Dynamic mechanical spectra of salep and casein mixtures

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Abstract In this study, a glucomannan based salep and casein were mixed in a solution at different concentrations (0–0.75% salep and 0–3.0% casein) according to the central composite rotatable design and their color, steady shear and dynamic shear rheological properties were characterized. All studied parameters were affected by the salep and casein levels significantly. Salep increased the apparent viscosity, consistency coefficient, storage modulus and complex modulus of the solution compared to casein and an interaction was observed between the salep and casein which shows a significant increasing effect on the parameters. Frequency dependency increased with the increase of casein in the mixtures. Regression equations having quite high coefficients of determination were constructed for the estimation of studied parameter levels. Optimization performed with desirability function showed that the maximum levels for the rheological parameters could be achieved by more increment of salep level in the solutions because sole effect of casein on the rheological parameters was limited compared to salep.

Keywords Salep · Casein · Rheology · Response surface methodology · Optimization

Introduction

Salep is a glucomannan based hydrocolloid produced by milling the dried tubers of certain wild terrestrial *Orchidaceae* species (Kaya and Tekin 2001). East Mediterranean countries are the main place in the world for the collection of salep and it is produced on an average 20 tons in Turkey every year (Hossain 2011; Kurt and Kahyaoglu 2014). Salep is a white powder which is commonly used as a traditional hot beverage called as salep drink in the winters in Turkey (Telcioglu and Kayacier 2007). Besides, it has a desired aroma and flavor in the drink product and it also serves as a thickening and stabilizing agent having a techno-functional properties for the food formulations especially for ice cream (Kurt and Kahyaoglu 2014) because the salep has a glucomannan structure which is a chain structured by glucose and mannose connected by β -(1 $\rightarrow$ 4) glycosidic bonds and it has a galactose substitution at the C-6 position (Farhoosh and Riazi 2007; Pourjavadi et al. 2013). Salep is a high molecular weight polymer called as hydrocolloid or gum and it interacts with water easily and strongly to create a thick solution. Due to this property, it is commonly used as a stabilizing agent in food industry for its thickening, gelling, stabilizing, texture-modification and film-forming properties (Farhoosh and Riazi 2007; Pourjavadi et al. 2013; Kurt and Kahyaoglu 2014). Because of unique functions and important properties, salep was used in different foods and model systems to characterize the rheological and techno-functional properties (Ayar et al. 2009; Tekinsen and Güner 2010; Yasar et al. 2009; Razavi and Karazhiyan 2009; Karaman et al. 2013a, b; Kaya and Tekin 2001; Kurt and Kahyaoglu 2014).

Casein micelle presents in the milk structure is a complex protein and a relatively large entity. It is a

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supramolecular association of individual casein sub-units namely α_{s1} , α_{s2} , β and κ caseins. These fractions present in a micelle structure (Schmidt 1982; Visser 1992; Bourriot et al. 1999). Protein-polysaccharide interactions are very important for the specific properties of the samples containing protein and polysaccharide. As is well known, biopolymer mixtures are used in the food industry because a desirable texture is easily performed by the mixed hydrocolloid systems (De Kruif and Tuinier 2001). These mixed hydrocolloid systems are used commonly in dairy products in which casein constitutes indeed the major protein components like ice cream (Swaigood 1982; Bourriot et al. 1999). Different studies aimed to investigate the interaction between casein and polysaccharides are present in the literature (Drohan et al. 1997; Langendorf et al. 1997; Augustin et al. 1999; Perissutti et al. 2002; Neiryneck et al. 2007; Li et al. 2012).

To the best of my knowledge, there is no published paper on the interaction between glucomannan based salep and casein. Only the study related to casein-konjac glucomannan blend was performed by Fan et al. (2015). They investigated the optimization of preparation process for casein-konjac glucomannan blend sol and its stability. The main aim of the current work was to investigate the effect of salep-casein mixing at different concentrations on color, steady shear and dynamic shear rheological properties of mixed salep-casein solutions. For this aim, response surface methodology-central composite rotatable design was used to determine the linear and interactive effect of the salep-casein levels on the studied parameters. This study will provide better understanding of the behavior of salep-casein complexation during mixing.

