Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 12, No. 1, 29-38, 2022
DOI:10.30495/JFBT.2022.18967 https://dorl.net/dor/20.1001.1.22287086.2022.12.1.4.3

Studies on Physicochemical and Structural Properties of Marshmallow (*Althaea officinalis*) Seed Mucilage

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Received: 12 May 2021 Accepted: 21 June 2021

ABSTRACT: Marshmallow (*Althaea officinalis*) belonging to *Malvaceae* family possesses mucilage containing cells in stem, petiole, petals and seeds showing antimicrobial activity, anti-inflammatory, immunomodulatory effects among others. In this study, in order to determine the characteristics of marshmallow seed mucilage, as a potential new source of hydrocolloid, some instrument methods (Scanning Electron Microscopy, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, etc.) were used and viscosity of mucilage was determined. SEM analysis showed that the mucilage had an amorphous structure and disordered particle size. Mucilage solution at pH of 7 had negative charge; zeta potential of -22.4 mV, electrical conductivity of -1.753 mS /cm and particle size being 255.1 d.nm. Its glass transition temperature (Tg) was 37.9 °C and the melting process started at 34.3 °C to 182.2 °C. An endothermal peak was observed at 92.6°C. Heat of infusion was 199.19 J/g. The most important functional groups identified by FTIR were an asymmetric stretching of double-bond (C=0) in the deprotonated carboxylated groups at 14.3 and 1627 cm^{-1} vibratory stretching ring of pyranose at 1280 cm^{-1} and 1115 cm^{-1} as well as glycoside bonds at 617 and 780 cm^{-1}. Marshmallow seed mucilage solution showed a shear–thinning (pseudoplastic) behavior and the most predominant elements found in the mucilage were carbon (26.59%), potassium (26.39%).

Keywords: *DSC, Marshmallow, NMR, SEM, Seed Mucilage, Viscosity.*

Introduction

In recent years' interest in use of natural polymers as gelling, thickening, stabilizing and emulsifier agents has increased (Zatz *et al*., 1989). In addition, hydrocolloids have many secondary functional properties, including body forming, film forming, purifying, clouding and foam stabilizing agents, crystallization inhibition, etc. (Glicksman, 1982). One of active compounds in plants is mucilage which is a high molecular weight biopolymer produced naturally during normal plant growth. The most common source of mucilage is seeds. However, it is also found in skin, leaves, flower, root, bulb, tubercles and fruits

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(Niknam, 1999). Hydrolysis of mucilage apart from uronic acid reveals pentose and hexose with the most common ones being arabinose, xylose, rhamnose, mannose, galactose and glucose (Moafeghy, 1992). Marshmallow (*Althaea officinalis*) belonging to Malvaceae family possesses mucilage containing cells in stem, petiole, petals and seeds showing antimicrobial activity, anti-inflammatory, immunomodulatory effects among others (Hashemluyan *et al*., 2010; Al-Snafi, 2013). Marshmallow extract has intense antioxidant activity as all studies demonstrated its antioxidant properties (Elmastas *et al*., 2004). Polysaccharides found in this plant possesses antioxidant property whith 69% antioxidant activity being resulted from its α-Tocopheral (Kardosava and Machova, 2006). As natural gums and mucilage these polymers are inexpensive available and bio compatible and they are being much attention as compared to synthetic and semi-synthetic compounds because they have lower toxicity more reasonable cost and a non–allergenic nature (Kulkarni *et al*., 2005). Hashemluyan carried out a study on the extraction and determination of terpene, phenols, and mucilage content in stem, leaves, plants and seeds of marshmallow (*Althaea officinalis*) and compared their mucilage percentage. The result showed that these organs contain high mucilage content; however, its seeds contain the highest mucilage percentage (Hashemluyan, 2010). In 2010, Rani and co-workers investigated the phytoconstituents of marshmallow seeds (Althea officinalis) and introduced three phytoconstituents, n-hexacos-2-enyl-1, 5-olide (altheahexacosanyl lactone), 2 β-hydroxycalamene (altheacalamene) and 5,6-dihydroxycoumarin-5-dodecanoate-6 β-Dglucopyranoside (altheacoumarin glucoside) along with known phytoconstituents such as lauric acid, β-sitosterol and lanosterol which their structures were revealed by optical analysis and chemical reactions (Rani *et al*., 2010). In 1983, Capek *et al*. extracted polysaccharides from marshmallow (Althea officinalis) root and examined their arabinans structure. According to their results, the water–soluble arabinans had a branched structure with some α- L- arabinofaranosyl branches that are linked by different bonds (1→5), (1→2) and (1→3) and some of the L-arabinosyl groups are involved in branches through O-2, O-3, and O-5. There was a good agreement between the results obtained by chemical methods and 13Cn-m-r. The derived polymer had structural properties similar to those of L-arabinan isolated from other plant sources (Capek *et al*., 1983). The aim of this study was to investigate the physicochemical and structural characteristics of marshmallow seed mucilage.

