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Molecular conformation and dilute solution properties of barley β-glucan: unveiling β-glucan as a highly flexible biopolymer under different processing conditions



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Abstract

Background The functional properties of food fluids containing hydrocolloids are influenced by temperature, soluble salts, pH and the presence of sugars. In this research, the properties of a dilute barley β -glucan (BBG) solution were evaluated in the presence of various factors. Different models were explored to determine the intrinsic viscosity of BBG.

Results The results indicated that the models of Higiro and Tanglertpaibul–Rao were more efficient in determining the intrinsic viscosity of BBG at different temperatures, different pH, the presence of sodium chloride, calcium chloride, and sucrose. Every 10 °C increase in temperature from 25 to 65 °C caused a decrease in intrinsic viscosity by 7.8, 10, 5.7 and 7.2%, respectively. In relation to the non-ionic structure of BBG, the presence of monovalent and bivalent salts had a negligible effect on reducing the intrinsic viscosity. The increase in pH from 3 to 7 caused a rise in intrinsic viscosity. But a further increase in pH, up to 9, caused a decrease in intrinsic viscosity. However, these changes were not significant and indicated that the non-ionic structure of BBG was independent of the pH. Since the constant b values of BBG were close to 1 at all temperatures, salt concentrations, different pH values and different sucrose concentrations, it can be assumed that the structure of BBG in the dilute range was close to the random coil conformation.

Conclusions The values of flexibility index and activation energy for BBG were calculated as 789.52 and 0.65×10^7 J/ kmole, respectively, which indicated that this hydrocolloid was highly flexible in different environmental conditions and it was independent of the processing temperature. Therefore, BBG can be recommended as a natural thickener in food-related fluids.

Keywords β-Glucan, Chain flexibility, Salt tolerance, Intrinsic viscosity, Molecular conformation

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Introduction

 β -Glucan is an important soluble fiber and the main component of the cell wall in barley endosperm cells. Barley β -glucan comprises a set of glucose chains with $\beta(1 \rightarrow 4)$ (70%) and $\beta(1 \rightarrow 3)$ (30%) bonds [1]. With β bonds, β -glucan is expected to have a sheet-like, cellulosic structure, which make it insoluble in water. However, the presence of β bonds between glucose molecules on carbon numbers 1 and 3, which are randomly present in its structure, usually create an irregularity and spatial obstruction in its structure and prevent the formation of large insoluble molecule plates similar to cellulose [2]. β-Glucan reduces bad cholesterol (LDL) and blood sugar by increasing the viscosity of the food in the digestive system, creating a gel-like network structure, entrapping part of the food glucose and bile acids in the small intestine and facilitating its excretion [3, 4]. Therefore, the daily consumption of β -glucan as a dietary fiber and food-drug is recommended by the US Food and Drug Administration [5].

 β -Glucan is known as a hydrocolloid because of its dispersed flow behavior in the aqueous phase. Solutions containing β -glucan can be used as a thickener in some food products such as sauces, ice cream and salads to increase their viscosity [6, 7].

In dilute solutions, macromolecule chains are separated from each other and remain without interaction with each other. Thus, exploring the behavior of these solutions can provide a deep insight into the flow behavior of macromolecules [8]. A complete scheme of the shape and structure of a macromolecule can be created by the evaluation of molecular and structural characteristics. In dilute solutions, macromolecules can be measured for their molecular weight, intrinsic viscosity, structural conformation, stiffness, coil and molecular shape [9]. It is expected that environmental factors such as temperature, pH and the presence of sugars and solutes in the environment, especially those that surround the macromolecule, have different effects on the shape and flow behavior of the macromolecule in dilute solutions. In this regard, previous research described the effect of these factors on the structural conformation and flow behavior of ionic and non-ionic hydrocolloids [10–15].

Previous research showed how an increase in temperature and the presence of salts can decrease the intrinsic viscosity. However, there is a knowledge gap in the effects of different pH values and sugar concentrations on the flow behavior of hydrocolloids in dilute solutions. Despite the unique structure of fibrous-polysaccharide and non-ionic β -glucan in barley, no amount of research has considered the flow behavior of this useful hydrocolloid in dilute environments so far. Accordingly, the purpose of this article was to evaluate a wide range of temperatures in food processing (25–65 °C), different environmental pH values (3–9), different concentrations of monovalent and bivalent salts and sucrose content on several hydrodynamic parameters (e.g., relative and intrinsic viscosity, stiffness, polymeric coil expansion, molecular conformation, coil overlap parameters, chain flexibility, coil radius, and coil volume). The ultimate aim was to evaluate the behavior of β -glucan macromolecules in dilute solutions.

Materials and methods

Preparation of barley flour

The sample of hull-less barley (cultivar 'Lut') was donated by SPII (Seed and Plant Improvement Institute) (Karaj, Iran). The content of β -glucan was determined by the specific enzymatic method using the mixed linkage Betaglucan assay kit (K-BGLU 07/11) provided by Megazyme International (Wicklow, Ireland). The barley kernels were grinded by a laboratory mill and sieved through a 0.50 mm screen. In brief, 200 g of barley flour was suspended in 800 ml of aqueous ethanol (80%, ν/ν) and stirred under reflux for 3 h. This procedure applied to removing the most of lipids and inactivates endogenous β -d-glucanases, which is responsible for the enzymatic hydrolysis of BBG chains during extraction, purification and storage. The liquid phase was separated by vacuum filtration and after that, the residue on filter paper dried at 40 °C for 12 h.