Materials and methods

Preparation of mixtures

To prepare the mixtures, the central composite rotatable design given in Table 1 was used. Thus, the corresponding amount of salep (Özselamoglu Food Co. (Kayseri, Turkey) in Table 1 for each run was added in 100 ml of 0.25 M NaCl at pH 7 heated up to 60 °C on a hot plate (Stuart, CB162, UK). After that, casein (Sigma Aldrich) was incorporated into the salep solution and the mixture was heated at 80 °C for 1 min and cooled down to room temperature. The final solution in a cover flask was hydrated overnight by mixing on the magnetic stirrer with 100 rpm. After the preparation of the samples, the analyses were performed immediately.

Color analysis

An automatic colorimeter (Konica Minolta, model CM-5, Mississauga, ON, Canada) was used for the measurement of color parameters. The samples were placed in liquid cuvette and inserted into the equipment. The color parameters were measured as L, a and b. The measurement was performed five times on same sample after the instrument was calibrated using its calibration procedure at the beginning of the each analysis.

Rheological measurements

Steady shear rheological properties

Steady shear rheological characteristics of salep-casein samples were determined using a stress/strain controlled rheometer (Thermo-Haake, Rheostress 1, Germany) equipped with a temperature-control unit (Haake, Karlsruhe K15 Germany). The measurements were carried out using a cone-plate configuration with a cone diameter of 35 mm and a gap of 0.140 mm between the cone and plate. Steady shear measurements were carried out in the range of 0.1–100 s⁻¹ shear rates at constant temperature (25 °C). A 0.85 ml of sample was placed between cone and plate and the measurement was started immediately. Total 25 data points were recorded at 10 s intervals during the shearing. Each measurement was replicated three times on three aliquots from the same mixture (each 0.85 ml) samples with two repetitions. The steady shear values recorded at a specified shear rate (50 s⁻¹, shear rate in mouth) were used to evaluate the steady shear properties (Bourne 2002). The apparent viscosity calculated as a function of shear rate was obtained directly from the software. Obtained data were fitted to Power law model (Eq. 1) using RheoWin Data Manager (RheoWin Pro V. 2.96, Haake, Karlsruhe, Germany) for calculation of consistency coefficient and flow behavior index values.

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

where η is the apparent viscosity (Pa s), K is the consistency coefficient (Pa sⁿ), $\dot{\gamma}$ is the shear rate (s⁻¹) and n is the flow behavior index (dimensionless).

Dynamic shear rheological properties

In order to determine the viscoelastic properties of samples, a stress/strain controlled rheometer equipped with peltier temperature control unit (Mars III, Karlsruhe, HAAKE, Germany) was used. To perform the rheological measurements, cone-plate geometry was (plate diameter 35 mm, and gap size 0.140 mm) used. Prior to frequency sweep test

Table 1 Second-order design matrix indicating the levels of coded and actual values for two variables

Runs	Coded level of variables		Actual level of variables	
	Salep level (X_1)	Casein level (X_2)	Salep level (%)	Casein level (%)
1	0	0	0.375	1.500
2	+1.414	0	0.750	1.500
3	0	0	0.375	1.500
4	+1	−1	0.640	0.439
5	−1	−1	0.110	0.439
6	−1	+1	0.110	2.561
7	0	+1.414	0.375	3.000
8	0	−1.414	0.375	0.000
9	0	0	0.375	1.500
10	+1	+1	0.640	2.561
11	−1.414	0	0.000	1.500

application, the linear viscoelastic region (LVR) of the samples was determined using stress sweep test. In LVR test, variation of dynamic mechanical spectra of samples versus increased stress was characterized. LVR of the samples was determined over a stress range of 0.1–10 Pa at constant frequency (1 Hz). In oscillatory frequency sweep test, dynamic mechanical spectra of the samples were evaluated in the frequency range of 0.1–10 Hz at constant stress (within the range of LVR) at constant temperature (25 °C). A sinusoidal stress or strain with an increasing frequency was applied to the samples and the elastic modulus G' , the viscous modulus G'' , complex modulus G^* and complex viscosity η^* were calculated as a function of frequency.

