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Research Article

Characterization of different orchid species and rheological properties of orchids solutions

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Abstract

This study investigated the physicochemical, bioactive, and rheological properties of salep obtained from ten different wild orchids collected from different regions of Turkey (*Anacamptis pyramidalis*, *Orchis isaura*, *Anacamptis palustris* subsp. *palustris*, *Orchis morio*, *Serapias vomeracea* subsp. *artemisiae*, *Orchis italica*, *Ophrys mammosa*, *Orchis sancta*, *Dactylorhiza euxina*, *Ranunculus ficaria* subsp. *calthifolius*, and commercial salep). Firstly, the salep samples were ground and their color, pH, bioactive components, and FTIR spectrum were determined. The color and pH properties of salep differed due to the different species ($p \leq 0.05$). Two strong peaks were observed at 1618 and 1422 cm^{-1} due to asymmetric and symmetric stretching of the ester groups in salep glucomannan. According to salep species, there was a significant difference in the TPC (total phenolic content), % DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, and CUPRAC values ($p \leq 0.05$), which were 5.29-26.84 mg GAE/g, 5.02-12.2%, and 0.81-3.82 mg TE/g, respectively. The FTIR findings revealed that the spectrum peaks did not change between commercial salep and other salep species. In the second part of the study, we prepared 1%, 2%, and 3% aqueous solutions of salep and examined their flow behavior, dynamic rheological properties, and 3-ITT (3-Time Interval Thixotropic Test) rheological behavior. A significant difference was observed between the values ($p \leq 0.05$). All salep solutions had a flow behavior index (n) value below 1, indicating that all samples exhibited shear-thinning behavior. The consistency coefficient (K) value significantly changed ($p \leq 0.05$) and was determined to be 0.003-2.91 $Pa.s^n$, 0.003-28.81 $Pa.s^n$, and 0.017-94.134 $Pa.s^n$ at salep concentrations of 1%, 2%, and 3%, respectively. Salep is a preferred stabilizer due to its functional properties, bioactive components, and polysaccharide structure. As a result, when the rheological properties of salep samples are evaluated, it is shown that especially *Anacamptis pyramidalis*, *Orchis morio*, *Orchis sancta* species can be used as stabilizers in the food industry.

Keywords: bioactive; methanol and ethanol extracts; physicochemical; rheological properties; salep

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Introduction

Orchids are wild plants with visually impressive and diverse group of plants in the Orchidaceae family, which is spread over wide geography in the world, with approximately 28000 varieties (Bazzicalupo *et al.*, 2023; Christenhusz , 2016). Orchids have bulbous and tuberous roots (Sandal and Söğüt 2010). Orchid tubers are usually beige, tubers are egg-shaped or forked, odorless, rough, and tasteless (Develi Işıklı *et al.*, 2015). Turkey has the highest number of orchid varieties in Europe and the Middle East (Tekinşen and Güner, 2010; Turkmen, 2021). 154 different types of wild orchids are grown in Turkey (Tekinşen and Güner, 2010). *Anacamptis pyramidalis, Dactylorhiza romana, Dactylorhiza osmanica* var. *osmanica, Himantoglossum affine, Ophrys fusca, Ophrys holosericea, Ophrys mammosa, Orchis anatolica, Orchis coriophora, Orchis italica, Orchis mascula* ssp. *Pinetorum, Orchis morio, Orchis palustris, Orchis simia, Orchis spitzelii, Orchis tridentata,* and *Serapias vomeracea* are used in the most common orchids tubera species in Turkey (Sandal and Söğüt, 2010) .

Salep is obtained by drying and grinding the tubers of orchid types that are in the Orchidaceae family (Caliskan *et al.*, 2023). Salep is used as a popular ingredient in some food products in Greece, Iran, Iraq, Albania, and Turkey (Charitonidou *et al.*, 2019). Salep is used in the production traditional beverage, ice cream, and pharmaceutical industries due to its functional properties (Georgiadis *et al.*, 2012; Gutiérrez, 2010; Kurt, 2021a). Different formulations of salep are consumed as a hot drink in Southeast Europe and the Middle East and as a popular and traditional beverage in Anatolia and Arab countries (Sandal and Söğüt, 2010).