BBG extraction and solution preparation

BBG was extracted using the method outlined by Hematian Sourki et al. [6] and Ji et al. [16]. At first, stock BBG solutions were prepared at concentration of 0.2% (w/v) by dispersing freeze-dried BBG powder in double-deionized water, while stirring at 70 °C for 60 min using a hot plate magnetic stirrer. Solutions were then cooled to 25 °C, and the concentration of each solution was adjusted with distilled water. Then, the solutions were prepared in appropriate amounts by adding different concentrations of salts (NaCl, CaCl₂), sucrose, HCl and NaOH in a pH range of 3–9. The samples were mixed using a roller mixer (SRT2, Stuart Scientific; Pars, Iran) and were retained overnight for a complete hydration of BBG. The filtration process discarded any insoluble residues via a methyl-cellulose membrane (Macherey-Nagel, Germany) with a pore size of 0.45 μ m, before measuring the intrinsic viscosity.

Intrinsic viscosity

Intrinsic viscosity of BBG solutions was determined using capillary viscometry [17]. A No. 75 Cannon-Ubbelohde semimicro dilution capillary viscometer (Cannon Instruments Co., USA) was immersed in a thermostatic water bath at a controlled temperature (± 0.1 °C). The flow time of sample solutions through capillary viscometer was measured with an accurate digital chronometer. The time taken for the bath temperature to reach equilibrium in each solution was 15 min. Using exactly 2 ml of solution sample, the system was manually diluted after generating at least three efflux time readings at each concentration. The intrinsic viscosity measurements were carried out at 25 °C. In the absence of co-solutes, however, it ranged from 25 to 65 °C. The conversion of sample viscosity to relative viscosity (η_{rel}) and specific viscosity (η_{sp}) was made possible by the following equations [18]:

$$\eta_{\rm rel} = \frac{\eta}{\eta_{\rm s}},\tag{1}$$

$$\eta_{\rm sp} = \eta_{\rm rel} - 1, \tag{2}$$

where η is the solution viscosity and η_s is the solvent viscosity.

The intrinsic viscosity was determined by extrapolating $\eta_{\rm sp}/C$ and $\ln \eta_{\rm rel}/C$ values to zero concentration through Huggins [17] and Kraemer [19] equations, respectively:

$$\frac{\eta_{sp}}{C} = [\eta] + k' [\eta]^2 C,$$
 (3)

where k' and C are the Huggins constant and solute concentration, respectively.

$$\frac{\ln\eta_{\rm rel}}{C} = [\eta] + \mathbf{k}''[\eta]^2 \mathbf{C},\tag{4}$$

where k'' is the Kraemer constant.

Previous research showed that in addition to the usual Huggins and Kramer models that calculate intrinsic viscosity based on intercepts of plots, if intrinsic viscosity is calculated from the slope of plots, more acceptable results can be obtained [11, 15, 20]. Accordingly, the following equations determined the intrinsic viscosity:

Tanglertpaibul–Rao's equation [21]:

$$\eta_{\rm rel} = 1 + [\eta] \mathcal{C}. \tag{5}$$

Higiro's equations [22]:

$$\eta_{\rm rel} = e^{[\eta]C},\tag{6}$$

$$\eta_{\rm rel} = \frac{1}{1 - [\eta]C}.\tag{7}$$

Molecular conformation of BBG

The following power equation (Eq. 8) estimated the molecular conformation. The *b* component was an indicator of polysaccharides' conformation, calculated from the slope of a double logarithmic plot of specific viscosity versus concentration [23]:

$$\eta_{\rm sp} = {\rm aC}^b. \tag{8}$$

Smidsrod–Haug stiffness, relative stiffness parameter and persistence length

Based on Fixman's theory, if the molecular weight of the polymer is assumed to be constant, the stiffness parameter can be calculated [24].

To evaluate the effects of ionic strengths of NaCl and CaCl₂ on the stiffness properties of BBG, intrinsic viscosity data were fitted versus the inverse square root of ionic strength ($I^{-0.5}$) using a linear-based equation as follows:

$$[\eta] = [\eta]_{\infty} + SI^{-0.5}, \tag{9}$$

where $[\eta]_{\infty}$ is the intrinsic viscosity at infinite ionic strength.

Since stiffness depends on molecular weight, Smidsrød and Haug [24] developed the relative stiffness parameter (*B*), which was calculated according to the following equation:

$$S = B([\eta]_{0,1})^{\nu}.$$
 (10)

Generally, the value of parameter ν is considered to range from 1.2 to 1.4. Therefore, a review of the available literature showed that the value of parameter ν was 1.3.

The value of $[\eta]_{0.1}$ is the intrinsic viscosity at an ionic strength of 0.1 M [25]. Low values of *B* are associated with stiff polymer backbones and vice versa [26]. Smidsrød and Christensen [27] proposed another characteristic titled "persistence length" ξ , which is a measure of the length over which the chain 'persists' in the direction of the first bond of the chain. The relation between relative stiffness and persistence length was calculated according to the following equation:

$$\xi = \frac{0.26}{B}.\tag{11}$$

Chain flexibility

The well-known Arrhenius model can be applied to predict the effect of temperature on viscosity of a polymer solution under C^* concentration (Newtonian region) [28]:

$$\eta = A e^{E_a/RT}, \tag{12}$$

where η is the viscosity (Pa. s), R is the universal law gas constant (J/mol K), A is the proportionality constant, E_a is the activation energy (J/mol), and T is the absolute temperature (K).

