Research article

Extraction, purification and assessment of galactomannan from fenugreek seeds

Jahnavi Teekanam[1], Shantkriti Srinivasan[2]*, Pavithra Uthayasooriyan[3], Usharani Subbiah[4], Balasubramani Govindasamy[5], Murugan Athiappan[6]

[1]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil, 626126, Tamil Nadu, India
[2]Dhanalakshmi Srinivasan University, Tiruchirappalli, 621112, Tamil Nadu, India
[3]Department of Biotechnology, Roever Engineering College, Perambalur, 621212, Tamil Nadu, India
[4]Department of Chemistry, S.I.V.E.T College, Chennai, 600073, Tamil Nadu, India
[5]Department of Product Development, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, 602105, Tamil Nadu, India
[6]Microbial Genomics Laboratory, Department of Microbiology, Periyar University, Salem, 636011, Tamil Nadu, India

(Received: February 2023 Revised: March 2023 Accepted: April 2023)

Corresponding author: **Shantkriti Srinivasan**. Email: shantkriti89@gmail.com

ABSTRACT

Introduction and Aim: Fenugreek (*Trigonella foenum graceum*) is extensively cultivated in several Asian nations. The leaves and seeds of this plant are well recognized for their potential against diabetes, some cancers and imparting immunity. They are often employed as adhesives and emulsifying agents. Polysaccharides can be found in abundance in fenugreek seeds. Galactomannan, a water-soluble polysaccharide, efficiently reduces the surface tension and increases the viscosity of liquids.

Materials and Methods: In this study, a simple method for extracting as well as purifying galactomannan was developed. It was produced from fenugreek plant's seeds and purified by centrifugation and isopropyl alcohol (IPA) spirit precipitation methods.

Results: The presence of carbohydrates by Molisch test and an absence of reducing sugar by Fehling's test was revealed. The pH of the purified galactomannan was 6.37, and its foaming capability was 14.28 %. The Galactomannan gum was found to possess 81 % emulsifying capacity. It revealed that the capacity to hold water was 1480% and the ability to hold oil was 268%.

Conclusion: In contrast to guar gum as well as the locust bean gum which are employed in various industries as thickeners, emulsifiers and stabilizers, galactomannan, produced in this study, is an effective and affordable method of stabilizing and emulsifying various products.

Keywords: Galactomannan; fenugreek; precipitation; emulsifying capacity; oil holding capacity.

INTRODUCTION

The fenugreek plant, scientifically known as *Trigonella foenum graecum* and belonging to the *Leguminosae* family, has the longest history in the medicinal plant category, originating primarily in India and Northern Africa but now widely available in other Asian countries such as China, Egypt, Pakistan, Turkey, and the Mediterranean (1). This plant's seed is a decent polysaccharide source; however, few have investigated its significance. Its utilization is confined to household remedies. Ethno-pharmacology studies show that it can be used to treat cancer (2, 3), basic chills, torn ligaments, mouth sores, diuretic, high blood pressure, abdomen aggravation, and dry lips, as well as being antimicrobial, antifungal, and hyper-cholesterolemic. The active principle in its leaves as well as seeds is utilized to make extracts or powders intended for therapeutic usage. An aqueous extract of *Trigonella foenum graecum* has been found to lessen oxidative stress in the kidneys of rats given morphine, demonstrating the herb's well-known anti-oxidative effects (4). Fenugreek seeds are composed of two cotyledons, with a wrinkled seed coat of a brownish yellow color, encircling an endosperm containing galactomannan polysaccharides. The major bioactive constituents of fenugreek seeds are: 3.6% moisture; 45 to 60% sugars and 25 to 30% protein; 7 to 9% ether extract; 5 to 7% steroid-containing saponins; 25 to 30% galactomannan; 20 to 25 % insoluble fiber; and 3 to 4 % ash (5). Fenugreek seeds have traditionally been used as a food source and for therapeutic purposes. Over time, there has been a growing demand to extract components from its seeds, which include polysaccharides used for storage as well as saponins. Similar to gums made from guar, tara, and locust beans (LBG), fenugreek gum (FG) has (-(1-4)-D mannose) in its core as well as -D-galactose at C6, however it is highly substituted. Most of the time, the ratio of mannose to galactose in FG is about 1, while it is 2, 3, and 4 for GG, TG, and LBG (6). The bulk polysaccharide component found in the seed endosperm is galactomannan which is stable, sticky, and very soluble in water. (Fig.1). Although, galactomannan biopolymer is available in various non-conventional plants (7), the plants from the *Leguminosae* family are preferred by scientists for research (8). It thickens and settles a variety of nutritional ingredients and is currently employed in the food sector as a rich carbohydrate storehouse (9).