Salep is used as a stabilizing agent in ice cream in north-east Mediterranean countries such as Greece and Turkey (Georgiadis *et al.*, 2012). Also today, fifty orchid species are used in traditional Chinese medicine, as well as Ayurvedic medicines in India (Bulpitt *et al.*, 2007). Although the physicochemical composition of salep varies according to the genus and species, it contains 7-61% glucomannan, 1-36% starch, 1-4% sugars, 0.5-1.5% nitrogenous substances, 2-10% ash, and 8-12% (Tekinşen and Güner, 2010) . Glucomannan is a non-toxic, water-soluble complex polysaccharide composed of straight glucose and mannose chains linked by beta β (1→4) glycosidic bonds (Bulut-Solak *et al.*, 2017). Glucomannan, usually referred to as salep gum, is frequently employed in the food industry due to its viscosity, aroma and flavor enhancing powers, gelling and stabilizing capabilities, and its capacity to influence the rheological characteristics of the product (Kayacier Dogan, 2006). The rheological characteristics of gums play a crucial role in the design, evaluation, and modeling of food processes. Additionally, these qualities serve as an indicator of the quality of the final product. (Marcotte *et al.*, 2001). Gum solutions are usually non-Newtonian pseudoplastic fluids and the apparent viscosity decreases with increasing shear rate, accordingly they demonstrate a shear-thinning flow behavior (Kayacier Dogan, 2006). The rheological properties of salep gum affects the quality, production process, production efficiency, and storage stability of the product (Wang *et al.*, 2012).

Plant phenolics are abundant in both edible and non-edible parts of plants and exhibit antioxidant activity. Phenolic compounds are stored in plants mainly in flowering tissues, leaves, stems, roots, or bark. Depending on the various parts of the plants, the antioxidant level may differ (Chand *et al.*, 2016; Velioglu *et al.*, 1998). The chemical composition of orchids includes carbohydrates, alkaloids, flavonoids, glycosides, bibenzyl derivatives, stilbenes, dihydrostilbenoids, phenanthrenes, terpenes, anthocyanins, and phenolic acids (Gantait *et al.*, 2021). Natural secondary metabolites of the plant are phenolics, and flavonoids are natural sources of antioxidants (Hürkan *et al.*, 2019). Previous research have demonstrated that orchids possess antioxidant properties and exhibit pharmacological, diuretic, anti-inflammatory, anti-carcinogenic, hypoglycemic, anti-rheumatic, and neuroprotective benefits (Gutiérrez, 2010; Sahaya Shibu *et al.,* 2012).

Several studies have been carried out on the rheological characteristics of salep, phenolic and antioxidation capacities, physicochemical properties (Farhoosh and Riazi, 2007; Şen *et al.*, 2018; Turkmen *et al.*, 2021), and chemical composition (Bulut-Solak *et al.*, 2017; Kurt Kahyaoglu, 2017) There is a limitation of research on the comprehensive physicochemical and bioactive characteristics of different wild types of

Orchidaceae spp. in Turkey, as well as a lack of detailed investigation on the flow behavior, dynamic and 3-ITT rheological properties of solutions derived from these species. The objective of this study is to evaluate the rheological properties of salep types in order to compare different types of flow behaviour with different concentrations. Additionally, the aim of this study was to determine phenolic and antioxidant capacities and some physicochemical properties of saleps obtained from tubers of different wild salep tubera. Therefore, in this study was undertaken to determine which salep species would be most suitable and functional for the food industry and cultivation.

Materials and Methods

Material

Raw material

The dried samples of ten different varieties of salep included in the study were gathered from various locations in Turkey and a commercial salep sample was obtained from a local market. Table 1 shows the codes for the 11 distinct types of saleps used in the study. In our previous study, the glucose and mannose contents of salep species were determined (Arslan *et al.*, 2023).