In some cases, researchers believe that E_a represents the chain flexibility of a polymer, so that high values of E_a indicate low chain flexibility [10, 29]. With an increase in temperature, polymer chain flexibility decreases [15]. According to the relationship between chain flexibility and E_a , in the event of replacing viscosity with intrinsic viscosity in the Arrhenius equation and calculating the natural logarithm of the two sides of the equation, a linear equation is thus obtained (Eq. 13), the slope of which is calculated as E_a to fit the data as the natural logarithm of intrinsic viscosity versus the inverse of the absolute temperature:

$$\ln\left[\eta\right] = \ln \mathbf{A} + \frac{E_a}{RT}.\tag{13}$$

Shape and voluminosity

Voluminosity (V_E) is the volume of the solvated polymer molecule. Therefore, it can provide useful information about polymer conformation in different solvents. Relative viscosity data were used for calculating the V_E , so that the Y data were calculated from the following equation and used against the concentration by drawing a graph. At different conditions of solvents, V_E can be calculated from the interception of the Y axis versus concentration (concentration=0):

$$Y = \frac{\eta_{\rm rel}^{0.5} - 1}{\left[C(1.35\eta_{\rm rel}^{0.5} - 0.1)\right]}.$$
 (14)

The relationship between intrinsic viscosity and V_E is calculated by another parameter called shape function (v) according to the following equation:

$$\eta] = V.V_E,\tag{15}$$

where V is the shape factor of the polymer molecule and can represent the different shapes of polymer particles in different solvents [30].

Coil radius and volume

Hydrodynamic coil radius was calculated according to a relation outlined by Antoniou et al. [30]:



Fig. 1 Relative viscosity of BBG as a function of concentration at **a** different temperatures, **b** pH, **c** sucrose concentrations, **d** NaCl concentrations and **e** different CaCl₂ concentrations

$$R_{coil} = \sqrt[3]{\frac{(3[\eta] \times M_{\rm w})}{(10\pi \times N_{\rm Av})}},\tag{16}$$

where $M_{\rm w}$ was the average molecular weight of the polymer, π was equal to 3.14159 and $N_{\rm Av}$ was Avogadro's number and equal to $6.022 \times 10^{23} {\rm mol}^{-1}$.

Assuming that the shape of the coil polymer particles was spherical, the coil volume was calculated according to the following equation:

$$V_{\rm coil} = \frac{4}{3}\pi R_{\rm coil}{}^3. \tag{17}$$

Coil overlap

To calculate coil overlap, $\log(\eta_{sp})$ was plotted against log $C[\eta]$, and the resultant curve was called the master curve. The slope of the master curve and the Berry number at all temperatures, sucrose concentrations, different pH and different concentrations of monovalent and bivalent salts were calculated from the master curve. According to the theory of De Gennes [31], the slope of the master curve in dilute (C < C*) and semi-dilute (C > C*) areas was in the range of 1.2–1.3 and close to 3.75, respectively.

Statistical analysis

All rheological measurements were performed in triplicate. For determining temperature, ions, salts and sugar effect on dilute solution properties of BBG, the experiments were designed according to a factorial test in the form of a completely randomized design. Duncan's multiple range test at 95% confidence interval was used to compare the mean values. The curve fitting toolbox of MATLAB programming software (R2013a, The Math Works, Inc., USA) was used to determine [η].

Results and discussion

Relative and intrinsic viscosity

Figure 1 shows changes in relative viscosity, parallel to the increase in BBG concentration, while also considering changes in temperature (a), pH (b), sucrose concentration (c), NaCl (d) and $CaCl_2$ (e) concentrations. The relative viscosity decreased significantly in response to an increase in temperature. Higher temperature and an increase in molecular mobility decreased the bonds