Materials and Methods

Marshmallow seeds were purchased from (Pakan-bazr Co, Isfahan, Iran). In order to extract Marshmallow seed mucilage a slightly modified method of Farahnaky *et al.*, (2013) was used. Marshmallow seed were ground and hydrated (seed powder: water, 1:50 w/v at room temperature for all night. The mixture was centrifuged with (Rotofix 32 A, Germany) at 4000 rpm for 20 min at 25 °C to separate the mucilage. The gum solution, was then separated, dried at 50 °C, ground and then packed in hermetic sealed plastic bags and kept in cool and dry place for later experiments. Determination of zeta potential, conductivity and particle size by using Malvern Zetasizer Nano series. Nano – Zs (Malvern Instrument Worcestershire, UK), zeta potential of 100 (g/L) solutions was measured through the disposable folded capillary cell (DTS 1060) at 25 °C. The pH value of solutions ranged from 6.29-6.69. The conductivity and particle size of samples were also measured. A differential scanning calorimeter (INNUO DSC-500B, China) was used for DSC

analysis of mallow seed mucilage. 5 mg sample was placed into a platinum cup and sealed. The temperature ranged from 20-300 °C under nitrogen atmosphere with a heating rate being 10°C /min (Stopic *et al*., 1997). FTIR spectra observed for the mucilage were recorded on a FTIR spectrometer (perkin elmer spectrum 400, USA). The dry powder was mixed with KBr and pressed to form pellets under mechanical pressure. The FT-IR spectra were obtained at 4000 and 400/cm (Sahoo *et al.,* 2010). Scanning Electron Microscope (SEM) (TESCAN Vega-3 SBU model, Czech Republic) with acceleration voltage of 5.0 kV was used. The samples were mounted on an aluminum stub with double-sided adhesive tape. The tape was attached to the stub and the sample powder was scattered carefully over its surface. The stub with the sample was then coated with a thin layer of gold to make the sample conductive. The specimen was subjected to SEM analysis (Nep and Conway, 2010). Quantitative elemental analysis and distribution of the elements (by X-ray mapping) in the Si/C composite was examined using the energy dispersive X-ray Spectroscopy (EDS) analyzer attached with the SEM. NMR spectra of 1H and 13C of mucilage were recorded in an NMR (250MHz) spectrometer (Bruker Avance model, Germany). The test mucoadhesive agent (100mg) was dissolved in D2O and chemical variations were reported in ppm against an internal standard TSP (3-trimethylsilylpropionic-2, 2, 3, 3, -d4 acid, sodium salt, 98% D) for 1H NMR and 1,4-dioxane (d 66.67 ppm) for 13C spectra. Proton NMR spectra were obtained at a base frequency of 250MHz, with 16 transitions and delay time 1.5 s and for 13C, the base frequency was 100MHz, with 3000 scans and delay time 2 s (Hamcerencu *et al*., 2008). The viscosity of 40 (g/L) solutions was measured by use of a rheometer (Anton paar, MCR300, CC27, Austria). The effect of shear rate on the apparent viscosity of hydrocolloid solution at 1-1000 S-1 was investigated. The data obtained from the measurements were subjected to univariate analysis one-way variance analysis (ANOVA) to determine significant differences among the samples and values were compared by use of Tukey's test defined at $P \leq 0.01$. All measurements were carried out in triplicate order and reported as mean ± SD from independent trials. Data analysis was performed by SPSS software (version 16; IBM Corporation, Armonk, NY, USA).