Table 1. Species and regions of 11 different types of salep samples

Sample code	Species	Regions
S1	*Anacamptis pyramidalis*	Yozgat
S2	*Orchis isaura*	Mersin
S3	*Anacamptis palustris* subsp. *palustris*	Muş
S4	*Orchis morio*	Adana
S5	*Serapias vomeracea* subsp. *artemisiae*	Muğla
S6	*Orchis italic*	Muğla
S7	*Ophrys mammosa*	Adana
S8	*Orchis sancta*	Kahramanmaraş
S9	*Dactylorhiza euxina*	Van
S10	*Ranunculus ficaria* subsp. *calthifolius*	Kahramanmaraş
S11	Commercial Salep	İstanbul

The glucose contents of salep species were 22.9, 0.7, 7.9, 31.1, 0.2, 5.9, 7.3, 13.2, 0.1, 0.07, 16.9, respectively and the mannose contents of salep species were 15.8, 0.1, 3.8, 15.0, 0.1, 1.7, 4.4, 3.2, 0.0, 0.0, 10.5, respectively (Arslan *et al.*, 2023).

Chemical materials

Ethanol, methanol, Folin Ciocalteu's phenol reagent, Na_2CO_3, $CuCl_2$, and NH_4Ac were supplied from Merck (Darmstadt, Germany) and gallic acid, 1,1-diphenyl-2-picrylhydrazyl, (±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox), catechin and neocuproine (Nc) were supplied by Sigma-Aldrich (Steinheim, Germany).

Methods

Analyses of salep samples

Color determination

Color analyses of salep samples were carried out using a color measuring instrument (CR-400 Chroma Meter, Konica Minolta, Japan), and measurements were taken in parallel from three different sites. For different salep types, the L* value represents brightness (0-100), the a* value shows color change value from red

(+) to green (-), and the b* value indicates color change value from yellow (+) to blue (-). Equations 1 and 2 were used to compute chroma (C*) and hue angle (h*) values.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

Extraction procedure

For the extraction of bioactive compounds from salep samples, each of the 11 different powdered salep samples was weighed 1 g and 20 mL of different solvents (pure water, 100% methanol, 100% ethanol, 80% ethanol, 80% methanol were added to them (Giri *et al.*, 2012). By optimizing the time and solvent of the samples, the optimum solvent ratios for extractions were found to be (80:20 v/v) for ethanol and (80:20 v/v) for methanol. Accordingly, 11 saleps were extracted with ethanol (80:20 v/v) and methanol (80:20 v/v). The samples were extracted in a shaking water bath for 2.5 hours at 25 °C, then by vortexing for 5 minutes. After the extracts, Mn 640 m-125 mm filter paper was passed. It was then passed through a 0.45 μm filter (Sartorius Stedim Biotech, Gottingen, Germany). The filtered extracts were removed at -18 °C for later analysis.

Bioactive properties

The total phenolic content (TPC) was determined according to the method described by Singleton and Rossi, 1965). Folin Ciocalteu's phenol reagent (1:10 v/v) and Na_2CO_3 (7.5 g/100 g) were added to diluted methanolic or ethanolic salep extracts (0.5 mL) in amounts of 2.5 mL and 2 mL, respectively. The mixture was then placed in a tube and maintained in a dark room at 25 °C for 30 minutes. A UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was used to measure absorbance at 760 nm. The phenolic content was calculated as mg gallic acid equivalents (GAE) per gram of salep (mg GAE/g salep).

1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the antioxidant capacity of methanolic or ethanolic salep extracts. The methanolic or ethanolic extract (0.1 mL) was mixed with 4.9 mL DPPH (0.1 mmol/L methanol). After 30 min incubation in a dark place at 25 °C, absorbance was measured at 517 nm with a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Singh *et al.*, 2002). The results were expressed as % AA.

Cupric Reducing Antioxidant Capacity (CUPRAC) assay was examined to determine the antioxidant capacity with the method specified by Apak *et al.*, 2004). 0.1 mL of methanolic or ethanolic salep extract was mixed with 1 mL solution of $CuCl_2$ (170.48 mg $CuCl_2.2H_2O$/100 mL water), 1 mL solution of Nc (0.156 g Nc/100 mL ethanol) and 1 mL solution of NH_4Ac (7.708 g NH4Ac/100 mL water) and then 1 mL distilled water was added to the mixture to complete the volume of the mixture to 4.1 mL at 25 °C, the mixture was incubated for 60 minutes. At 450 nm, the absorbance was measured with a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The antioxidant capacity values were represented as mg Trolox equivalent (TE) per gram salep (mg TE/g salep).