The dynamic mechanical spectra parameters of G' (elastic or storage modulus) and G'' (viscous or loss modulus) are calculated using the following equations (Steffe 1996):

$$G' = G^* \cos \delta \quad (2)$$

$$G'' = G^* \sin \delta \quad (3)$$

Equations of complex modulus G^* and complex viscosity η^* as following can be used to characterize the overall response of the sample against to the sinusoidal strain:

$$\eta^* = G^* / \omega \quad (4)$$

The frequency dependency (f) of G' and G'' for salep-casein mixtures at all combinations according to the Table 1 was fitted to the following equations (Steffe 1996; Tunick et al. 1993; Sharma et al. 2016).

$$G' = k_{\text{elastic}} f^n \quad (5)$$

$$G'' = k_{\text{viscous}} f^n \quad (6)$$

where n and k are constants and the n is the frequency dependency degree.

Statistical analysis

Design-Expert version 7.0 (Stat-Ease Inc., Minneapolis, USA) and JMP version 9.0.2 (SAS Institute, Inc., Cary, USA) were used for the computational work including designation of experimental points, randomization and fitting of the second-order polynomial models as well as optimization.

Results and discussion

Color properties

Table 2 shows the mean values for the color parameters of the salep-casein mixture samples. The highest L value (91.72) was observed for sample containing 0.38% salep and no casein (run 8) while the lowest value (55.37) was for the sample prepared with 0.64% salep and 2.56% casein

Table 2 Mean values for the color of salep-casein mixtures

Runs	Color properties		
	L	a	b
1	64.92 ± 0.61	2.91 ± 0.02	14.00 ± 0.17
2	59.94 ± 0.41	2.52 ± 0.02	12.71 ± 0.10
3	67.58 ± 0.44	2.71 ± 0.02	14.07 ± 0.10
4	80.21 ± 0.05	1.09 ± 0.00	9.59 ± 0.01
5	87.97 ± 0.00	0.43 ± 0.01	6.24 ± 0.01
6	61.13 ± 0.16	3.59 ± 0.01	14.97 ± 0.03
7	57.93 ± 0.30	3.60 ± 0.03	14.60 ± 0.01
8	91.72 ± 0.01	0.23 ± 0.00	3.88 ± 0.01
9	68.93 ± 0.16	2.50 ± 0.01	13.86 ± 0.01
10	55.37 ± 0.08	3.14 ± 0.02	13.82 ± 0.04
11	75.49 ± 0.01	1.73 ± 0.01	12.09 ± 0.01

(run 10). It is clear from that the salep solution is more transparent compared to casein solution and their brightness in the mixtures was higher than those of their sole solution. Similarly, redness (a) and yellowness (b) values of the samples were in the range of 0.23–3.60 and 3.88–14.97, respectively and the lowest a and b values were in salep solution (run 8). Figure 1 shows the change in the color parameters (L, a and b) of salep-casein mixtures depending on the processing levels [salep (X_1) and casein (X_2)] and Table 3 shows the effects of the processing variables on the studied parameters. It is clear from the figure that salep and casein increased the L values of the samples significantly (Table 3, $p < 0.05$) and it is seen that the casein had the most decreasing effect on the L

values because the lowest L values were observed in the majority of experimental runs including high level of casein (Table 2). Interactive effect of the salep-casein mixtures (X_1X_2) was found to be insignificant while the quadratic effect of casein (X_2^2) showed a dominant effect on the brightness of the samples (Table 3). Similarly, redness values were affected by the salep and casein significantly ($p < 0.05$). Increase in both of salep and casein increased the a values of the samples and the most increasing effect was for the casein because at constant level of salep, casein provided a huge and significant increment on the redness values of the samples (Fig. 1). Interactive and quadratic effects of salep and casein on the redness values were determined to be significant (Table 3,

Fig. 1 Change in color properties of samples depending on salep and casein levels

