

INVESTIGATING FUNCTIONAL CHARACTERISTICS OF WATER-SOLUBLE POLYSACCHARIDE ISOLATED FROM BALANGU SEED (*LALLEMANTIA ROYLEANA*) GUM

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Abstract In this study, the influence of molecular weight (MW) was measured on functional characteristics of Balangu seed (*Lallemantia royleana*) gum (BSG) fractions. Firstly, BSG fractionated by precipitation method using ethanol basis on MW. Two fractions called precipitate (PER) Balangu and supernatant (SUPER) Balangu were obtained as the highest and lowest MW fractions, respectively. Then the physicochemical properties (uronic acid, protein and molecular weight) were investigated for BSG and fractions. Moreover, the rheological characteristics of BSG and fractions emulsions were determined. The results showed, the value of MW for Balangu, PER-Balangu and SUPER-BSG were 3120 kDa, 6130 kDa and 2050 kDa, respectively. All the emulsions established shear-thinning behavior (1%, w/w). SUPER- Balangu was obtained lower storage moduli (G') and loss moduli (G''), which showed as the best uniform emulsion. The present of high uronic acid content (20.35%) and protein content (10.8%) of SUPER-Balangu led its increase emulsifying activity. PER-Balangu emulsion contains more poly-dispersed oil droplets with larger size which may be due to low protein content (6.03%). According to the results the most uniform emulsion related to SUPER-Balangu which can be a replacement for some of the plant hydrocolloids used in food products.

Key words: Balangu seed gum, molecular weight, emulsion, rheology, linear viscoelastic.

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Introduction. Formulation of the problem

Polysaccharides are kind of natural macromolecular polymer usually composing of more than ten monosaccharides among glycosidic linkages in each line or branched chain, with a molecular weight (MW) virtually thousands to millions [1]. Another macromolecule, which are dissoluble in water and can form viscous dispersions or gels, are known as hydrocolloids (gum) [2]. Due to their environmental sustainability, suitable price, biocompatibility, biosafety, biodegradability and their versatile physicochemical feature, these natural polymers have superiority over synthetic polymers [3]. For instance, through forming suspensions, escalating viscosity and strengthening other rheological attributes, they present improved attributes to aqueous solutions [4].

Analysis of recent research and publications

Dynamic rheology is one of the methods for analyzing viscoelastic behavior of polysaccharides solutions / dispersions and gels [5]. There are some reports related to this concept done by other researchers such as *Lepidium perfoliatum* seed gum [6], gum tragacanth [7], *Alyssum homolocarpum* seed gum [2] and basil seed gum [8].

Balangu seed (BS) also is known as *Lallemantia royleana* grows in different areas of Europe, Asia and Middle East, particularly in Iran [9]. Some features of the seed of BS as following: the color of seed is almost dark-brown, the seeds are smooth and if they will be

exposure to water, they will become coated in a clear and voluminous mucilage which give a bland and spicy taste [10].

The BS is widely used by Iranian in traditional medicine due to its nutritional and human health effects, also the seed is conventionally used in some foods and drinks like bread and "Tokhme Sharbati" (a type of beverage) [11]. BS powder has been also used as tonic medication and therapy for psychopath illness [12].

BS gum (BSG) contains carbohydrates, proteins, ash, moisture and uronic acid respectively at 75.87, 2.71, 8.24, 8.51 and 20.33 %w/w [13]. Various chemical compounds have been reported for Balangu that due to different plant growth conditions, purification and extraction methods [14]. BSG contains monosaccharides analyzed with anion exchange chromatography including arabinose, galactose, rhamnose, xylose and glucose respectively at 37.88, 33.54, 18.44, 6.02 and 4.11% [13]. BSG solution at 1% (w/w, 20°C) indicated a high pseudoplastic behavior over an in the range of shear of 0.01 -1000 1/s [11].

The aim of current study was investigated the rheological and functional characteristics including droplet size measurement and optical microscopy of BSG and fractions.

Research materials and methods

Materials. BS was purchased from a local market in Bojnord, Iran in May 2018 and cleaned for dust and broken seeds. Ethanol (96%) was obtained from Pars

Alcohol Company (Isfahan, Iran). Other chemical applied in this study had an analytical grade (Merck, Darmstadt, Germany).