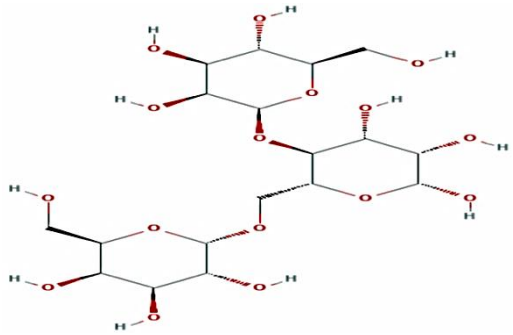

Fig. 1: Structure of galactomannan

Latest research suggests that galactomannan gum may be surface dynamic. It has been established that steady emulsions having a generally small bead measure (3 mm) can be shaped by utilizing cleaned Galactomannan gum. According to a study, the gum contains 0.8 % protein, which prevents hydrophilic gum from performing the task of surface action alone (10). Galactomannan gum additionally demonstrated the highest settling qualities in an oil-in-water colloidal system among five gums under study and eleven industrial gums. Since there has not been much research on the use of galactomannan in the food industry, a detailed analysis of its physicochemical properties as well as structure would provide light on its fundamental nature and support efforts to promote its potential application in the food and nutrition industry (11). Thus, the present study aimed to extract and purify galactomannan gum and evaluate its physical properties. The fenugreek seeds were simply processed to obtain galactomannan. Isopropyl alcohol in addition to water were used as solvents for extraction, and centrifugation was used to clean the substance. Its stability, emulsification, as well as ability to contain both water and oil were evaluated.

MATERIALS AND METHODS

Sample material

The dried seeds of fenugreek plant utilized in this investigation were bought at the neighbourhood market.

Extraction and purification of polysaccharides

Galactomannan was extracted and purified by the techniques depicted in Fig. 2 and by protocol followed by Kristjansson *et al.*, with some alteration (12). Fenugreek seeds were washed in distilled water, dried, and crushed. They were then immersed in a 5 % NaCl solution that had been pre-acclimatized to a pH range of 3 to 5, and allowed to settle for 48 hours at 37 °C with intermittent stirring. The sample was then put through a 10-minute, 10,000-rpm, 4-degree Celsius centrifugation process. Unrefined gum material comprising crude galactomannan was isolated as a supernatant. The IPA spirit (10 % ethanol with 90 % isopropanol) was mixed with unrefined galactomannan in a 3:1 proportion by volume, followed by active mixing and vigorous stirring in a homogenizer. The gum, including galactomannan, was then collected by centrifuging the mixture for seven minutes at 4°C and 6000 rpm. In order to precipitate this gum once more, a new portion of isopropyl alcohol was added after it had been thoroughly dispersed in freshwater. This process was repeated three times to produce a whiter-looking product that was also notably devoid of salts as well as acidic ingredients. A 5 % salt solution was used to rinse the seed particles till all of the gum was removed from the seeds. Using an oven drier set to 50°C, the generated gum was dehydrated under vacuum.