Table 3 Significance of the regression models (F values) and the effects of variables on color, steady and dynamic shear rheological properties of salep-casein mixtures

	df	Color parameters			Steady shear rheological parameters				Dynamic shear rheological parameters			
		L	a	b	η_{50}	σ	K	n	G'	G''	G^*	η^*
Linear												
b ₁	1	41.11 ^a	5.22 ^b	5.57 ^b	777.7 ^a	777.4 ^a	60.03 ^a	7.50 ^a	3.29 ^a	125.7 ^a	44.79 ^a	44.81 ^a
b ₂	1	322.6 ^a	294.9 ^a	463.4 ^a	97.03 ^a	96.98 ^a	25.75 ^a	9.53 ^a	9.32	43.34 ^a	28.98 ^a	28.98 ^a
Interaction												
b ₁₂	1	0.26	7.25 ^a	23.89 ^a	36.43 ^a	36.42 ^a	22.52 ^a	7.07 ^a	7.67 ^a	20.10 ^a	15.49 ^a	15.48 ^a
Quadratic												
b ₁₁	1	0.10	10.48 ^a	13.18 ^a	0.000	0.000	0.23	6.75 ^a	5.31 ^c	1.08	6.01 ^b	6.02 ^b
b ₂₂	1	21.46 ^a	19.81 ^a	138.3 ^a	60.93 ^a	60.90 ^a	2.78	1.02	1.40 ^a	13.89 ^a	7.23 ^a	7.22 ^a
Lack of fit	3	0.87	1.01	30.41	0.99	1.00	6.40	19.59	4.38	2.63	3.51	3.51
Pure error	2											
Total model	10	77.33 ^a	66.25 ^a	126.23 ^a	195.59 ^a	195.49 ^a	22.22 ^a	6.18 ^a	5.87 ^a	41.61 ^a	21.60 ^a	21.61 ^a
SD		1.96	0.21	0.46	0.016	0.11	0.12	0.10	0.22	0.12	0.16	0.063
Mean		70.11	2.22	11.80	0.23	1.66	0.46	0.75	0.64	0.84	0.96	0.38
C.V%		2.79	9.25	3.91	6.77	6.77	26.71	13.43	35.01	13.89	16.46	16.46
R ²		0.987	0.985	0.992	0.994	0.995	0.957	0.861	0.855	0.977	0.956	0.955

^a $p < 0.01$ ^b $p < 0.05$ ^c $p < 0.1$

$p < 0.05$). Morales and van Boekel (1998) reported that heating of casein and sugar solutions caused browning. Due to browning, redness values became high. For the b values, similar results were observed and increment in the level of salep and casein increased the yellowness values of the samples significantly (Fig. 1; Table 3, $p < 0.05$). Yılmaz et al. (2013) reported the physicochemical and steady shear rheological properties of salep and starch mixtures and they found that the turbidity which is a parameter related to opacity and transparency of the solution was affected by the level of salep and starch and it was concluded that the turbidity of final mixtures increased with the increase of salep and starch in the solutions. The constructed models for the color parameters could be used effectively because of their high coefficients of determination ($R^2 > 0.985$).

Steady shear rheological properties

All salep-casein mixture samples showed non Newtonian pseudoplastic behavior. Figure 2 shows the steady shear rheograms of some samples prepared according to the experimental design (Table 1). As can be seen from the figures, increase in the shear rate caused an increase in shear stress and decrease in apparent viscosity values of the samples. Depending on the levels of the processing variables in the solutions, the values for shear stress and

apparent viscosity changed significantly ($p < 0.05$) and also, rheological behavior was affected by the level of the processing variables, especially, salep as for run 11 in Fig. 2 because the decrease in the salep level in the solution caused a change in the structure and the behavior started to change. Steady shear rheological parameters of the salep-casein mixture samples were calculated using Power law model and tabulated in Table 4. Due to decrement in apparent viscosity values depending on the increase in the shear rate, apparent viscosity at 50 s^{-1} was considered because this value is important for the sensorially (Bourne 2002). Apparent viscosity values of the samples were in the range of $0.002\text{--}0.257 \text{ Pa s}$. The highest apparent viscosity value was determined in the sample containing 0.75% salep and 1.50% casein (run 2) while the lowest one was in the sample having 1.50% casein and no salep. Shear stress values recorded during the shearing ranged between $0.08\text{--}12.86 \text{ Pa}$ and the lowest and highest values were in the same solution for the lowest and highest apparent viscosity and so, a significant and very high positive correlation ($r = 0.999$) was determined between shear stress and apparent viscosity of the sample. As is known, shear stress and apparent viscosity are recorded as a function of shear rate for the pseudoplastic fluids. Differences of shear stress values among the samples can be seen in Fig. 2. Consistency coefficient and flow behavior index of the samples calculated using Oswald–