Gum Extraction. BSG was extracted at optimized conditions (water/seed ratio 59:1, pH 7, temperature 85°C and entire extraction time 20 min), according to method described by Mohammad Amini (2007) [15]. Segregation of mucilage from the inflated seed was attained through scraping method. The seeds were crossed through an extractor equipped with a rotating plate which scraped the mucilage layer on the seed surface. The extracted solution was filtered and afterward purified completely by mixing with three volumes of 96% ethanol to precipitate polysaccharide. The precipitated polysaccharide was dissolved in water and dried overnight in an air forced laboratory oven at 38°C. The dried gum was then milled (Magic Bullet, Model 6350M, Korea), sieved (mesh 120 micron), packed and kept in cool and dry condition.

Fractionation. The method of polysaccharide fractionation from BSG was set according to Francuskiewicz [16] which is on the basis of MW using ethanol precipitation. For this purpose, BSG solution (0.1% w/w) was prepared at laboratory temperature for about 24 hrs until complete dissolution. Then various volumes of ethanol (10–90% v/v) were added drops wise to the sample at constant agitation rate on stirrer. The precipitate (PER-Balangu) and supernatant (SUPER-Balangu) fractions which were known as high and low MW fractions were obtained after centrifugation for 10 min at 12,000 g (Hettich, Germany). The fractions were dried then milled (Magic Bullet, Model 6350M, Korea), sieved (mesh 120 micron), packed and kept at cool and dry condition (24 hrs, 4°C).

Chemical composition measurement. The AOAC method was measured the protein content of Balangu fractions [17]. The uronic acid content of Balangu fractions were determined by m-hydroxyphenyl colorimetric technique by D-galacturonic acid as standard [18].

Determination of molecular weight. The MW was measured with Zetasizer Nano ZS (Malvern Instruments, England) according to method described by Mohammad Amini and Razavi [10].

Emulsion preparation. At first, Gum solutions (0.3%, w/w) were prepared at laboratory temperature for about 24h on roller mixer until complete dissolution. Then, sunflower oil (30%, w/w) was added drop wise to the solution at constant agitation rate on stirrer at 2000 rpm (Alfa 860, Iran). Then, the emulsions were homogenized at 20000 rpm with Ultra Turrax (IKA T 25, Germany) for 6 min. Sodium azide (0.02%, w/w) was appended to emulsion as antimicrobial agent.

Rheological measurements. The GmbH Physica-MCR 301 (Germany) rheometer (cone plate system with 50 mm diameter, 4° angle and 0.206 mm gap) was employed to conduct rheological tests (20°C).

Flow behavior. The shear rate between 0.01 and 300 s⁻¹ was used to investigate flow behavior (Eq. (1)) and was determined by fitting of the data (shear stress/shear rate) with the Herschel-Bulkley model (20°C).

$$\tau = \tau_{0H} + (\dot{\gamma})^{n_H} k_H \quad (1)$$

Where, the shear stress, consistency coefficient (Pa.sⁿ), shear rate, flow behavior index (unitless) and the yield stress (Pa), respectively, are shown by τ , k_H , $\dot{\gamma}$, n_H and τ_{0H} [19].

Dynamic shear mode tests

Stress sweep. The shear stress state between 0.01 and 10 Pa, stress sweep measurements were used to achieve of the linear viscoelastic (LVE) zone, permanent frequency (1 Hz, temperature 20°C) [19].

Frequency sweep. The frequency sweep test was evaluated [19] at frequency in the range of 0.1-10 Hz and at a steady stress (0.1 Pa), which was in LVE zone (20°C).

Light microscopy. Light microscopy was used to determine the emulsions microstructure (Olympus DP12, Tokyo, Japan). The samples were diluted 10 times with sodium dodecyl sulfate (SDS, 0.1% w/v) solution the microscope slide entrusted a drop of emulsion and covered with a coverslip. The image was obtained by a microscope camera (Olympus DP12, Japan) [20,21]. The images were ameliorated with a smart sharp filter of Adobe Photoshop (Adobe, v.9CS2). During 4 weeks storage, the particle size analyzer were measured (25°C) with Image J software (NIH, Version 1.4 J, USA).