Results and Discussion

Zeta potential indicates the surface and sub – surface charge of suspended particles. It is an indicator of colloid solutions stability. If the particles contained in suspensions have very high or very low zeta potential. They will show the tendency towards repulsion and aggregation was reduced (Acedo-Carrillo *et al.,* 2006). As a general rule, the stability or un stability of a suspension may be determined by zeta potential. Particles are stable when their zeta potential is > 30 mV and / or < -30 mV. Although some exceptions have been reported (Wang *et al*., 2010). Zeta potential is an indicator for measurement of pure charge of emulsion particles often representing the charged part of the biopolymer (Jindal *et al*., 2013). Various factors including pH, ion strength, type and concentration of protein and polysaccharide macromolecules affect surface charge, electrophoretic movement and complex surface charge, electrophoresis movement and complex zeta potential (Khoshmanzar *et al*., 2013). As shown in Figure 1 marshmallow mucilage solution (1mg/ml) showed negative charge at pH 7 and a zeta potential of -22.4 mV showing low stability due to its low zeta potential.

Particle size and particle size distribution of hydrocolloids are important parameters for the solubility and strength of

an emulsion (Mathur, 2012). Particle size (as average diameter, Z- average) in 1 mg/ml marshmallow seed mucilage solution passed 0.45 micron filter is shown in Figure 2 Particle z- average of marshmallow mucilage aqueous dispersion was 255.1 d.nm, while its PDI (polydispersity index) was 0.66. The results reveal micro particles with high polydispersity. Researchers reported that particle size in gums affects their swelling in water due to the surface changes exposed to water, in turn, influence, the inherent viscosity as well as molecular mass of gums. Also particle size also affects the swelling rate and molecular weight of guar gum (Wang *et al*., 2003).

Electrokinetic measurement is one way to study complex surface chemical exchange-processes at the mineral surface/liquid interface. All electro kinetic phenomena are related to the development of electrical double-layer at particles/electrolyte interface. Additionally, electrophoretic movement of suspended particles depends on zeta potential. It has been concluded that conductivity in any solution is a function of the concentrations of electrolytes, ions as well as electrical charges leading to an increase in electrical conductivity. Electrical conductivity and dielectric constant increase with heat and electrical stress with these changes being due to the decomposition of compounds and subsequent formation of compounds having smaller molecules. Given the previous studies it could certainly said that ZP has a great effect on mucilage properties (Singh and Bothara, 2014). marshmallow seed mucilage solution (1

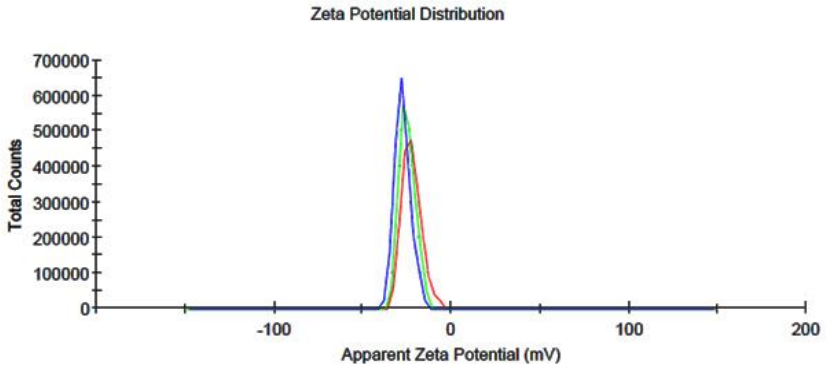


Fig. 1. Zeta potential data of marshmallow seed mucilage.

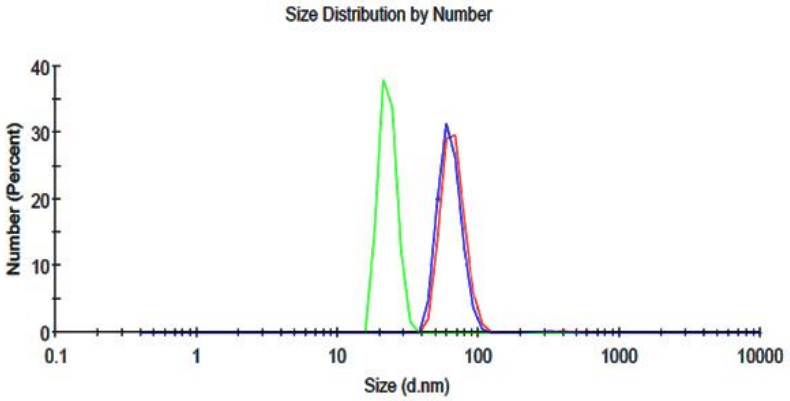


Fig. 2. Particle size data of marshmallow seed mucilage.

mg/ml) has an electrical conductivity value of -1.753 mS/cm.