Fourier transform infrared (FTIR) spectroscopy

The Fourier transform infrared (FTIR) spectra of salep samples were recorded on a spectrophotometer (Perkin Elmer, Model Spectrum Two, Ohio, USA) that was fitted with a Miracle Single-Reflection Diamond ATR device in the wavelength range of 4000-500 cm^{-1}.

Salep solution preparation

11 different varieties of salep were used to prepare salep solutions with concentrations ranging from 1.0 to 3.0%. First, salep was dissolved in water at a certain concentration and mixed in a magnetic stirrer for 1 hour to thoroughly hydrate.

Rheological analysis of salep solutions

Mayonnaise samples were subjected to rheological measurements (flow behaviour, viscoelastic properties, 3-ITT, and emulsion stability) using a rheometer (MCR 302, Anton Paar, North Ryde, Australia) outfitted with a parallel-plate arrangement to shear (probe: PP50 and gap:0.5 mm) at 25 °C. The PP50 probe has 50 mm diameter (Anton Paar, North Ryde, Australia). All measurements were carried out in triplicate.

The flow behaviour of mayonnaise samples were assessed at shear rates varied from 0.1 s-1 to 100 s^{-1}. After being fitted to the model parameters, the flow behaviour rheological data were generated by the Power law model and nonlinear regression.

$$\tau = K \times \gamma^{n} \tag{3}$$

In Equation 3, the τ represents the shear stress (Pa), K presents the consistency coefficient (Pa.sn), γ is the shear rate (s^{-1}), and n is the flow behavior index.

The viscoelastic behaviour of mayonnaise samples was conducted using a parallel plate configuration. By performing an amplitude sweep test at 0.1% and 100% strain, the linear viscoelastic zone was first identified. The frequency sweep test was conducted with 0.1-64 s^{-1} angular velocity (ω) based on the results. Using the samples' angular velocities as a starting point, the storage modulus (G′) and loss modulus (G″) of the samples were determined. With the help of nonlinear regression and the power law model, the viscoelastic parameters were computed.

$$G' = K \times (\omega)^{n'} \tag{4}$$

$$G'' = K \times (\omega)^{n''} \tag{5}$$

In Equation 4 and 5, the G′ and G″ values present storage modulus (Pa) and loss modulus (Pa), ω names angular velocity value (s^{-1}), K′, K″ shows consistency coefficient values (Pa.s^{n}) and n′, n″ are flow behavior index values.

Three Time Interval Time Test (3-ITT) were conducted for mayonnaise samples at the constant shear rate of 0.5 s^{-1} and variable shear rate 150 s^{-1}. The shear rate value applied at the second time period was determined using the linear viscoelastic region. The samples' linear viscoelastic region terminates at 55 s^{-1}. During the first-time interval, the mayonnaise samples were exposed to 100 s of at a 0.5 s^{-1} shear rate. In the second time interval, 150 s^{-1} was subjected to the recommended shear force for 40 seconds. By exposing the samples to a low shear rate in the first-time interval, the dynamic rheological behavior in the second time interval was studied in the third time interval. For this reason, the change in viscoelastic solid structure (G′) of salad dressing samples was assessed. A second-order structural kinetic model was used to simulate how samples behaved throughout the third time period.

$$\left[\frac{G'-G_e}{G_0-G_e}\right]^{1-n} = (n-1)\times k \times t - 1 \tag{6}$$

In Equation 6, G′ and G″ values present storage modulus (Pa) and loss modulus (Pa) in the 3rd time interval; G_0 represents an initial storage modulus (Pa) in the 3rd time interval, G_e denotes the equilibrium storage modulus (Pa); and k denotes the thixotropic velocity constant (Atik *et al.*, 2021; Toker *et al.*, 2015).

Statistical analysis

All analytical measurements were made in triplicate. The results were reported as arithmetic mean values with standard deviation ranges. Nonlinear regression analysis was used to determine the rheological analysis findings using the power-law model parameters. The coefficient of determination (R^2) was used to evaluate the model's applicability. STATISTICA (StatSoft, Inc., Tulsa, OK) was used to conduct the nonlinear regression analysis.