Tabl	e 1	Intrinsic viscosit	y values determine	d by	five model	ls for	BBG at	t different	concentrations
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Treatment	Huggins		Kraemer		Tanglertpaibul and Rao		Higiro 1			Higiro 2					
	[η]	R ²	RMSE	[η]	R ²	RMSE	[η]	R ²	RMSE	[η]	R ²	RMSE	[η]	R ²	RMSE
Temperature	e (°C)														
25	5.4778	0.9988	0.0449	5.7499	0.9670	0.0401	8.1124	0.9854	0.0435	6.1859	0.9994	0.0059	4.7661	0.9908	0.0346
35	4.7006	0.9991	0.0396	5.0023	0.9632	0.0675	7.4458	0.9832	0.0455	5.7028	0.9985	0.0094	4.4248	0.9938	0.0278
45	4.1517	0.9980	0.0531	4.4314	0.9918	0.0305	6.6167	0.9814	0.0448	5.1333	0.9975	0.0113	4.04	0.9967	0.0189
55	4.2214	0.9896	0.0938	4.4137	0.9283	0.0571	6.1388	0.9855	0.0357	4.8395	0.9984	0.0083	3.8562	0.9951	0.0210
65	3.9935	0.9882	0.0879	4.1696	0.9143	0.0455	5.7675	0.9852	0.0371	4.4925	0.9986	0.0078	3.5418	0.9946	0.0224
рН															
3	5.1474	0.9786	0.1556	5.2525	0.9872	0.0171	7.3947	0.9834	0.0480	5.5473	0.9992	0.0071	4.2182	0.9911	0.0352
5	4.7129	0.9763	0.2112	5.071	0.9151	0.0902	7.5474	0.9747	0.0604	5.6859	0.9963	0.0154	4.3654	0.9964	0.0228
7	5.3172	0.9964	0.0787	5.6125	0.9765	0.0279	8.0603	0.9836	0.0505	5.9912	0.9993	0.0068	4.5131	0.9889	0.0416
9	5.0834	0.9938	0.0954	5.361	0.9431	0.0383	7.6521	0.9835	0.0498	5.6944	0.9993	0.0068	4.2971	0.9897	0.0394
Sucrose (%)															
10	5.0026	0.9975	0.0693	5.3221	0.9476	0.0656	7.8984	0.9832	0.0505	5.8976	0.9989	0.0087	4.464	0.9897	0.0396
20	4.6742	0.9872	0.1306	4.9532	0.8839	0.0745	7.1208	0.9822	0.0485	5.3849	0.9986	0.0093	4.1319	0.9932	0.0300
30	4.7556	0.9956	0.0678	4.9707	0.9574	0.0298	6.9227	0.9859	0.0412	5.2723	0.9994	0.0058	4.062	0.9909	0.0331
40	4.4191	0.9970	0.0523	4.6135	0.9343	0.0468	6.4388	0.9959	0.0485	4.9841	0.9993	0.0058	4.1319	0.9932	0.0300
NaCl (mM)															
5	5.2355	0.9981	0.0678	5.6067	0.9701	0.0574	8.4236	0.9815	0.0546	6.2738	0.9987	0.0096	4.7427	0.9914	0.0373
25	5.5025	0.8873	0.4605	5.1486	0.9266	0.0963	7.8515	0.9810	0.0581	5.805	0.9948	0.0200	4.3766	0.9903	0.0417
50	4.7374	0.9734	0.3999	5.188	0.9379	0.1890	8.4172	0.9707	0.0801	6.2986	0.9934	0.0253	4.8331	0.9982	0.0200
100	5.1635	0.9995	0.0407	5.4726	0.9854	0.0422	5.7261	0.9858	0.0356	6.057	0.9992	0.0088	3.6589	0.9903	0.0294
CaCl ₂ (mM)															
5	5.3752	0.9931	0.1441	5.6703	0.9698	0.0501	8.2212	0.9832	0.0587	6.1401	0.9989	0.0100	4.651	0.9943	0.0344
25	5.0926	0.9980	0.0823	5.4085	0.9934	0.0322	8.0833	0.9828	0.0589	6.059	0.9986	0.0113	4.6092	0.9950	0.0318
50	4.5118	0.9835	0.2242	4.8413	0.9548	0.1032	7.3538	0.9775	0.0621	5.6208	0.9960	0.0180	4.38	0.9988	0.0142
100	5.1439	0.9978	0.0859	5.4598	0.9968	0.0218	7.3538	0.9775	0.0621	6.0883	0.9986	0.0111	4.38	0.9988	0.0142



Fig. 2 Effect of temperature on the intrinsic viscosity ([n]) of BBG



Higiro et al. [32] stated that intrinsic viscosity is a measure of the hydrodynamic volume taken by macromolecules. This consumed volume is strongly dependent on the size and structure of macromolecule chains in solvents. In dilute solutions, polymeric chains are generally separate and independent of each other, meaning that intrinsic viscosity is exclusively dependent on the size and conformation of macromolecule chains in different solvents [32].

In this research, intrinsic viscosity was calculated by two methods: the intercept of plots by Eqs. 3 and 4 and the calculation method used on the slope of the plots by Eqs. 5, 6 and 7. A suitable linear extrapolation was observed for Eqs. 3 and 4, which indicated the appropriate application of these models in determining the intrinsic viscosity of BBG under different temperatures and chemical conditions.

Table 1 shows the intrinsic viscosity values of BBG calculated by the above methods under different temperatures (25, 35, 45, 55 and 65 °C), different pH values (3, 5,



Fig. 3 Intrinsic viscosity of BBG as a function of the inverse square root of ionic strength (25 °C)

7 and 9), different concentrations of sucrose (10, 20, 30 and 40%) and different concentrations of NaCl (5, 25, 50 and 100 mM) as a monovalent salt and different concentrations of CaCl₂ (5, 25, 50 and 100 mM) as a bivalent salt. The results showed that all equations can be used in different conditions to determine the intrinsic viscosity of BBG. This was proven by the high values of the coefficient of determination (R^2) and the small values of RMSE. However, based on the coefficient of determination and RMSE, it seems that the equations based on the slope of plots (5, 6 and 7) led to a more effective fitting of data pertaining to the intrinsic viscosity of BBG. In this regard, Yousefi et al. [15] Fathi et al. [20], Sherahi et al. [33] and Koocheki et al. [34] reported that determining intrinsic viscosity based on the slope of plots led to a higher coefficient of determination and a lower standard error, compared to the method based on the intercept of plots for sage seed gum, Prunus armeniaca gum exudates (PAGE), Descurainia sophia seed gum and Lepidium perfoliatum seed gum, respectively. The intrinsic viscosity of BBG at 25 °C in deionized water was 580.359 ml/g, which is higher than other polysaccharides such as pectin (313.31 ml/g) [35], konjac (565 ml/g) [36], and dextran (49.1 ml/g) [30].