Fig. 2 Apparent viscosity rheograms of salep and casein mixtures versus shear rate **Table 4** Mean values for the steady and dynamic shear rheological properties of salep-casein mixtures

Runs	Steady shear rheological parameters					Dynamic shear rheological parameters			
	η_{50}	σ	K	n	R^2	G'	G''	G^*	η^*
1	0.075 ± 0.00	3.73 ± 0.07	0.314 ± 0.01	0.63 ± 0.00	0.999	0.44 ± 0.04	0.99 ± 0.03	1.08 ± 0.04	0.17 ± 0.00
2	0.257 ± 0.02	12.86 ± 0.92	0.793 ± 0.06	0.69 ± 0.00	0.993	1.18 ± 0.19	2.75 ± 0.29	2.99 ± 0.34	0.48 ± 0.05
3	0.083 ± 0.00	4.13 ± 0.22	0.352 ± 0.01	0.62 ± 0.00	0.999	0.36 ± 0.03	0.96 ± 0.07	1.03 ± 0.08	0.16 ± 0.01
4	0.216 ± 0.01	10.82 ± 0.53	2.041 ± 0.12	0.43 ± 0.00	0.996	2.58 ± 0.11	3.61 ± 0.13	4.44 ± 0.17	0.71 ± 0.03
5	0.005 ± 0.00	0.27 ± 0.01	0.007 ± 0.00	0.92 ± 0.01	0.999	0.38 ± 0.10	0.14 ± 0.07	0.40 ± 0.12	0.06 ± 0.02
6	0.005 ± 0.00	0.23 ± 0.00	0.005 ± 0.00	0.99 ± 0.03	0.999	0.20 ± 0.07	0.04 ± 0.03	0.21 ± 0.07	0.03 ± 0.01
7	0.007 ± 0.00	0.36 ± 0.11	0.021 ± 0.01	0.75 ± 0.07	0.994	0.03 ± 0.01	0.09 ± 0.05	0.10 ± 0.05	0.02 ± 0.01
8	0.064 ± 0.00	3.21 ± 0.07	0.298 ± 0.01	0.60 ± 0.00	0.999	0.17 ± 0.02	0.75 ± 0.05	0.77 ± 0.05	0.12 ± 0.01
9	0.065 ± 0.00	3.27 ± 0.03	0.227 ± 0.01	0.67 ± 0.00	0.999	0.17 ± 0.02	0.71 ± 0.05	0.73 ± 0.04	0.12 ± 0.01
10	0.072 ± 0.01	3.58 ± 0.33	0.057 ± 0.03	1.04 ± 0.13	0.988	0.04 ± 0.03	0.47 ± 0.05	0.47 ± 0.09	0.07 ± 0.01
11	0.002 ± 0.00	0.08 ± 0.00	0.002 ± 0.00	0.93 ± 0.08	0.987	0.65 ± 0.38	0.20 ± 0.17	0.68 ± 0.41	0.11 ± 0.07

de-Waele were also given as steady shear properties of the samples in Table 4. Consistency coefficients of the samples were in the range of 0.002–2.041 Pa sⁿ while the flow behavior index of the samples ranged from 0.43 to 1.04. The flow behavior index showed that the flow behavior characteristic of the mixtures changed significantly depending on the salep-casein concentration in the sample. As could be understood from the results that sole casein solution showed non-Newtonian behavior because the flow behavior index was not unity. With the addition of salep, non-Newtonian character started to be dominant. Bourriot

et al. (1999) reported similar behavior for the casein and they stated that the casein suspension showed Newtonian flow behavior with a low viscosity. In this study, viscosity of sole casein solution was determined to be very low compared to galactomannan based salep solution. Similarly, casein solution's viscosity was determined to be quite low compared to galactomannan solution (Bourriot et al. 1999). Increase in the concentration of casein increased the viscosity of the final solution. As stated by Bourriot et al. (1999), casein level increase in the mixture of casein and galactomannan concluded a dramatic increase in the final