Results and Discussion

Color evaluation

Color parameters (L*, a*, and b*) express brightness/ light-darkness (L*), redness/blueness (a*), green/yellowness (b*). The color parameters of the powdered salep species and the powdered commercial salep sample are also shown in Table 2. L*, a* and b* values of powdered salep samples vary between 88.55-73.30, 5.01-1.71 and 16.32 -7.68, respectively. In a study conducted by Kurt *et al.* (2017), the L* brightness value of *Serapias vomeracea* species was found to be 70.47±0.00 and the L* value of *Orchis sancta* salep species to be 75.10±0.24 (Kurt Kahyaoglu, 2017). In this study, S5 coded *Serapias vomeracea* subsp. The L* value of the Artemisiae species was 73.30±0.11 and the L* value of the S8 coded *Orchis sancta* species was 84.53±0.44. The differences are thought to be due to the subspecies of the species and the changes in the morphological characteristics of the salep species due to growing and regional factors.

Table 2. Color parameters of salep samples

Salep	L*	a*	b*	C*	h*
S1	79.89±0.01c	5.01±00.4a	16.32±0.01a	17.07±0.01a	72.98±0.01b
S2	86.26±0.01b	3.51±0.15de	13.99±0.01b	14.40±0.01b	76.33±0.05a
S3	80.67±0.05c	4.05±0.01bc	13.46±0.21bc	14.05±0.20bc	73.27±0.23b
S4	87.08±0.02a	3.21±0.00ef	13.38±0.05bc	13.76±0.05bc	76.49±0.04a
S5	73.30±0.11e	4.81±0.01a	13.26±0.01bc	14.10±0.01b	70.09±0.01c
S6	80.43±0.23c	3.61±0.11cde	12.03±0.23d	12.56±0.25d	73.31±0.18b
S7	88.25±0.19a	2.98±0.09f	12.42±0.02d	12.77±0.05d	76.52±0.36a
S8	84.53±0.44b	3.85±0.14cd	13.055±0.40cd	13.61±0.44cd	73.59±0.09b
S9	78.21±0.02d	3.76±0.11cd	11.06±0.35e	11.68±0.36e	71.20±0.04c
S10	73.86±0.01e	4.37±0.07b	12.60±0.01d	13.34±0.01cd	70.88±030c
S11	88.55±0.25a	1.71±0.08g	7.68±0.02f	7.87±0.01f	77.43±0.65a

Results are given as mean ± standard deviation. Different letters in the same column indicate that there is a significant difference between the samples at the p≤0.05 level. S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris*, S4: *Orchis morio,* S5: *Serapias vomeracea* subsp. *artemisiae*, S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial Salep

Salep species are obtained from different wild *Orchidaceae* species in various regions, so they do not have a standard chemical composition and flower, leaf and tuber morphologies of salep species also differ (Hürkul *et al.*, 2020). The variations in the different color parameters of salep stem from the other species. In addition to being effective in the morphology of the species of orchid from which salep is obtained, the region where it grows plays an effective role in the structure and qualities of salep (Arslan *et al*., 2023).

Bioactive properties

Phenolic compounds are secondary metabolism products found in the chemical structures of plants (Minh *et al.*, 2016). The amount of phenolic compounds in the plant varies depending on various factors such as temperature, UV light, the region where it grows and genetic diversity (Ling and Subramaniam, 2007). The total phenolic content (TPC) of the samples extracted using methanol and ethanol from salep species is shown in Table 3. TPC content is 25.84±0.18-5.82±0.14 mg GAE /g dry matter. The TPC content for ethanol extracts varies between 26.09±0.18-5.29±0.33 mg GAE/g dry matter. The species with the highest TPC value for both extracts was found in salep belonging to the S8 coded *Orchis sancta* species. TPC values varied between species and it was concluded that the results differed statistically (P≤0.05). It can be inferred that ethanol extracts have higher TPC than methanol extracts. Hürkan *et al.* (2019) investigated the bioactive components of six different types of salep extracted with 4 different solvents, n-hexane, chloroform, methanol and water. TPC obtained by methanol extracts of *Anacamptis morio, Anacamptis pyramidalis, Neotinea tridentata, Ophrys mammosa, Ophrys lutea* and *Ophrys speculum* salep orchids is 11.31±0.79, 9.52±0.32, 4.46±0.19, respectively, 37.20±0.51, 13.10±0.86, 16.37±0.26 mg GAE/g dry matter (Hürkan *et al.*, 2019).