The effect of different temperatures on the intrinsic viscosity of BBG is shown in Fig. 2. According to Fig. 2, an increase in temperature from 25 to 65 caused a significant decrease in the intrinsic viscosity of BBG from 8112.4 to 5767.5 ml/g. The effect of temperature on the intrinsic viscosity of BBG showed that with every 10 °C increase in temperature, the intrinsic viscosity decreased by 8.2%, 11.1%, 7.2% and 6%, respectively. In this regard, Amini and Razavi [10] reported that as the temperature increased from 20 to 30, 40 and 50 °C, the intrinsic viscosity of BSG decreased by 30.84%, 24.10%, and 15.12%, respectively. The same pattern was observed in polysaccharides, including cellulose, Tara gum, *Melissa officinalis* seed gum, and exopolysaccharide from *Virgibacillus salarius* BM02, where the



Fig. 4 Intrinsic viscosity ([η]) of BBG at different concentration of sucrose

decrease in intrinsic viscosity resulted from an increase in temperature [37–40]. However, different results have been reported in several studies. Sometimes, increasing the temperature reportedly caused an increase in intrinsic viscosity [41, 42] or it had no effect on intrinsic viscosity [43]. The decrease in intrinsic viscosity by an increase in temperature can be attributed to the provision of the required enthalpy for the polymer–solvent mixing process, so that the increase in temperature causes phase separation and the polymer moves inside the solvent at a faster rate [10].

Changes in intrinsic viscosity per pH are presented in Table 1. With an increase in the pH from 3 to 9, there were no significant changes in the intrinsic viscosity of BBG. The results showed that the intrinsic viscosity of BBG was independent of the pH of the solution. The lack of change in the intrinsic viscosity of BBG against changes in pH indicated the non-ionic structure of BBG. Accordingly, as the pH changed, the polymeric chains of BBG did not change and were actually not ionized, so their absorption capacity and the interaction between chains and the solvent did not change. Jowkarderis and van de Ven [44] reported that the intrinsic viscosity of cellulose nanofibers increased in response to an increase in pH. They reported that the lowest intrinsic viscosity of cellulose nanofibers corresponded to pH values lower than 3.5, where almost all carboxyl groups were protonated.

Figure 3 shows the ionic strength of the monovalent (NaCl) and bivalent (CaCl₂) salts and how they affect the intrinsic viscosity of BBG. As can be seen, although the intrinsic viscosity decreased with an increase in ionic strength, this decrease was not significant (p < 0.05). It is also observed that the effect of ionic strength of NaCl on the intrinsic viscosity of BBG consists of two sections. At very low ionic strengths, it is constant and increasing the ionic strength does not

Table 2 The values of Berry number (C[η]), exponent b (slope of log η_{sp} vs. log C), Huggins and Kramer constant of BBG at different conditions

Treatments		Berry number	b	R ²	K _H	κ
Temperature (°C)	25	0.145-0.580	1.229	0.9993	1.05	0.16
	35	0.128-0.541	1.266	0.9996	1.41	0.32
	45	0.114-0.511	1.260	0.9990	1.53	0.38
	55	0.112-0.503	1.212	0.9988	1.15	0.23
	65	0.105-0.532	1.203	0.9988	1.05	0.17
рН	3	0.132-0.620	1.195	0.9979	0.86	0.054
	5	0.129-0.582	1.253	0.9976	1.36	0.25
	7	0.142-0.637	1.233	0.9989	1.04	0.13
	9	0.135-0.636	1.225	0.9986	1.01	0.12
Sucrose concentration (%)	10	0.135-0.608	1.260	0.9993	1.24	0.22
	20	0.125-0.590	1.260	0.9993	1.14	0.18
	30	0.125-0.589	1.215	0.9990	0.98	0.12
	40	0.116-0.548	1.217	0.9995	1.06	0.18
NaCl (mM)	5	0.143-0.605	1.270	0.9992	1.31	0.24
	25	0.143-0.642	1.188	0.9959	0.78	0.03
	50	0.135-0.538	1.296	0.9977	1.90	0.48
	100	0.139-0.624	1.249	0.9994	1.20	0.20
CaCl ₂ (mM)	5	0.143-0.607	1.232	0.9987	1.1	0.16
	25	0.137-0.583	1.256	0.9993	1.29	0.25
	50	0.124-0.526	1.257	0.9976	1.56	0.37
	100	0.139-0.588	1.253	0.9992	1.26	0.23

affect the intrinsic viscosity of BBG, while it changes linearly for higher ionic strengths. A similar trend has not been observed in previous studies. More research will be needed to better understand this behavior. The rate of decrease in intrinsic viscosity of BBG against the increase in ionic strength was more profound in CaCl₂ salt, compared to NaCl, which indicates a greater effect of bivalent salts on the absorption of free water around the polymer. Ultimately, this reduces water absorption by the polymer. Amini and Razavi [10] reported that more cross-molecular bonds are formed in bivalent salts, which reduces the solvency and intrinsic viscosity of macromolecules. In this regard, similar results were reported on the reduction of intrinsic viscosity against the increase in ionic strength of salts for various natural polymers, including k-carrageenan [45], xanthan gum [46], L. sativum gum [47], Balangu seed gum (BSG) [10] and Melissa officinalis seed gum [39].

A decrease in the intrinsic viscosity of macromolecules in contrast to the increase in ionic strength can be attributed to the protective effect of charges on the macromolecular chains of polymers. By increasing the concentration of ions and thus the ionic strength, the protective effect of ions dominates the interactions between macromolecule and solvent, thereby causing a decrease in molecular repulsion between macromolecule chains and ultimately a decrease in intrinsic viscosity.