viscosity of the solution. It is clear from that a synergistic interaction occurred between the glucomannan based salep and casein. Sittikijyothina et al. (2010) reported that the 6.5 % wt of β -lactoglobulin solution exhibited Newtonian behaviour while the galactomannan solutions behaved as shear-thinning with a Newtonian region in the lower shear rate range for all concentrations studied. They also stated that the mixed systems behaved differently from the individual components. Neiryneck et al. (2007) reported that increasing concentration of guar gum which is a galactomannan type hydrocolloid increased the viscosity of guar gum-sodium caseinate dominantly compared to casein and the flow behavior index approached to unity in the absence of guar gum because of the casein behavior was close to Newtonian and decreased with the increase of guar gum level in the solution. Perissutti et al. (2002) performed an investigation on the rheological properties of mixture prepared with a galactomannan from *Mimosa scabrella* milk and its derived proteins like sodium caseinate and purified casein fractures and they reported that galactomannan and sodium caseinate showed a good synergistic interaction. The main mechanism behind this influence was attributed to an increase of solution ionic strength due to the presence of proteins, and there can also be a contribution of the synergy of the galactomannan–milk protein system. The main reason between the interaction of galactomannan and casein is a transition from the behavior of a macromolecular solution to that of a slightly structured system when casein added to the galactomannan solution (Bourriot et al. 1999). A significant correlation was determined between the consistency coefficient and apparent viscosity values ($r = 0.812$). It was seen from the Table 3 that the linear effect of salep and casein concentration showed a

significant effect ($p < 0.05$) on all steady shear rheological parameters (shear stress, apparent viscosity, consistency coefficient and flow behavior index). According to the Fig. S1 (Supplementary 1), a clear and huge increase was observed in shear stress, apparent viscosity and consistency coefficient levels with the increase of salep level in the mixture compared to casein. Increase in salep caused a decrement in the flow behavior index values as expected due to the negative correlation ($r = -0.726$) between the consistency coefficient and flow behavior index. Interaction between salep and casein was also determined to be very significant for all steady shear parameters ($p < 0.05$). It was concluded that there is a structural interactive association between the glucomannan based salep and casein molecules. Table 5 shows the constructed regression models for the steady shear rheological parameters. As is seen, the models could be effectively used because of quite high coefficients of determination ($R^2 > 0.861$).

Dynamic shear rheological properties

Dynamic shear rheological parameters of the salep-casein mixtures namely loss modulus, elastic modulus, complex modulus and complex viscosity recorded at 1 Hz during frequency sweep test were tabulated in Table 4. Storage modulus values which shows the elastic character of the sample were in the range of 0.03–2.58 Pa while the loss modulus values showing the viscous character of the sample ranged between 0.04 and 3.61 Pa. The highest loss and storage modulus values were determined for the sample containing 0.64% salep + 0.43% casein (Table 4). Bourriot et al. (1999) reported that loss modulus and storage modulus of guar gum and casein solution in one-

Table 5 Regression equations for the color, steady and dynamic shear rheological parameters of salep-casein mixtures

Parameters	Regression equations ^a	R ²
L	$Y_L = 100.2 - 22.18X_1 - 22.57X_2 + 1.78X_1X_2 + 3.71X_{12} + 3.39X_{22}$	0.987
a	$Y_a = -1.18 + 5.09X_1 + 2.57X_2 - 0.98X_1X_2 - 3.98X_{12} - 0.34X_{22}$	0.985
b	$Y_b = 0.23 + 14.99X_1 + 10.91X_2 - 4.01X_1X_2 - 10.03X_{12} - 2.03X_{22}$	0.992
Shear stress (Pa)	$^bY_{SS} = -0.5 + 5.97X_1 + 1.07X_2 - 1.20X_1X_2 + 0.01X_{12} - 0.33X_{22}$	0.994
Apparent viscosity (Pa s)	$^bY_{AV} = -0.07 + 0.84X_1 + 0.15X_2 - 0.17X_1X_2 + 0.00X_{12} - 0.05X_{22}$	0.995
Consistency coefficient (Pa s ⁿ)	$^bY_{CC} = -0.43 + 3.10X_1 + 0.41X_2 - 1.04X_1X_2 - 0.36X_{12} - 0.08X_{22}$	0.957
Flow behavior index	$Y_{FBI} = 1.20 - 2.27X_1 - 0.19X_2 + 0.48X_1X_2 + 1.57X_{12} + 0.04X_{22}$	0.861
Loss modulus (Pa)	$^bY_{LM} = 0.29 - 0.12X_1 + 0.48X_2 - 1.10X_1X_2 + 3.08X_{12} - 0.10X_{22}$	0.855
Storage modulus (Pa)	$^bY_{SM} = -0.12 + 2.60X_1 + 0.58X_2 - 0.93X_1X_2 + 0.73X_{12} - 0.16X_{22}$	0.977
Complex modulus (Pa)	$^bY_{CM} = 0.21 + 1.33X_1 + 0.61X_2 - 1.10X_1X_2 + 2.31X_{12} - 0.16X_{22}$	0.956
Complex viscosity (Pa s)	$^bY_{CV} = 0.09 + 0.53X_1 + 0.24X_2 - 0.44X_1X_2 + 0.92X_{12} - 0.06X_{22}$	0.955