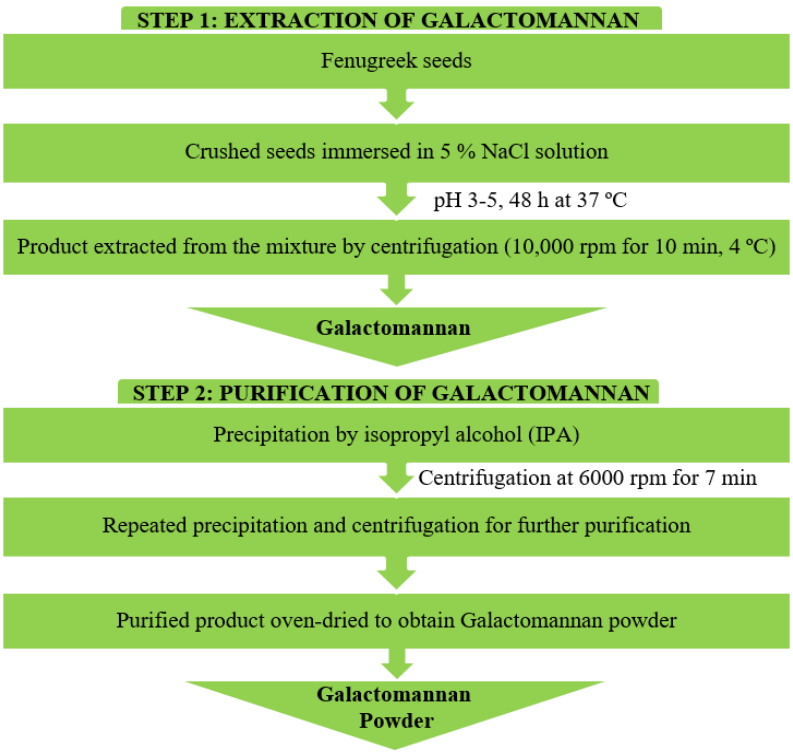

Fig. 2: Extraction and purification process for Galactomannan

Qualitative analysis of carbohydrates

Molisch test

Two millilitres of the sample galactomannan solution and two drops of the alpha-naphthol reagent were poured into a test tube. A dropper was used to pour concentrated sulphuric acid along the tube's edges while it was kept in a slanting posture. Carbohydrates can be detected by the development of a violet colour.

Fehling's test

Identical parts of Fehling A and B solutions were combined with two millilitres of the galactomannan sample in a test tube before being placed in a pot of boiling water for a few minutes. The existence of reducing sugars in the test group was indicated by the development of a yellow or brownish-red cuprous oxide precipitate.

Assessment of various activities

Emulsifying capacity

The emulsification capability of Galactomannan was investigated according to Brummer *et al.*, with some modification (13). Standard available coconut oil (four millilitre) was blended to forty millilitre of hydrocolloid suspension (0.5 % weight/volume), which was then homogenized for one minute at 10,000 rpm. Centrifugation was then performed for 5 mins at 6000 rpm. The Emulsifying Capacity (EC) was determined as:

$$EC = x_v \times 100 / t_v$$

Here, x_v refers to the emulsion volume while t_v denotes the total volume

Water and oil holding capacity

The protocol prescribed by Robertson *et al.*, with certain changes was utilized to measure the sample's water as well as oil holding capacity (OHC) (14). 250 mg of the material was mixed with twenty-five millilitres of either distilled water or coconut oil bought in retail. After mixing, it was allowed to sit at room temperature for one hour. For five minutes, the mixture was then centrifuged at 10,000 rpm. The residual material was quantified following centrifugation. The following formula was used to calculate the capacity to hold both water and oil:

HC (%) = (weight gained by the sample / dry weight of the sample) × 100

Foaming property

According to Temelli, the foaming capacity was examined (15). The volumes were measured in advance and subsequently after the test, which involved dissolving the 1.25 g of the sample in 50 mL of distilled water and violently shaking the mixture for 5 minutes. The equation utilized to determine the percentage of volume increase is as follows:

Percentage increase in volume = $[(V_2 - V_1)/ V_1] \times 100$
Here, V_1 and V_2 refer to the volume of solution before and after mixing.

RESULTS

Extraction and purification of galactomannan

This study's altered approach for extracting as well as purifying galactomannan was quite successful. The crude extract of galactomannan with some impurities was obtained after centrifugation (Fig. 3). But to remove the impurities from crude galactomannan, further purification was done by isopropyl alcohol precipitation. A customary whitish sticky galactomannan was finally obtained after purification steps and the comparative quality of the biopolymer was accounted for in the literature (Fig. 4). The acquired galactomannan had a high dissolving ability in water. The strategy was very effective in extracting a good yield of galactomannan biopolymer.