DSC is a useful method for investigation of physical and chemical changes occurring during heating process in the polymers. This technique is based on the thermal changes. When a sample is subjected to thermal changes (received or lost thermal energy), it experiences a physical change such as phase transition. The amount of heat entered (or exit) the sample is different from the heat entered (or exit) a standard (Skoog *et al*., 2011). Changes including melting, crystallization, freezing and glass transition all being the indicators of compounds identification may be evaluated by this method (Haines *et al*., 1998). Glass transition temperature (Tg) is the temperature at which density, hardness and stiffness of polymer increase and its elongation percentage decreases drastically, i.e. it is the intermediate temperature between melted and hard states of matters. Above the Tg, secondary bonds between molecules are considerably weaker than thermal movement, since the polymer becomes rubbery and acquires a certain elasticity and plastic deformation without fracture occurs (Mathot *et al*., 1994; Maglic *et al*., 1984; Höne *et al*., 1996). Thermogram of marshmallow seed mucilage is given in Figure. 3. Suggesting that the melting process started at 34.3 °C to 182.2 °C. An endothermal peak indicating the melting process was observed at 92.6 °C suggesting its amorphous structure with the heat of fusion was found to be 199.19 J/g.

FTIR is widely used for describing the molecular polymer and investigating the type and number of functional groups in the structure. FTIR was used for understanding the functional groups present in the marshmallow seed mucilage (Baxter *et al*., 1992). Characterization using FTIR is often due to the identification of functional group and how they are connected to the structure of polymer. As shown in Figure. 4, The peak observed at 3430 cm-1 may be attributed to the symmetrical vibrations of carbon – hydrogen bond (-C-H) in the aliphatic chain of methylene group (-CH2) as well as acid carboxylic functional group (-COOH). The spectra observed at 1627d cm-1 are related to symmetrical vibration of carboxyl double-bond (C=O) in deprotonated carboxylate groups. The mucilage spectrum reveals that the bands found at 1000-1300 cm-1 (1115 and 1280 cm-1) are related to the vibrations of oxygen – carbon (-C-O), while the band observed at 617 and 780 cm-1 demonstrates the simple bonds of carbon – carbon (C-C) and out – plane vibrations of hydrogen – carbon (=C-H). FTIR revealed the presence of carboxyl groups likely used as the position where bivalent cautions are connected (Bramhachari *et al*., 2007)

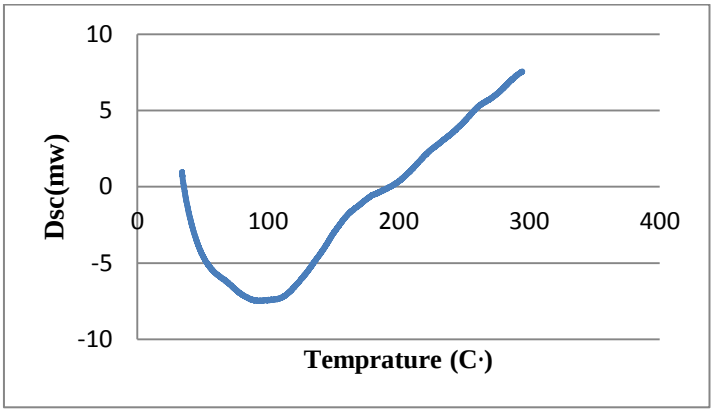


Fig. 3. Differential Scanning Calorimetry (DSC) Characterization of marshmallow seed mucilage Using DSC analyzer.