^aFinal equation in terms of actual factors

^bSquare root transformation was applied

component system showed a strong variation depending on the frequency with lower storage modulus and higher loss modulus. The change in the viscoelastic parameters showed that the behavior of galactomannan was a macromolecular type and with the addition of casein, a suspension type behavior occurred compared to solution and so, a solid like behavior resulted from the structuration of the medium (Bourriot et al. 1999). Complex modulus which is the function of both loss and storage modulus ranged between 0.10 and 4.44 Pa. Complex viscosity which shows the dynamical consistency of the samples was in the range of 0.02–0.71 Pa. Figure 3 shows the dynamic mechanical behavior of the mixture samples. As can be seen from the figure, increase in the frequency increased the loss modulus and storage modulus values of the samples and depending on the processing levels, increase in the parameters changed significantly ($p < 0.05$). On the contrary, frequency increase caused a significant decrement in the complex viscosity values of the salep-casein mixtures (Fig. 3). The Fig. 3 also showed that the storage modulus increased at a faster rate than the loss modulus and this resulted in a crossover point at high frequency levels. It was reported that the presence of $G' - G''$ crossover point during the frequency sweep test was demonstrated by soft gels formed by entangled polymer networks or physical gels with weak

bond strengths (Sharma et al. 2016; Stading and Hermansson 1990; Tunick et al. 1993). It was observed that the linear effect of salep and casein in the mixtures showed a significant effect on the dynamic mechanical parameters namely loss modulus, storage modulus, complex modulus and complex viscosity significantly (Table 3, $p < 0.05$). In addition to that, interaction between the salep and casein showed a significant effect on the dynamic shear rheological parameters (Table 3, $p < 0.05$). Figure 4 illustrates the change in the dynamic shear rheological parameters depending on the salep and casein concentration. Similar to steady shear parameters, the most increasing effect was observed for the salep compared to casein and increase in the salep level in the mixture increased the loss modulus, storage modulus, complex modulus and complex viscosity significantly ($p < 0.05$). Table 5 shows the constructed regression models for the steady shear rheological parameters. As is seen, the models could be effectively used because of quite high coefficients of determination ($R^2 > 0.855$).

Frequency dependency of samples was determined using the Eqs. 5 and 6 and the calculated model parameters for elastic and viscous moduli were given in Table 6. The degree of frequency dependency was given by n in the equations. As can be seen from the table, for elastic modulus of the samples, the highest n value (6.125) was calculated for the samples containing salep (0.375%) and casein (1.5%) while the lowest value was in the sample containing salep (0.640%) and casein (0.439%). According to the results, frequency dependency decreased with the increase of salep level in the mixtures while the casein concentration increment caused an increase in frequency dependency for elastic modulus of the samples. For loss modulus values of the samples, the n value showing the frequency dependency changed significantly according to the processing variables levels. The lowest n value was for the sample containing salep (0.640%) and casein (0.439%). Similar to storage values, frequency dependency decreased with the increase of salep level in the mixtures while the casein level increase caused an increase in frequency dependency for viscous modulus of the samples.