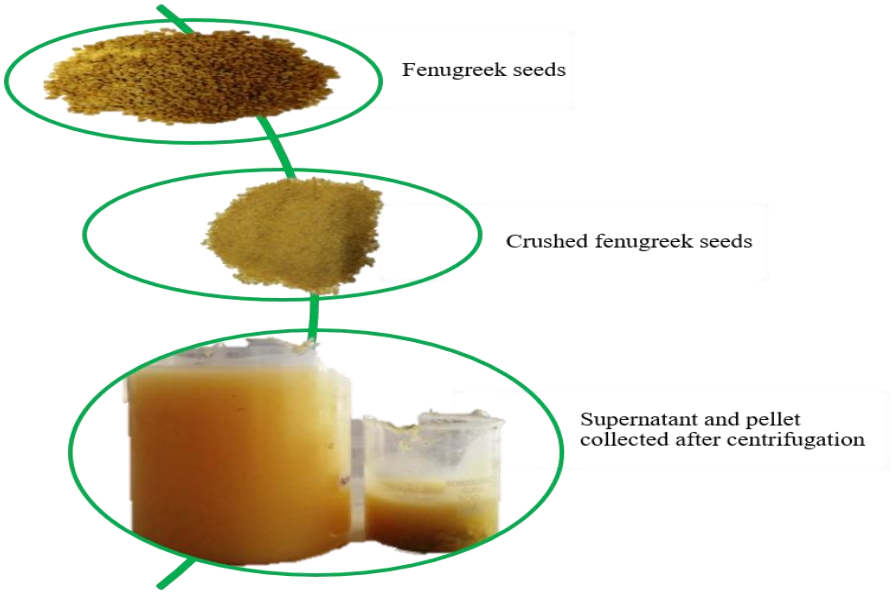

Fig. 3: Extraction of galactomannan

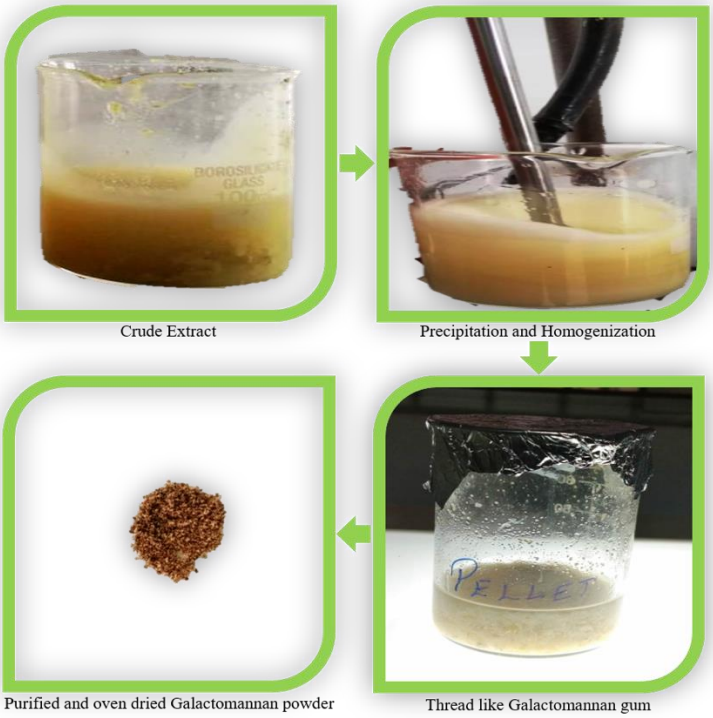

Fig. 4: Purification of galactomannan

Qualitative analysis

Molisch test

The highly sensitive Molisch test for detecting the presence of carbohydrates was carried out. In this study, the formation of a violet layer indicated the presence of carbohydrates (Fig. 5a).

Fehling's test

By turning a deep blue copper (II) solution into a red precipitate of insoluble copper (I) oxide, which is an indication of the existence of reducing sugars in the sample. This test showed negative results due to an absence of brownish red precipitate. This indicated the absence of reducing sugar (Fig. 5b).