The effect of different concentrations of sucrose on changes in the intrinsic viscosity of BBG in Fig. 4 showed that by increasing the concentration of sucrose from 10 to 40%, the intrinsic viscosity of BBG decreased by 18.48% (from 7.9 to 6.44 dl/g). There was no significant difference between the effects of 20 and 30% sucrose concentrations. However, with the increase in sucrose concentration from 30 to 40%, the intrinsic viscosity of BBG decreased significantly. Amini and Razavi [10] reported that, on average, every 10% increase in sucrose concentration decreased the intrinsic viscosity of BSG by 8%, which was much higher than that of BBG. Fathi et al. [20], Yousefi et al. [15] and Behrouzian et al. [11] also presented similar results regarding the decrease in intrinsic viscosity of PAGE, sage seed gum and cress seed gum against the increase in sucrose concentration. Due to their osmotic properties and high hydrophilicity, sugars usually compete with macromolecules in absorbing water and render free water unavailable to macromolecules. This usually decreases the swelling of macromolecule chains and increases their inter-accumulation, thereby reducing the intrinsic viscosity.

Huggins constant

Huggins constant values are provided in relation to changes in concentration, temperature, pH, sucrose and

different salts in Table 2. This parameter indicates the intermolecular interactions between polymeric macromolecule chains in dilute solutions. Huggins constant depends on the expansion rate of the polymeric coil and indicates the molecular structure of the polymer. It determines how the polymer expands in a dilute solution. In the case of flexible polymers in a good solvent, the Huggins constant can range from 0.3 to 0.4. However, values from 0.5 to 1 indicate a lack of complete expansion of the macromolecule in a suitable solvent. Values greater than 1 indicate the unsuitability of the solvent. In fact, at values greater than 1, the solvent cannot fully expand the macromolecule.

The results showed that the Huggins constant was greater than 1 at all temperatures, pH values (except pH 3), sucrose concentrations (except 30%), NaCl salt concentrations (except 25 mM) and all CaCl₂ concentrations. This indicates that the BBG molecular coil does not fully expand in distilled water. β -Glucan has $\beta(1 \rightarrow 4)$ and $\beta(1 \rightarrow 3)$ bonds, which gives the polymer a semisheet structure. The presence of $\beta(1 \rightarrow 3)$ bonds causes irregularity in the polymeric structure and prevents the formation of parallel planes. As a result, a spatial prevention is created in these areas and the solvent (i.e., distilled water) can penetrate into spaces between the polymeric chains and hydrate them. In the linear regions of the BBG structure, where only $\beta(1 \rightarrow 4)$ bonds exist, such a spatial prevention is not observed, and the BBG has a celluloselike structure that is insoluble in water. Therefore, BBG does not completely dissolve in distilled water and is only hydrated in parts of its structure. High values of the Huggins constant confirm that BBG cannot be dissolved completely in water.

In Table 2, the Huggins constant increased in response to the rise in temperature from 25 to 45 °C. Thereafter it decreased as the temperature increased from 45 to 65 °C. The best temperature for a complete dissolution of BBG in distilled water is 70 °C. In fact, at high temperatures, the necessary energy is provided for a complete molecular expansion and the penetration of the solvent into spaces between the macromolecule chains. Amini and Razavi [10] reported that the Huggins constant of BSG decreased in response to the increase in temperature, indicating an increase in molecular associations in the polymer structure.

An evaluation of the effect of different pH values showed that the lowest and highest Huggins constant values were observed at pH values 3 and 5, respectively. The Huggins constant decreased with an increase in pH from 5 to 9. According to the results, it can be concluded that the best pH for the dissolution of β -glucan in deionized water is pH 3 and then alkaline pH. In acidic and alkaline



Fig. 5 Master curve for BBG determined at a selected temperatures, b different pHs, c sucrose concentrations, d NaCl concentrations and e different concentration of CaCl₂

pH, there is a possibility of depolymerization of BBG chains and thus the molecular weight decreases. The breaking of the molecular chain of BBG due to acidic and alkaline pH values reduces the prevalence of cellulose-like units in its structure, and therefore the solvency of BBG increases [6].

The Huggins constant decreased with the increase in sucrose concentration from 10 to 30%. Amini and Razavi [10] reported that the Huggins constant decreased to less than 0.3, which indicates a less optimal condition

of the solvent. In fact, the competition between sucrose molecules and macromolecules reduces the interactions between the macromolecule chains and the solvent.

At all concentrations of monovalent and bivalent salts, the Huggins constant was higher than the control sample (i.e., the sample without salt at 25 °C). In fact, the presence of salts and the occurrence of osmosis and water absorption removed the free water which was necessary for the complete dissolution of BBG. Thus,

the BBG macromolecule chains are not fully expanded and are not completely dissolved in water.

Molecular conformation by coil overlap parameters

According to Fig. 5, the master curve shows BBG at a slope of 1.23. According to Fig. 5, there is only one slope for the BBG solution and it shows that the BBG solution is in the dilute range. The results showed that the slope of the master curve of BBG was between 1.19 and 1.29 at all temperatures, pH values, sucrose concentrations, and salt concentrations, thereby indicating that they were all in the dilute range (Table 2).

If the Berry number is more than 1, the molecular entanglement of macromolecules occurs [11]. In fact, in semi-dilute areas, the Berry number is between 1 and 10. The results showed that the Berry number of BBG was 0.11–0.58 at different temperatures, 0.13–0.64 at different pH values, 0.12–0.61 at different sucrose concentrations, and 0.12–0.64 at different salt concentrations, all of which were less than 1 and in the dilute range (Table 2).