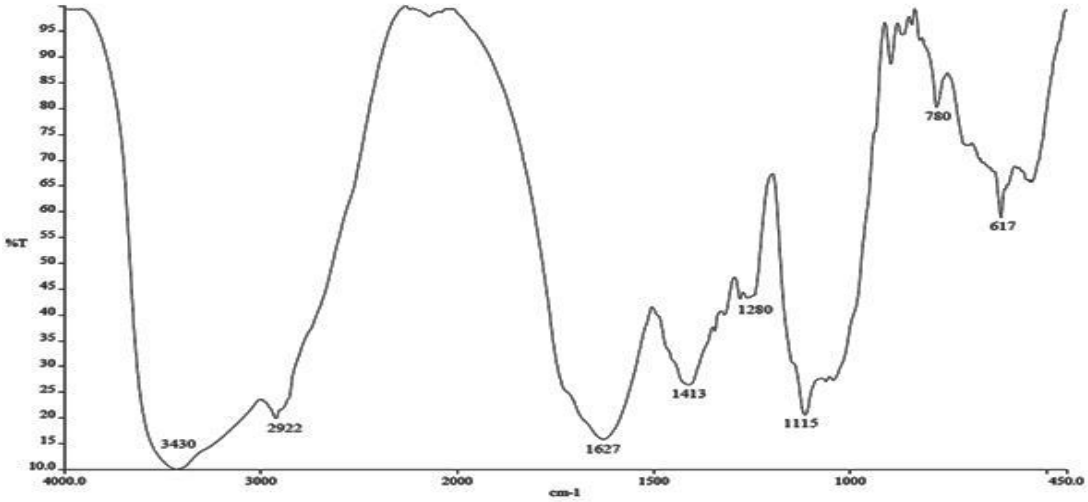


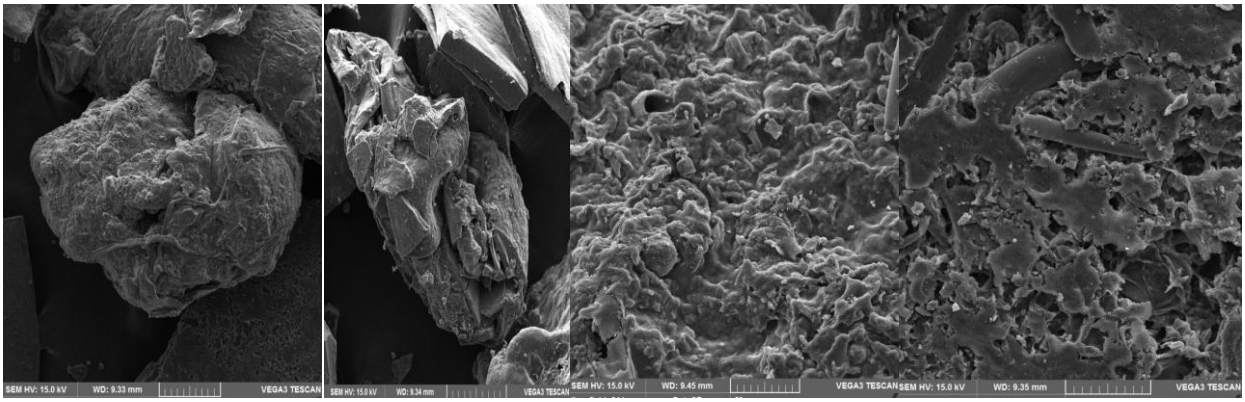
Fig. 4. FTIR spectral characterization of marshmallow seed mucilage using thermo scientific FTIR spectrophotometer.

SEM is an appropriate method for physical observations and morphological evaluations (Qi *et al*., 2005). In the present study, the micrograph of different levels of marshmallow seed mucilage suggests its amorphous nature with its surface characteristics may be attributed to the hydration capacity of mucilage. SEM analysis also indicates that the mucilage had different particle size and disordered morphology suggesting its fiber nature (Figure 5a and b), although the surface structure and/or topography of mucilage might be affected by the method of extraction or purification (Qian *et al*., 2009). It has been stated that particle size and specific surface area of gum and mucilage affect their hydration behavior in turn influence the inherent viscosity and molecular mass. Wang empirically reported that particle size influenced the rate of water absorption as well as molecular mass of guar some gum rich in galactomannan (Wang *et al*., 2002). A very rough surface with large wrinkle was observed likely caused by partly collapsed polymer gel network when drying (Figure 5c), In some part a continuous system but with a disordered and uneven structure having less roughness, rounded edges and different particle size was observed (Figure 5d), The analysis of elements may determine some chemical elements as minerals contained in the sample such as carbon, hydrogen, nitrogen and sulphur quantitatively and qualitatively. The results obtained by EDS showed the contents of elements as follows: C, N, O, Mg, S, Cl, K, and Ca at 26.59%, 5.34%, 18.32%, 2.04%, 4.22%, 9.40%, 26.39% and 7.69% respectively.