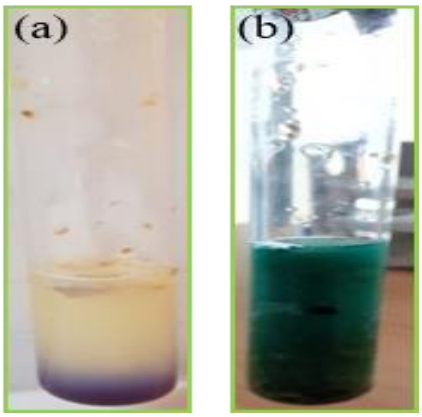

Fig. 5: (a). Molisch test (b). Fehling's test

Assessment of various activities

Emulsifying capacity (EC)

The emulsifying capacity for the purified galactomannan was 81.0 %. To further understand how galactomannan gum affects emulsions, duplicate emulsions with various amounts of this glue were created. The emulsion containing different concentrations of galactomannan gum (0.25, 0.5, and 0.1 %) demonstrated an emulsifying action which extended with increasing gum fixation up to 0.5 %, in contrast to guar gum. The strength of emulsions made with varied concentrations such as 0.25, 0.5, in addition to 0.1 % weight by volume of galactomannan gum appears to be economically reliable. It was revealed that galactomannan gum revealed good effects on emulsion modification. After two weeks of storage at 25 °C, the stability of this galactomannan gum emulsion with 0.5 % was found to be higher than with 96.5 %. Besides, suspensions with 0.25 % and 0.1 % of galactomannan gum could retain the emulsification strength till 95 % and 90 % respectively for more than fourteen days, separately.

Water holding capacity (WHC) and oil holding capacity (OHC)

The WHC was determined to be 1480 % and the OHC was found to be 268 %. The gum's ability to retain water and its wetness once equilibrium has been established under a specific environment make up the gum's water-holding limit. In this investigation, galactomannan gum had a greater oil holding capacity than the guar gum.

Foaming property

The arrangement of foams is caused by the whipping of gums. The creation of a stable system that could hold small solute particles was said to be the cause of this foaming. The foaming property of purified galactomannan was 15 %. The foam limit and

steadiness of watery scatterings of galactomannan gum were determined to be the important factors. Interestingly, galactomannan gum effectively maintained the stability of the foam volume even after two hours, whereas the foam strength of guar gum decreased. This characteristic facilitates its use as a stabilizer in foamy beverages, such as beer or even cappuccino.

DISCUSSION

In the literature, very low yield of this plant-derived adhesive is reported as it might be affected by the procedure utilized for extraction of biopolymer. The enzymatic technique produced 5.14 %, which was the highest β-glucan yield (16). It is acceptable to say that the present study produced high yield of galactomannan, which is essential for an efficient usage of this biopolymer.

Biochemical tests were performed to identify the nature of the biomolecules produced. Its dehydration with sulfuric acid is the basis of Molisch's test, which results in the formation of a violet ring as a result of an aldehyde condensation with two molecules of naphthol. The presence of aldehydes was determined using Fehling's assay.

Due to their inability to disperse or be weakened by the expansion of oil, these emulsions were found to be of the oil-in-water type (17). These hydrocolloids' emulsifying abilities may be due to their fundamental makeup, as to the manner in which their substituents and proteins are put together, which may lend them a hydrophobic quality (11, 13). In the literature under similar conditions, galactomannan gum was shown to possess greater emulsion soundness than mash gelatin of sugar beet whose emulsion strength was 70.1 % for more than one day (18). It was also high for some basic nutrition hydrocolloids, for example, methylcellulose, oat gum, guar gum, gum Arabic, gelatin, flaxseed, and beetle bean gum (11, 19). The effect of galactomannan gum as an emulsion is credited to its steric capacity for balancing out the drops of oil in an emulsion averting mixture (12). The lower emulsion limit and solidity could be caused by the arrangement's interfacial strain and the size of the polymers' subatomic loads.