The constant values of b, which is the slope of the power-law curve (Eq. 8), are presented in Table 2. If the constant value of b is greater than 1, it indicates the random coil conformation in dilute solutions. However, several researchers have reported that when the constant value of b is less than 1, a rod-like conformation of macromolecules is expected [48]. According to Table 2, the constant values of b ranged between 1.19 and 1.30 at all temperatures, pH values, different sucrose concentrations and different concentrations of monovalent and bivalent salts. Since the b constant of BBG was close to 1, it can be assumed that the BBG structure in the dilute regions approximated the random coil conformation. In this regard, Behrouzian, et al. [11] reported that the constant value of b in cress seed gum was 1.08, which suggested that the structure of



Fig. 6 An Arrhenius-type plot for chain flexibility parameter of dissolved BBG in deionized water

this gum was in the dilute range between random coil and rod-like conformation. Higiro et al. [22] reported that the b constant value of locust bean gum was 1.234, which indicates the random coil structure of this gum in dilute ranges. Fathi et al. [20] reported that the constant b values of PAGE, without any additives, were 0.95-0.99 at 25-65 °C. This indicated the rod-like conformation of this gum in dilute regions. They reported that adding different sugar concentrations, NaCl salt and CaCl₂ salt caused an increase in constant b values of PAGE to 1.04-1.16, 1.12-1.22 and 1.15-1.20, respectively, indicating a change in the conformation of this gum from rod-like to random coil conformation.

Salt tolerance on stiffness parameters

Considering the non-ionic structure of BBG, it seems that the electrostatic force is not an effective factor in changing its conformation and only affects the structure of BBG through competition for the absorption of free water. By drawing a curve based on an equation reported by Smidsrød and Haug [24], and by plotting intrinsic viscosity against the square root of ionic strength($I^{-0.5}$), the stiffness and intrinsic viscosity were calculated for BBG at infinite ionic strength ($[\eta]\infty$) under the effect of sodium and calcium ions (Fig. 3). The low values of the coefficient of determination (R^2) showed no

 Table 3
 The coil radius and coil volume of BBG at different conditions

Treatments		R _{coil} (nm)	V _{coil} (nm) ³		
Temperature (°C)	25	28.37	95,620.23		
	35	27.18	84,131.04		
	45	26.14	74,851.37		
	55	26.00	73,623.31		
	65	25.49	69,344.33		
рН	3	27.47	86,836.40		
	5	27.29	85,172.44		
	7	28.13	93,248.98		
	9	27.70	89,007.16		
Sucrose concentration (%)	10	27.70	89,001.58		
	20	27.02	82,586.98		
	30	27.00	82,470.34		
	40	26.36	76,734.44		
NaCl (mM)	5	28.21	93,998.08		
	25	28.21	94,045.16		
	50	27.66	88,681.46		
	100	27.94	91,368.23		
CaCl ₂ (mM)	5	28.24	94,376.16		
	25	27.86	90,561.26		
	50	26.93	81,798.59		
	100	27.94	91,341.63		

suitable linear relationship for the Smidsrød equation in the case of Na⁺ ions ($R^2 = 0.43$), whereas this value was higher in the case of calcium ions ($R^2 = 0.84$). The results showed that $[\eta] \infty$ for BBG in the presence of sodium and calcium ions was 544.6 and 516 ml/g, respectively. Amini and Razavi [10] reported $[\eta]\infty$ values of 591.26 and 164.80 ml/g for BSG in the presence of sodium and calcium ions, respectively. The values of stiffness (S) for BBG in the presence of sodium and calcium ions were 0.020 and 0.039, respectively, which were much lower than the values indicated for BSG (0.346 and 0.507) as reported by Amini and Razavi [10]. Mohammadifar et al. [26] reported a value of 0.6 for the stiffness (S) of tragacanthin, which was much higher than the stiffness of BBG. The low stiffness value of BBG compared to other reported gums and hydrocolloids indicates a greater flexibility of its structure compared to other gums, regardless of molecular weight. Accordingly, a higher BBG stiffness in the presence of calcium ion, compared to sodium ion, indicated a decrease in its flexibility in the presence of bivalent salts, compared to monovalent salts.

Since stiffness largely depends on the molecular weight of macromolecules, it is not a suitable parameter for evaluating the stiffness or flexibility of macromolecules. Therefore, Smidsrød and Haug [24] developed the "relative stiffness parameter" (B), which is independent of molecular weight. In the presence of sodium and calcium ions, B values of BBG were 0.002 and 0.004, respectively. Mohammadifar et al. [26] reported that low values of B indicate the stiffness of a polymeric structure and vice versa. They reported that tragacanthin had a B value of 0.013. In the presence of sodium and calcium ions, the B value for PAGE gum was 2.57 and 4.24, respectively, which was much higher than that of BBG [20].

Based on an equation proposed by Smidsrød and Christensen [27], the persistence length of BBG in the presence of sodium and calcium ions was 121.62 and 61.96 nm, respectively. High ξ values confirmed the random coil conformation of the β -glucan macromolecule chain. Mohammadifar et al. [26] reported that tragacanthin had a ξ value of 14–27 nm, which was much lower than that of BBG.