Optimization for the parameters

To determine the maximum and minimum values for the studied parameters depending on the processing variables (salep and casein), a multiple response optimization procedure was applied using desirability function. Table 7 shows the results of the maximum and minimum parameter values. As can be seen, the minimum brightness, redness and yellowness values (54.84, 1.24 and 8.98, respectively) would be at 0.64% salep + 0.44% casein mixture while the maximum values (68.20, 2.78 and 14.10, respectively)

Fig. 3 Dynamic mechanical spectra rheograms of salep and casein mixtures versus frequency

Fig. 4 Effect of salep and casein on dynamic shear rheological parameters along with the second-order polynomial model equations predicting effects of the variables

would be at 0.14% salep and 1.78% casein mixture. So, increment in the color parameters especially in the brightness values would be achieved by the decrement in the salep concentration as stated before. For the steady shear rheological parameters, the minimum apparent viscosity and consistency coefficient values would be at 0.18% salep and 2.56% casein while the maximum values would be at 0.64% salep and 1.46% casein. As can be seen, the major increment for the steady shear parameters would be achieved by the huge increase in the salep concentration in the mixture. Similar results were determined for the dynamic shear rheological parameters for the samples. The minimum levels for the loss modulus, storage modulus, complex modulus and complex viscosity would be at 0.34% salep and 2.56% casein while the maximum values

would be at 0.64% and 0.44% casein. It is quite clear from the optimized results that the maximization is possible with the major increase of the salep compared to casein in the mixture (Table 7).

Conclusion

Mixture of glucomannan based salep and casein resulted in significant effect on the color, steady shear and dynamic shear rheological properties of the final solution. Response surface methodology was effectively used to describe the effect of salep and casein levels on the studied parameters. The results indicated that salep and casein showed a significant effect on the parameters and an interaction between

Table 6 Frequency dependency model constants of salep-casein mixtures

Runs	$G' = k_{\text{elastic}} f^n$			$G'' = k_{\text{viscous}} f^n$		
	k	n	R^2	k	n	R^2
1	0.118	1.735	0.970	0.935	0.749	0.869
2	1.199	0.823	0.972	2.956	0.556	0.987
3	1.2×10^{-5}	6.304	0.996	0.197	1.433	0.781
4	2.491	0.502	0.941	3.325	0.353	0.967
5	0.038	3.333	0.990	1.406	1.719	0.967
6	2.01×10^{-4}	6.125	0.992	0.011	4.162	0.986
7	0.003	4.469	0.999	7×10^{-5}	6.951	0.997
8	0.007	3.321	0.992	0.522	1.029	0.970
9	4×10^{-4}	4.603	0.993	0.609	0.837	0.991
10	0.034	2.609	0.999	0.538	0.702	0.951
11	0.020	4.195	0.998	3.266	1.199	0.955

Table 7 Multiple response optimization procedure applied to find optimum values for the parameters of salep-casein mixtures

Parameters	Multiple response optimization							
	Minimization				Maximization			
	Minimum levels	Salep (%)	Casein (%)	Desirability	Maximum levels	Salep (%)	Casein (%)	Desirability
Color properties								
L	54.84	0.64	0.44	0.514	68.20	0.14	1.78	0.638
a	1.24				2.78			
b	8.98				14.10			
Steady shear rheological properties								
Shear stress (σ)	0.375	0.18	2.56	0.622	9.35	0.64	1.46	0.622
Apparent viscosity (η_{50})	0.008				0.186			
Consistency coefficient (K)	0.035				0.767			
Flow behavior index (n)	0.526				0.644			
Viscoelastic properties								
Storage modulus (G')	0.054	0.34	2.56	0.902	1.855	0.64	0.44	0.895
Loss modulus (G'')	0.210				3.253			
Complex modulus (G^*)	0.235				3.751			
Complex viscosity (η^*)	0.037				0.597			

the salep and casein was observed. The main and major increasing effect for the steady shear rheological parameters namely apparent viscosity and consistency coefficient and dynamic shear rheological parameters namely storage modulus and complex viscosity was performed by the salep compared to casein in the prepared mixture solutions. Effective regression models were constructed to estimate the studied parameters for the different salep and casein concentrations with quite high coefficients of determination.

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