The proportion of water or oil held per gram of the sample was used to express the water holding capacity (WHC) as well as the oil holding capacity (OHC). It is crucial to evaluate the WHC of gum in light of the parameters of capacity. It is also imperative for choosing an adhesive based on its water-holding properties in correlation to its economic potential for applications in nutrition, beautification, as well as a therapeutic agent. As opposed to guar gum, some findings demonstrated that galactomannan gum was less adept at holding water (20). The retention of moisture in these polysaccharide structures may be caused by the proximity of a huge amount of hydroxyl assemblies and residual proteins (21). Because proteins have a strong relationship with polar constituents and exhibit a hydrophilic association through hydrogen holding, the water holding limit is dependent on capillary, pore measurement, and charges on the protein atoms. Close examination of galactomannan revealed the existence of proteins as well as minerals, making galactomannan gum ideal for use as a stabilizer in specific food items.

Non-polar gum particles that can attract oil beads and cause an unnatural condition of oil retention are to blame. This characteristic affected the texture of food in a variety of ways (22). The synthetic and structural properties of hydrocolloids, such as the ratio and location of hydrophobic to hydrophilic assemblies within the hydrocolloid structure, are largely responsible for their ability to contain oil (23). The extracted gum substance will be very helpful in retaining the fat molecules that might encourage lubricity, a liquefying effect, as well as plasticity in the finished products (24).

The arrangement of foams is caused by the whipping of gums. The foam limit is related to an atom's ability to reduce the surface pressure of the air/water contact, and it is specifically related to surface strain. Apart from the polysaccharide, the concentrate's glycoprotein combination with protein may have contributed to this foaming, which is characteristic for galactomannan gum. This enables the galactomannan to lessen surface strain and show even more foaming capacity (25). Galactomannan's stability makes it easier to use in food items which consists of foam, such as mousse, various drinks, meringues as well as marshmallows.

CONCLUSION

The type of technique used for extraction as well as purification has a significant impact on the physiological properties of galactomannan. In this study, galactomannan was extracted and purified by centrifugation and IPA (isopropyl alcohol) spirit precipitation. Our modified strategy was fruitful in expelling the majority of the galactomannan’s debasements with a decent ultimate yield. Galactomannan showed good functional properties, including the ability to emulsify, hold water and oil, and foam, in addition to having a fair amount of carbohydrates but no reducing sugar. Galactomannan is better than gums that are already on the market because it is cheap and has good properties. It could be used as an emulsifier, thickener, and stabilizer in industry.