Chain flexibility parameters

According to Fig. 6, the Arrhenius plot shows the logarithm of the intrinsic viscosity of BBG versus the inverse of the absolute temperature. The slope of the curve E_a/R describes the flexibility of the BBG macro-molecule chain. The values of flexibility index and activation energy for BBG were 789.52 and 0.65×10^7 (J/kmole), respectively. The flexibility index value for BBG was higher than the values reported for chitosan (488) [49], *Alyssum homolocarpum* seed gum (618) [9], cellulose diacetate (645) [50]. However, it was lower than

the values reported for sage seed gum (3046) [15], chitosan (2300) [51], xanthan (1100) [52], BSG (1156) [10] and PAGE (997) [20]. A comparison of the flexibility index of BBG with that of other gums and hydrocolloids shows that this hydrocolloid (BBG) has a better relative flexibility than hard hydrocolloids such as chitosan. The activation energy of BBG was close to the value reported for PAGE 0.83×10^7 (J/kmole) [20], which is lower than the values reported for BSG 1×10^7 [10], chitosan 1.5×10^7 [53] and sage seed gum 2.53×10^7 [15]. The lower activation energy indicates the independence of the physicochemical and rheological properties of hydrocolloids to temperature. Therefore, considering the low activation energy of BBG, this hydrocolloid can be used suitably as a thickener in food formulations that are affected by high temperatures.

Coil radius and volume

The changes in coil radius and coil volume of BBG varied, depending on the different temperatures, pH values, sucrose concentrations, monovalent and bivalent salts (Table 3). The coil radius and coil volume decreased significantly in response to the increase in temperature. The presence of monovalent and bivalent salts of sodium chloride and calcium chloride reduced the coil radius and the coil volume. Increasing the concentration of sucrose also significantly reduced the coil radius of BBG. The lowest and highest coil radius was observed at 65 $^{\circ}$ C and 25 °C, respectively, without the presence of any additives. Beyond 65 °C, the highest coil radius was observed in the presence of 40% sucrose. According to the results, it can be concluded that temperature has a greater effect on the compression and reduction of the coil radius, compared to the effects of sugars, salts, and pH values.

The results showed that the changes in coil radius and coil volume were similar to the changes in intrinsic viscosity per the temperature. With an increase in temperature from 25 to 65 °C, the coil radius decreased by 10% and, thus, the intrinsic viscosity also decreased by 27%. These results were consistent with previous reports. For instance, Amini and Razavi [10] reported that increasing the temperature caused a decrease in the intrinsic viscosity, as well as a decrease in the coil radius and coil volume of BSG. In this regard, Fathi et al. [20] reported that by increasing the temperature from 25 to 65 °C, the coil radius of PAGE gum decreased by 11%, which was similar to the results of the current study. According to the nature and chemical structure of water and polar solvents, it seems that increasing the temperature causes a relative increase in intermolecular bonds and compression of the macromolecule conformation. The increase in temperature reduces the stability of hydrogen bonds between macromolecule-solvent and

macromolecule–macromolecule. This causes a relative increase in intermolecular bonds and the compression of the macromolecule structure, associated with a decrease in the coil radius and coil volume of the macromolecule [10]. The effect of monovalent and bivalent salts as well as sucrose on the reduction of coil radius and coil volume can be explained by the presence of ions and sugars which adversely affected the competitiveness of macromolecules for free water. As a result, the macromolecule chains accumulated and compressed inward. Thus, the coil radius and coil volume of BBG decreased in the presence of salts and sucrose.

Conclusion

All of the proposed models in this research were suitable for calculating the intrinsic viscosity of BBG, although the equations based on slope (Tanglertpaibul-Rao, Higiro 1 and Higiro 2) were more effective in fitting the intrinsic viscosity of BBG at different temperatures, pH values, different sucrose and salt concentrations. An increase in temperature decreased the intrinsic viscosity of BBG. Similarly, an increase in temperature decreased the coil radius and coil volume of BBG by reducing the stability of hydrogen bonds. Changes in the pH did not have a significant effect on the changes in intrinsic viscosity, probably because of the non-ionic structure of BBG which is independent of changes in the pH of the environment. Increasing the concentration of bivalent calcium chloride salt, compared to monovalent sodium chloride salt, had a greater effect on reducing the intrinsic viscosity of BBG. Bivalent salts are usually more capable of absorbing free water and making it inaccessible to BBG macromolecules. Similar to salts, sucrose decreased the intrinsic viscosity of BBG through changes in osmotic pressure and binding with free water. When the Berry number was greater than 1, together with a high persistence length, the random coil conformation was in the dilute range. The non-ionic structure of BBG was the reason why the changes in ionic concentration around this macromolecule had no significant effect on its stiffness. Accordingly, the stiffness of BBG was lower than those reported in the case of several other gums and hydrocolloids, regardless of molecular weight. This can be a reason for the high flexibility of BBG, compared to other gums. The high values of the flexibility index and the low activation energy of BBG, compared to several other hydrocolloids, suggested the high flexibility of this hydrocolloid and its independence of temperature. Therefore, BBG can be used effectively as a thickener and stabilizer in food emulsions that are processed and stored at different temperatures.

Abbreviations

BBG	Barley β-glucan
LDL	Low-density lipoprotein
FDA	US Food and Drug Administration
SPII	Seed and Plant Improvement Institute
RMSE	Root mean square error
PAGE	Prunus armeniaca Gum exudates
BSG	Balangu seed gum

Author contributions

AHS designed the experiment. MAH prepared the samples and performed the experiments. AHS performed the experiments, analyzed data, and wrote the paper. AHS and MAH reviewed and checked all the details. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Declarations

Not applicable.

Ethics approval and consent to participate

Consent for publication

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Competing interests

No competing interests declared by the authors.

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