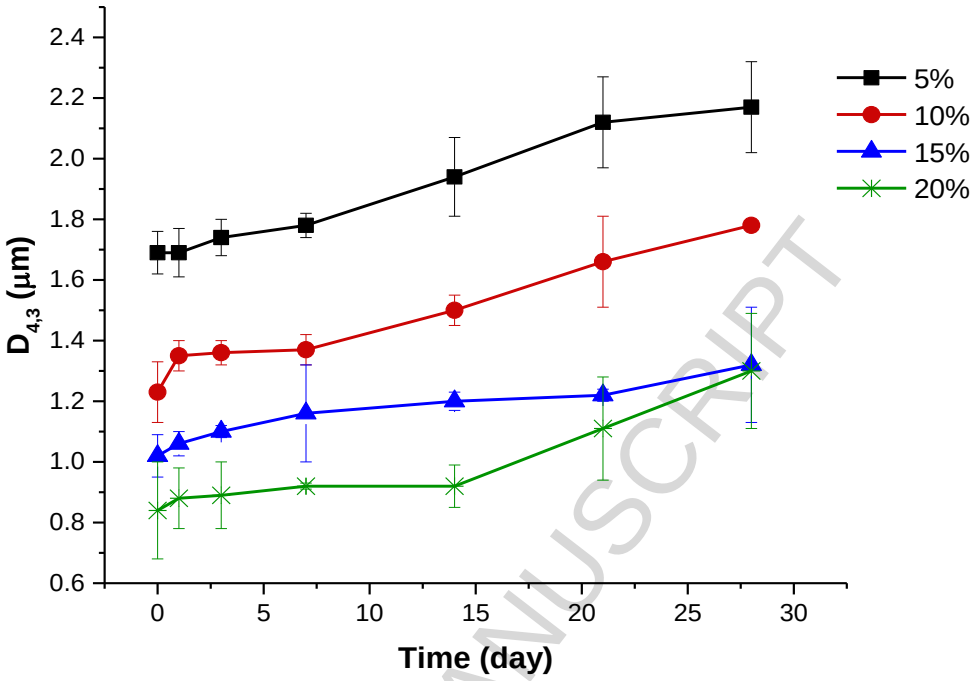
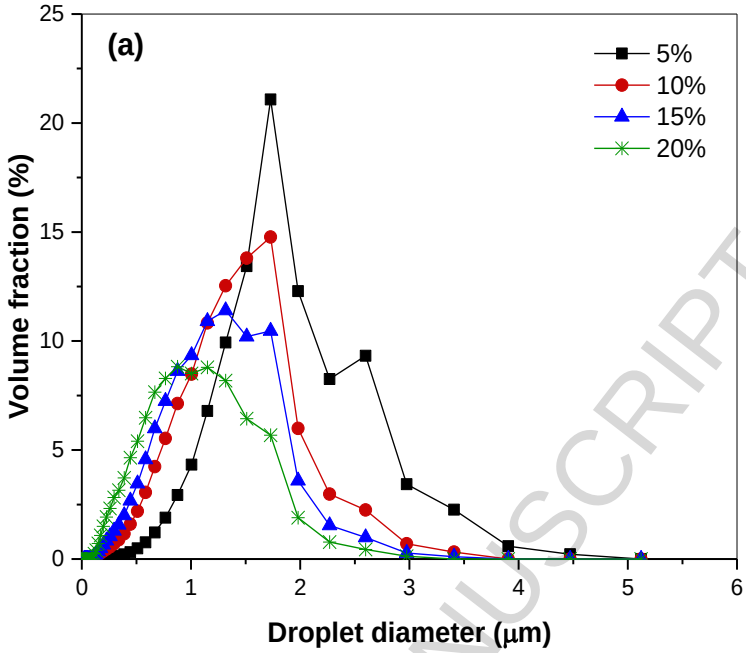