Kotiloğlu *et al.* (2020) reported that the total amount of phenolic substance belonging to the Georgica species *Dactylorhiza romana* subsp. was found to be 21.40±0.68 by extracting with methanol and 24.91±0.95 mg GAE/g extract by extracting with ethanol (Kotiloğlu *et al.*, 2020). In another study investigating the phytochemical components of the *Dactylorhiza chuhensis* species, the root, leaf and tubera (salep) of different parts of the orchid were investigated and the total phenolic content of the tuber structure of the orchid species was 13.9±0.6 mg GAE/dry matter (Dalar *et al.*, 2015). In the same study in which the antioxidant activity of the orchid species were studied, the ORAC antioxidant activity method was studied, and it was concluded that the orchid *Dactylorhiza chuhensis* had more or less antioxidant activity of salep tuber<root<flower<leaf (Dalar *et al.*, 2015). This shows us the result that the leaves and flower parts of orchids may have more antioxidant activity than the salep tuber.

Table 3. Bioactive components of different salep species in 80% ethanol and methanol solution

Salep	TFM (mg GAE/g)		DPPH (%AA)		CUPRAC (mg TE/g)	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
S1	16.29±0.515c	16.62± 0.43c	8.98±1.68bc	9.53±0.77bcd	1.78±0.23bc	1.94±0.08cde
S2	8.09±0.43f	8.91±0.28f	5.61±0.39de	6.43±0.77e	1.37±0.15bc	1.99±0.04cde
S3	18.54±0.24b	21.21±0.39b	6.89±0.64cd	7.39±0.32cde	1.71±0.25bc	2.06±0.09cde
S4	10.29±0.12e	11.82±0.35e	5.61±1.81e	6.89±0.90de	1.16±0.24bc	1.77±0.12de
S5	16.32± 0.51c	16.44±0.51cd	8.71±0.13b	9.03±1.74bcd	3.61±0.14a	3.82±0.81bcd
S6	18.35±0.33b	20.21±0.65b	7.93±0.32bc	9.44±0.64b	1.66±0.47bc	3.27±0.66bc
S7	10.05±0.14e	11.40±0.13e	5.02±0.84e	6.43±0.52de	0.81±0.16c	1.71±0.12de
S8	26.09±0.18a	26.84±0.18a	11.04±1.48a	13.22±0.58a	4.11±0.32a	5.45±0.16a
S9	14.12±0.26d	15.33±0.41d	8.89±0.26ab	9.97±1.29cde	1.93±0.05b	2.08±0.10cde
S10	7.93±0.01f	8.06±0.01f	10.72±0.39a	11.49±1.48a	4.25±0.38a	4.55±0.00b
S11	5.29± 0.33g	5.82±0.14g	6.79±0.77cd	8.40±0.34bc	1.74±0.09b	1.84±0.33e

Results are given as mean ± standard deviation. Different letters in the same column indicate that there is a significant difference between the samples at the p≤0.05 level. S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris*, S4: *Orchis morio,* S5: *Serapias vomeracea* subsp. *artemisiae*, S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial Salep

The total phenolic compounds in foods gives an idea about the hydroxyl groups that are effective in antioxidant activity. There is mostly a linear correlation between the total phenolic content of foods and their antioxidant activity (Prior *et al.*, 2005). In the study, antioxidant activity salep species as percentages DPPH

scavenging activity varied between 5.02-11.04% and 6.43-13.22% for methanol and ethanol extracts, respectively. DPPH scavenging activities were found to be statistically differed ($p \leq 0.05$). The percentage DPPH scavenging capacity of commercial salep and the antioxidant activity values of salep of the S3 coded type were not statistically different ($p > 0.05$). The antioxidant activity values calculated by the CUPRAC method were found to be 0.81-4.25, 1.71-5.45 mg/g dry matter for methanol and ethanol, respectively. When the results were evaluated statistically, there was no statistically significant difference in CUPRAC antioxidant capacity of methanol extracts of S1, S2, S3, S4 and S6 samples ($p > 0.05$), but it was concluded that the difference between species was statistically significant ($p \leq 0.05$). In general, it was observed that the TPC and antioxidant activities of the samples extracted with ethanol were higher than the methanol extracts.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is used as an easy and practical method without any pre-processing for the estimation of the glucomannan content in salep (Acemi *et al.*, 2019). FT-IR results of Salep species are shown in Figure 1. According to the FT-IR results, the hydroxyl groups (-OH) of the broad band sugar rings of the salep samples between 3500-3000 cm^{-1} show the presence (Kurt Kahyaoglu, 2015). Peaks in the 3000-2800 cm^{-1} regions are an indication of C-H stretching (Kurt Kahyaoglu, 2017). Two strong peaks were observed at 1618 and 1422 cm^{-1} due to asymmetric and symmetric stretching of the ester groups in salep glucomannan (Pourjavadi *et al.*, 2013).