Apparent viscosity of 40 (g/L) marshmallow seed mucilage solution is given in figure. 6 apparent viscosity decreased from 0.296 to 0.0147 Pa as hear rate increased from 0.1 to 1000 s-1 showing dependency on the shear rate suggesting that the mucilage is pseudoplastic.

^{1}H and ^{13}C NMR spectral characterization of marshmallow seed mucilage are shown in Figure 7 and Figure 8 respectively. According to Table 1, all compounds in certain amount are present in the mucilage extracted from marshmallow seed mucilage, which is a non–starch polysaccharide. The higher integral value or the greater peak area the higher the amount of that type of proton in the compound. Among the suggested compounds, rhamnose was more prominent. The peak area for protons bound to second carbon type, (-C-H) in aliphatic chain is greater because it has a greater integral area. On the other hand, first carbon type, methyl

carbon (-CH3) is present only in the structure of rhamnose being visible in that spectrum. In addition to (-C-H) peaks in H-NMR spectra, the peaks observed for the proton of hydroxyl group (-O-H) and carboxyl (-COOH), having a great area, suggest the presence of all above compounds. Given the presented structures, the most predominant proton groups were methyl first carbon type – bound proton, second carbon type – bound proton, and oxygen – bound proton known as hydroxylic proton (-O-H) which is an alcoholic, hydroxylic or carboxylic type.



(a) (b) (c) (d)

Fig. 5. Scanning electron microscopy of marshmallow seed mucilage at different magnification using (TESCAN Vega-3 SBU model, Czech Republic).

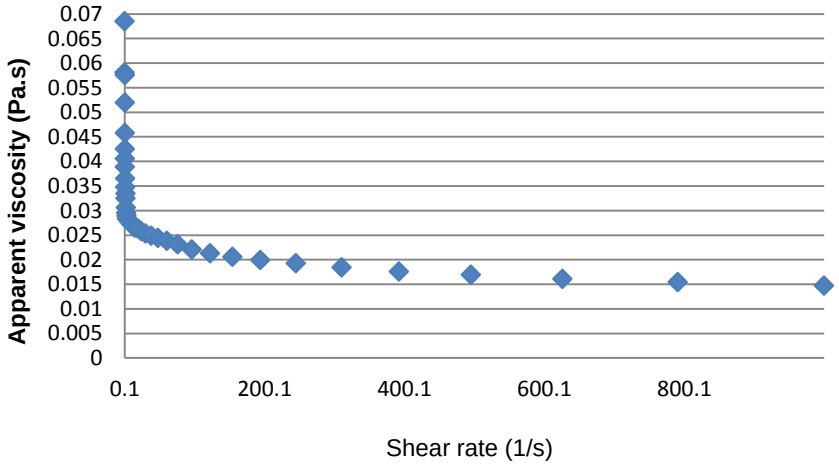


Fig. 6. The apparent viscosity of 40 g/L marshmallow seed mucilage solution at shear rate of 0.1-1000 1/s.

Table 1. Position, Area and kind of peaks in 1H-NMR spectra

Peak height (Integral)	**2.019**	**0.936**		**1.000**	**2.815**		**1.452**	**1.438**		
Chemical shift (ppm)	1.034[a]	1.062[b]	1.636[c]	2.134[d]	2.918[e]	2.983[f]	3.626[g]	3.827[h]	3.855[i]	4.604[j]
Glucuronic acid (Gu)	-	-	-	-	+	+	+	+	+	+
Galactronic acid (Ga)	-	-	-	-	+	+	+	+	+	+
Galactose (G)	-	-	-	-	+	+	+	+	+	+
Rhamnose (Ra)	+	+	+	-	+	+	+	-	+	+
Arabinogalactan (Ar)	-	-	-	-	+	+	+	+	+	+

[a,b,c]: Hydrogen of methyl ($-CH_3$) group in structures
[e,f,g,h]: Hydrogen bonded carbon ((II) type) (-C-H-) in structures
[i,j]: Hydrogen bonded oxygen in hydroxyl group (-O-H-) in structures