Droplet size measurement. The oil droplets diameter of emulsions were also measured by laser diffraction particle size analyzer (Shimadzu SALD-2101, Japan) instantly after production [21].

Statistical analysis. All the measurements were prepared in duplicates. The results were performed as means \pm standard deviation (SD). Thus, the data were determined using the software SPSS (2013). Statistical differences were analyzed by variance (ANOVA) and Duncan's multiple range test (P values <0.05). Rheological parameters were analyzed using MATLAB (2015a).

Results of the research and their discussion

Chemical composition. The values of protein and uronic acid for Balangu and fractions are shown in Table 1 which shows the following order: SUPER-Balangu > Balangu > PER-Balangu.

Table 1 – Chemical composition of Balangu, PER-Balangu and SUPER-Balangu

Samples	Yield	Protein	Uronic acid
Balangu	19.95±0.12	8.41±0.11 ^b	15.16±0.34 ^b
PER-Balangu	62.72±1.20	6.03±0.12 ^c	9.75±0.20 ^c
SUPER-Balangu	37.28±0.48	10.8±0.14 ^a	20.35±0.35 ^a

*Different letters display significant differences at p<0.05

Molecular weight. The value of MW for Balangu, PER-Balangu and SUPER-Balangu were 3120 kDa, 6130 kDa and 2050 kDa, respectively. Naji-Tabasi et al. [22] obtained the value of MW for Basil seed gum was 2320 kDa.

Rheological measurements

Flow behavior. Emulsions tendency to instability due to physicochemical factors such as coalescence, flocculation, creaming, Ostwald ripening or phase inversion. The stable of these dispersed systems is mainly measured with their physical stability [23]. Table 2 shown the Herschel–Bulkley model parameters.

The high coefficients of determinations (R^2) ranged between 0.981 and 0.985 which indicated the good fitness of this model. The shear-thinning behavior when the values of flow index (n_H) were less than 1 in all emulsions (Table 2).

Shear-thinning behavior has different advantages in food industry which becomes the industrial operations favorable as pumping and mixing [24]. The main factor for this behavior may be belonged to the existence of non-adsorbed gums [25]. Moreover, it may be possible to shearing during the rheology measurements disappear some of the flocs existence in the emulsion, thus viscosity decreased [26].

Table 2 – Flow behavior of SUPER-Balangu, PER-Balangu and Balangu emulsions

Parameters	Balangu	PER-Balangu	SUPER-Balangu
τ_{0H} (Pa)	2.94 ± 0.38 ^a	2.38 ± 0.27 ^b	1.02 ± 0.02 ^c
Pa.s ⁿ (k_H)	1.25 ± 0.42 ^a	1.04 ± 0.36 ^a	0.29 ± 0.01 ^b
n_H	0.36 ± 0.01 ^a	0.32 ± 0.05 ^a	0.27 ± 0.03 ^a
R^2	0.985	0.983	0.981
RMSE	0.38	0.31	0.23

^aDifferent letters display significant differences at $p < 0.05$

The k_H value of Balangu emulsion are summarized in Table 2 which shows the following order: Balangu (1.25 Pa.sⁿ) > PER-Balangu (1.04 Pa.sⁿ) > SUPER-Balangu (0.29 Pa.sⁿ) (Table 2).

Table 3 – Linear viscoelastic parameters of SUPER-Balangu, PER-Balangu and Balangu emulsions ($G'_{(LVE)}$: storage modulus; $G''_{(LVE)}$: loss modulus; $\tan \delta_{LVE}$: loss tangent; τ_Y : yield stress; τ_f : flow-point stress and G_f : $G' = G''$: corresponding modulus)