Fig. 4



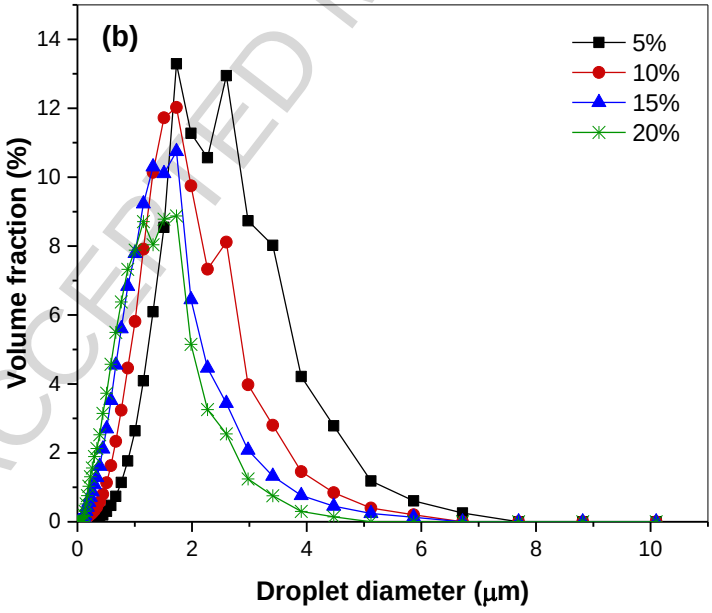
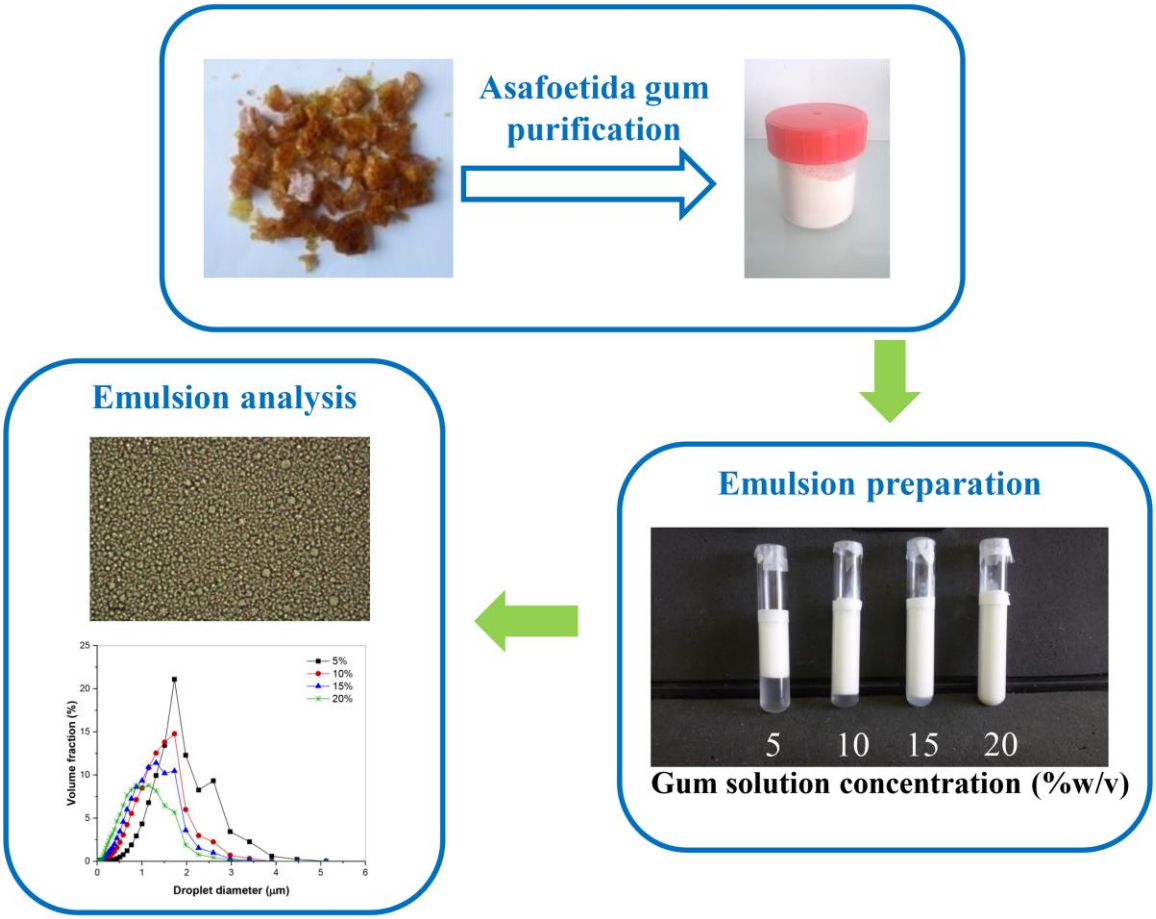


Fig. 5

Graphical abstract



Conflict of interest:

The authors declare no conflict of interest.

Highlights:

- Physicochemical properties of Asafoetida gum were affected by extraction variables.
- Time, temperature, ethanol/gum ratio and pH were studied as extraction parameters.
- Ethanol/gum ratio was the most effective factor.
- Emulsions contained 80:20 of Asafoetida gum solution (20 %w/v) to oil were stable.