According to Table 1, 13C-NMR peaks observed for Suggested compounds were revealed and C-13 indicates the all compounds given the observed peaks. The peaks appeared at 26-31 ppm indicate first carbon type, methyl carbon (-CH3), only being found in rhamnose structure. The peaks observed at 63 – 100 ppm related to CII where hydrodxy groups are connected to it at different positions being referred to as alcoholic carbon (-C-OH) or acid carbon (-COOH). This type of carbon is found in all compounds are shown in Table 2, appeared in the respective spectrum. Only the peak for aldehyde carbon (-C=O), commonly found at 200 ppm, did not appeared likely due to peaks overlapping and/ or the impurities in the matter.

Conclusion

The results indicated that the mucilage has an amorphous structure with different particle size showing a non–Newtonian shear–thinning (pseudoplastic) behavior. Marshmallow seed mucilage has microprticles (255.1 d.nm) with high polydispersity (PDI =0.66). It has low stability because of having low zeta potential (-22.4mV). However, it may be used for stabilization of negatively charged colloid systems or sedimentation of positively charged system due to possessing of negative charges. Its Tg was 37.9 °C being attributed to the amorphous structure of the mucilage and the heat of infusion was 199.19 J/g. The most important functional groups identified by FTIR were an asymmetric stretching of double-bond (C=0) in the deprotonated carboxylated groups at 14.3 and 1627 cm^{-1} vibratory stretching ring of pyranose at 1280 cm^{-1} and 1115 cm^{-1} as well as glycoside bonds at 617 and 780 cm^{-1}.

Fig. 7. 1D ^{1}H NMR Spectral characterization of marshmallow seed mucilage using bruker advance II 250 NMR spectrophotometer.

Table 2. Position and Area of peaks in C-NMR spectra

	Chemical shift (ppm)							
Glucuronic acid (Gu)	-	-	-	-	-	+	+	+
Galactronic acid (Ga)	-	-	-	-	+	+	-	+
Galactose (G)	-	-	-	-	+	+	+	+
Rhamnose (Ra)	+	+	-	-	+	+	+	+
Arabinogalactan (Ar)	-	-	-	-	+	+	-	-

[a,b,c]: Carbon of methyl ($-CH_3$) and reveal (-C) of methyl in structure
[e,f,g,h],: Carbon ((II) type) bonded hydroxyl group and/or carboxylic acid (-COOH) in aliphatic chain in structure

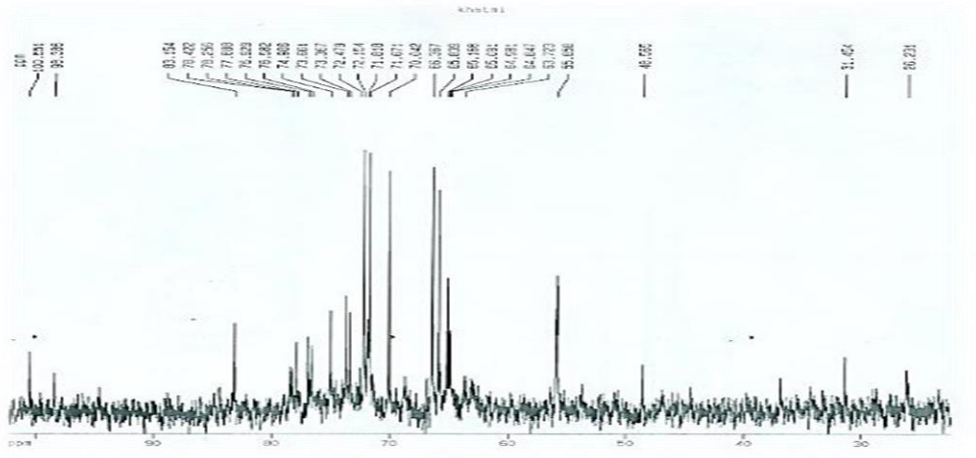


Fig. 8. 1D ^{13}C NMR Spectral characterization of marshmallow seed mucilage using bruker advance II 250 NMR spectrophotometer.

Acknowledgements

This paper was supported by the Department of Food Science and Technology of Islamic Azad University, Sabzevar, Iran.

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