Emulsion	$G'_{(LVE)}$ (Pa)	$G''_{(LVE)}$ (Pa)	$\tan \delta_{LVE}$	τ_Y (Pa)	τ_f (Pa)	G_f (Pa)
Balangu	24.32 ± 0.29 ^a	7.01 ± 0.29 ^a	0.28 ± 0.01 ^b	1.45 ± 0.48 ^a	2.82 ± 0.22 ^a	6.48 ± 0.18 ^a
PER-Balangu	22.30 ± 0.52 ^a	5.38 ± 0.62 ^b	0.24 ± 0.03 ^b	1.06 ± 0.39 ^a	2.02 ± 0.34 ^b	6.26 ± 0.07 ^a
SUPER-Balangu	6.32 ± 0.46 ^b	1.82 ± 0.32 ^c	0.49 ± 0.04 ^a	0.63 ± 0.28 ^b	0.98 ± 0.32 ^c	2.83 ± 0.18 ^b

^aDifferent letters display significant differences at $p < 0.05$

The yield stress (τ_Y) is characterised in shear stress test at the limit LVE. As long as the yield point is more than the stress, no significant change occurs in the internal structure [32]. The highest and lowest value of τ_Y were related to Balangu (1.45 Pa) and SUPER-Balangu (0.63 Pa) (Table 3). The flow point (τ_f) and corresponding modulus which indicates the transition from solid to liquid like behavior [33] were determined

Yield stress is showed as minimum shear stress for flowing. During storage or transportation, yield stress is a considerable quality control factor [27]. This parameter has a practical importance in gum industry because of maintaining various components in food formulations [13,28]. As shown in Table 2, the highest and lowest amount of yield stress values (τ_0) were revealed for Balangu (2.94 Pa) and SUPER-Balangu (1.02 Pa), respectively, which indicates high stability of Balangu emulsion against creaming.

Dynamic rheological

Stress sweep. The LVE region measured with stress sweep (stress between 0.01 to 10 Pa and temperature 20°C). The storage modulus values were more than loss modulus values in all emulsions that shows their elastic behavior (Table 3). Moreover, the storage modulus of Balangu and PER-Balangu emulsions were more than SUPER-Balangu emulsion (Table 3).

Strong colloids solutions compared to weak gum solutions maintain longer at linear mode [29]. The strain value scarcely exceeds 0.1 for colloidal gels in the limit LVE, a larger region of LVE with the strain ≥ 1 is for normal biopolymer gels [30].

The storage modulus characterizes the non-dissipative section of the mechanical characterizes for a material and indicates the elastic properties, while loss modulus represents the dissipative section of the mechanical characterizes and indicates the viscous behavior of material [24].

All measured emulsions had $\tan \delta$ (G''/G') between 0.28 and 0.49 (Tables 3). A $\tan \delta < 1$ shows mostly elastic behavior. Also, $\tan \delta > 1$ shows mostly viscous behavior, while the greater $\tan \delta$ than 0.1 indicates that sample is not true gel, which have a behavior between a true gel and a concentrated biopolymer [31]. The weakest gel structure was measured by emulsion related to SUPER-Balangu, as it obtained the highest $\tan \delta_{LVE}$ (Table 3).

as 2.82 and 2.02 Pa respectively for Balangu and PER-Balangu emulsions.

The yield stress results of dynamic shear test are in agreement with results of the yield stress determined with steady shear test (Tables 2–3). The corresponding modulus (G_f) shows as an indicator of behavior strength in flow point [34]. The highest and lowest value of G_f were obtained for Balangu (6.48 Pa) and SUPER-Balangu (2.83 Pa), respectively (Table 3).

Frequency sweep. Frequency sweep information is used to characterize/classify dispersions. The most common classification is that of: dilute solution, entanglement network system, weak gel and strong gel [29,30]. Dynamic rheological of parameters of emulsions (0.1 Pa, 20 °C) are displayed in Table 4.

The storage modulus was higher than loss modulus (0.1–10 Hz) and no crossover point showed, which

indicated solid like behavior of emulsions. The elastic modulus of Balangu emulsion was higher than PER-Balangu and SUPER-Balangu emulsions ($p < 0.05$).

According to table 4, $\tan \delta$ values were between 0.29 and 0.58, which indicated the weak gel structure of Balangu emulsions. The lowest and highest η^* value related to SUPER-Balangu (0.72 Pa.s) and Balangu (5.31 Pa.s) and respectively (Table 4).