ACKNOWLEDGMENT

Authors thank the management of Dhanalakshmi Srinivasan University, Tamil Nadu, India and Kalasalingam Academy of Research and Education, Tamil Nadu, India for their support and all the laboratory facilities.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Jiang, J.X., Zhu, L.W., Zhang, W.M., Sun, R.C. Characterization of galactomannan gum from fenugreek (*Trigonella foenum-graecum*) seeds and its rheological properties. Int J Polym Mater. 2007; 56(12):1145-1154.
2. Chau, C.F., Huang, Y.L. Characterization of passion fruit seed fibres-a potential fibre source. Food Chem. 2004; 85(2):189-194.
3. Gupta, S.K., Kalaiselvan, V., Srivastava, S., Saxena, R., Agrawal, S.S. *Trigonella foenum-graecum* (Fenugreek) protects against selenite-induced oxidative stress in experimental cataractogenesis. Biol Trace Elem Res. 2010; 136(3):258-268.
4. Krishnan, S.S.A/P., Jeppu, A.K., Halim, S., Alkesah, T.S., Kumar, K.A., Soni, G. Renal protective effect of *Trigonella foenum-graecum* seed extract on morphine withdrawal in rats. Biomedicine. 2022; 42(6):1172-1177.
5. Kang, L.P., Zhao, Y., Pang, X., Yu H.S., Xiong, C.Q., Zhang, J., *et al.*, Characterization and identification of steroidal saponins from the seeds of *Trigonella foenum-graecum* by ultra-high-performance liquid chromatography and hybrid time-of-flight mass spectrometry. J Pharm Biomed Anal. 2013; 74:257-267.
6. Sav, A.R., Meer, T.A., Fule, R.A., Amin, P.D. Investigational studies on highly purified fenugreek gum as an emulsifying agent. J Dispers Sci Technol. 2013; 34(5):657-662.
7. Cerqueira, M.A., Souza, B.W., Simões, J., Teixeira, J.A, Domingues, M.R.M, Coimbra, M.A., *et al.*, Structural and thermal characterization of galactomannans from non-conventional sources. Carbohydr Polym. 2011; 83:179-185.
8. Campia, P., Ponzini, E., Rossi, B., Farris, S., Silvetti, T., Merlini, L., Brasca, M., Grandori, R., Galante, Y.M. Aerogels of enzymatically oxidized galactomannans from leguminous plants: Versatile delivery systems of antimicrobial peptides and enzymes. Carbohydr Polym. 2017; 158:102-111.
9. Stephen, A.M., Phillips, G.O. Food polysaccharides and their applications. CRC press; 2016.
10. Garti, N., Madar, Z., Aserin, A., Sternheim, B. Fenugreek galactomannans as food emulsifiers. LWT-Food Sci Technol. 1997; 30(3):305-311.
11. Huang, X., Kakuda, Y., Cui, W. Hydrocolloids in emulsions: particle size distribution and interfacial activity. Food Hydrocoll. 2001; 15(4-6):533-542.
12. Kristjansson, M., Eybye, K.L., Mhlanga, E.G. The effect of drying methods on functionality properties and odour profile of purified fenugreek gum extracted with a novel extraction method. Annu Trans Nord Rheol Soc. 2012; 20:297-302.
13. Brummer, Y., Cui, W., Wang, Q. Extraction, purification and physicochemical characterization of fenugreek gum. Food Hydrocoll. 2003; 17(3):229-236.
14. Robertson, J.A., de Monredon, F.D., Dyssele,r P., Guillon, F., Amado, R., Thibault, J.F. Hydration properties of dietary fibre and resistant starch: a European collaborative study. LWT-Food Sci Technol. 2000; 33(2):727-729.
15. Temelli, F. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J Food Sci. 1997; 62(6):1194-1201.
16. Ahmad, A., Anjum, FM., Zahoor, T., Nawaz, H., Ahmed, Z. Extraction and characterization of β-d-glucan from oat for industrial utilization. Int J Biol Macromol. 2010; 46(3):304-309.
17. Rwan, J.H., Shen, J.H. Comparison of emulsifying properties of chitosans of different deacetylation degrees and their application on producing salad dressing. Taiwan J Agric Chem Food Sci. 2006; 44(1):41-53.
18. Yapo, B.M., Robert, C., Etienne, I, Wathelet, B., Paquot, M. Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chem. 2007; 100(4):1356-1364.
19. Zeng, W.W., Lai, L.S. Characterization of the mucilage isolated from the edible fronds of bird's nest fern (*Asplenium australasicum*). Food Hydrocoll. 2014; 40:163-172.
20. Chang, Y.H., Cui, S.W., Roberts, K.T., Ng., Wang, Q. P.K. Evaluation of extrusion-modified fenugreek gum. Food Hydrocoll. 2011; 25(5):1296-1301.
21. Naqvi, S.A., Khan, M.M., Shahid, M., Jaskani, M.J., Khan, I.A., Zuber, M., Zia, K.M. Biochemical profiling of mucilage extracted from seeds of different citrus rootstocks. Carbohydr Polym. 2011; 83(2):623-628.
22. Thanatcha, R., Pranee, A.J. Extraction and characterization of mucilage in *Ziziphus mauritiana* Lam. Int Food Res J. 2011; 18(1):201-212.
23. Phimolsiripol, Y., Siripatrawan, U., Cleland, D.J. Weight loss of frozen bread dough under isothermal and fluctuating temperature storage conditions. J Food Eng. 2011; 106(2):134-143.
24. Rincón, F., Muñoz, J., De Pinto, G.L., Alfaro, M.C., Calero, N. Rheological properties of *Cedrela odorata* gum exudate aqueous dispersions. Food Hydrocoll. 2009; 23(3):1031-1037.
25. Naji, S., Razavi, S., Karazhiyan, H. Effect of freezing on functional and textural attributes of cress seed gum and xanthan gum. Food Bioprocess Technol. 2013; 6(5):1302-1311.