Table 4 –Dynamic rheological of parameters for SUPER-Balangu, PER-Balangu and Balangu emulsions measured by frequency sweep test*

Emulsion	G' (Pa)	G'' (Pa)	η^* (Pa.s)	Tan δ	Slope of η^*
Balangu	34.28±0.52 ^a	12.38±0.63 ^a	5.31±0.07 ^a	0.36±0.03 ^b	-0.93 ±0.03 ^a
PER- Balangu	29.34±0.41 ^b	8.64±0.82 ^b	3.15±0.09 ^b	0.29±0.07 ^b	-0.95±0.04 ^a
SUPER- Balangu	4.18±0.82 ^c	2.43±0.15 ^c	0.72±0.04 ^c	0.58±0.04 ^a	-0.73±0.07 ^b

*Different letters display significant differences at $p < 0.05$

The slope of the η^* curves is between -0.73 and -0.95 (Table 4). Morris [35] reported that the η^* slope near - 0.8, which is showed of a “weak gel” with polysaccharide structure formed using chains of overlapping and entangling flexible random coil.

The frequency dependency of storage moduli (G') and loss moduli (G'') of Balangu and fractions were described by power-law functions ($G' = a\omega^b$ and $G'' = c\omega^d$), where ω is oscillatory frequency, b and d are shows of power law's storage modulu (G') and loss

modulu (G''), respectively (Table 5). The systems were performed values of $b > 0$ as a physical gel, $b = 0$ as a covalent gel, $b < 0$ as an elastic gel, whereas b close to 1 as a viscous gel [6].

According to table 5, b and d values are near to zero, so G' and/or G'' does not change with frequency (Table 5). Balangu emulsion shows high elastic behavior of emulsion. The lowest and highest values of b and d were related to Balangu and SUPER-Balangu.

Table 5. Power-law parameters measured for the storage moduli (G') and loss moduli (G'') of SUPER-Balangu, PER-Balangu and Balangu emulsions*

Emulsion	G' = $a\omega^b$				G'' = $c\omega^d$			
	a	b	R ²	RMSE	c	d	R ²	RMSE
Balangu	34.46±0.31 ^a	0.06±0.02 ^b	0.974	0.439	7.23±0.34 ^a	0.19±0.02 ^b	0.996	0.283
PER- Balangu	22.35±0.48 ^b	0.13±0.03 ^b	0.961	0.534	4.21±0.52 ^a	0.23±0.06 ^b	0.992	0.394
SUPER- Balangu	2.25±0.76 ^c	0.23±0.04 ^a	0.98	0.196	0.41±0.29 ^b	0.42±0.04 ^a	0.995	0.297

*Different letters display significant differences at $p < 0.05$

Particle size. The literature shows that the interaction activity of polysaccharide gums is due to the existence of surface active molecules in the crude gum, such as proteins [36]. Droplets size of O/W is measured by two fundamental processes occur during preparation: coalescence of the recently formed droplets and atomisation in the dispersed phase to small droplets. Some addition like emulsifiers and stabilizers effects processes, by: a) decrease of surface tension, which causes the conversion of dispersed phase to small droplets and b) formation of an interfacial biopolymer layer, which inhibits coalescence phenomenon [37].

The main factor parameter of food products based on emulsion is the mean diameter (MD) of oil droplets, which is an indicator of central tendency for distribution, and standard deviation (SD), which is an indicator of width of the distribution [38]. The droplet size of Balangu emulsions display in Table 6 which shows the following order: PER-Balangu (8.45 μm) > BSG (5.11 μm) > SUPER-Balangu (3.54 μm).

The average of particle size and deviation of all emulsions increased during storage (Table 6). The greatest increase in particle visibility was observed for PER-Balangu emulsion. During storage, the SUPER-Balangu emulsion had the smallest particle size which made the emulsion more uniform. The high ability of SUPER-Balangu emulsion to stabilize oil droplets is probably due to various factors, such as the presence of high protein (10.8%) and higher electrostatic charges due to high uronic acid content (20.35%) on the surface of dispersed oil droplets. According to earlier researches, the lower the emulsion particle size, the higher will be the encapsulation efficiency [39].

Light microscopy. Figure 1 shows microstructure of Balangu emulsions which also confirms that SUPER-Balangu has more uniform emulsion with small droplets compared to the other fractions. In contrast, PER-Balangu emulsion contains more poly-dispersed oil droplets with larger size which may be due to low protein content.

Table 6. Particle size of SUPER-Balangu, PER-Balangu and Balangu emulsions (0.3% w/w) during 4 weeks storage at temperature 25°C*

Storage time (day)	Particle size analyzer (μm)	Balangu	PER-Balangu	SUPER- Balangu
After the production **	MD (μm)	$5.11 \pm 0.17^{\text{B}}$	$8.45 \pm 0.21^{\text{A}}$	$3.54 \pm 0.37^{\text{C}}$
	SD (-)	0.23	0.37	0.21
After the production ***	MD (μm)	$3.05 \pm 0.28^{\text{Bb}}$	$4.1 \pm 0.14^{\text{Ac}}$	$1.81 \pm 0.19^{\text{Cb}}$
	SD (-)	0.32	0.45	0.29
Week 1 **	MD (μm)	$3.39 \pm 0.36^{\text{ABab}}$	$4.3 \pm 0.41^{\text{Ac}}$	$2.08 \pm 0.32^{\text{Bb}}$
	SD (-)	0.52	0.64	0.32
Week 2 **	MD (μm)	$3.61 \pm 0.12^{\text{ABab}}$	$4.46 \pm 0.37^{\text{Abc}}$	$2.36 \pm 0.32^{\text{Cb}}$
	SD (-)	0.68	0.76	0.39
Week 3 **	MD (μm)	$3.84 \pm 0.34^{\text{Ca}}$	$4.8 \pm 0.17^{\text{Bb}}$	$2.72 \pm 0.27^{\text{Dab}}$
	SD (-)	0.77	1.54	0.42
Week 4 **	MD (μm)	$3.89 \pm 0.42^{\text{Ca}}$	$5.11 \pm 0.38^{\text{Ba}}$	$2.93 \pm 0.18^{\text{Da}}$
	SD (-)	0.91	2.46	0.56

*Different letters display significant differences at $p < 0.05$.

**Particle size analyzer

***Image processing technique (MD \pm SD)

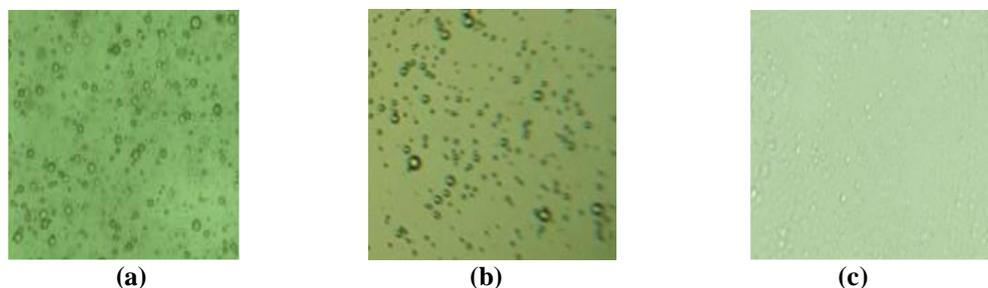


Figure 1. Microstructure of Balangu (a), PER-Balangu (b) and SUPER- Balangu (c) emulsions

Conclusions

In this study, investigated the rheological properties of BSG and fractions. Results showed that all the emulsions established shear-thinning behavior (1%, w/w). SUPER-Balangu was obtained the lower consistency coefficient (R^2), storage moduli (G') and loss moduli (G''), which showed the best uniform emulsion. The present of high uronic acid content (20.35%) and protein content

(10.8%) of SUPER-Balangu led its increase emulsifying activity. PER-Balangu emulsion contains more poly-dispersed oil droplets with larger size which may be due to low protein content. According to the results the most uniform emulsion related to SUPER-Balangu which can be a replacement for some of the plant hydrocolloids used in food products.

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