The 1732, 1635, 1413, 1375, 1247, 1151, 1076, 1053, 1027, 873, and 812 cm^{-1} peaks have been reported in samples as ester carbonyl groups (C=O), mainly originating from the glucomannan acetyl group. It has been stated in studies that 873 and 812 cm^{-1} peaks may be glucose and mannose. The points most strongly associated with peak areas to assess glucomannan content were reported in the literature at 1732 1635, 1246, 1154, 1027, 873, and 812 cm^{-1} (Acemi *et al.*, 2019). It shows the fingerprint region for carbohydrates between 800 and 1200 cm^{-1} (Kurt Kahyaoglu, 2017). In another study, glucose and mannose-related peaks in the spectrum were reported at ~870 cm^{-1} and ~800 cm^{-1} (Chua *et al.*, 2012). According to Figure 1, salep species showed similar spectrum peak in the literature and all species showed similar spectrum peaks.

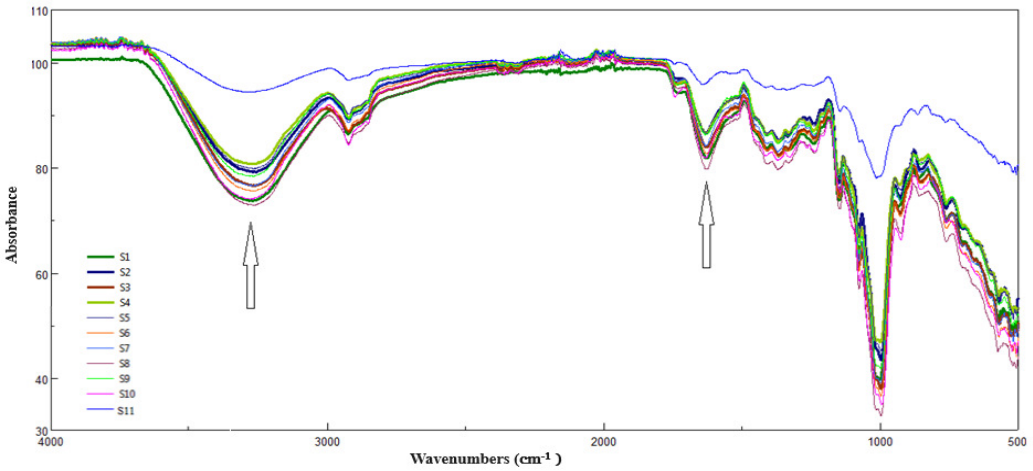

Figure 1. FTIR spectrum

S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris*, S4: *Orchis morio,* S5: *Serapias vomeracea* subsp. *artemisiae*, S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial Salep

Rheological analysis of salep solutions

Flow behavior of salep solutions

According to the result obtained from the analysis of salep aqueous solutions containing 11 different types of salep at 1-2-3%, the graph of shear stress versus shear rate shows that salep aqueous solutions exhibited non-Newtonian rheological characteristic (Figure 2). Salep aqueous solutions are a non-Newtonian flow behavior type in which a decreasing increase in viscosity is observed depending on the increasing shear rate. As seen in Figure 2, the highest shear stress values corresponding to the changing shear rates in all salep aqueous solutions belong to samples 1 and 4. It has been reported in the literature that water or milk-based salep solutions show pseudoplastic flow behavior and their viscosity decreases with increasing shear rate, due to weak physical bonds, electrostatic and hydrophobic interactions, increased alignment speed of molecules, and decrease in intramolecular injection (Farhoosh Riazi, 2007; Kurt, 2021b; Kurt Kahyaoglu, 2015).

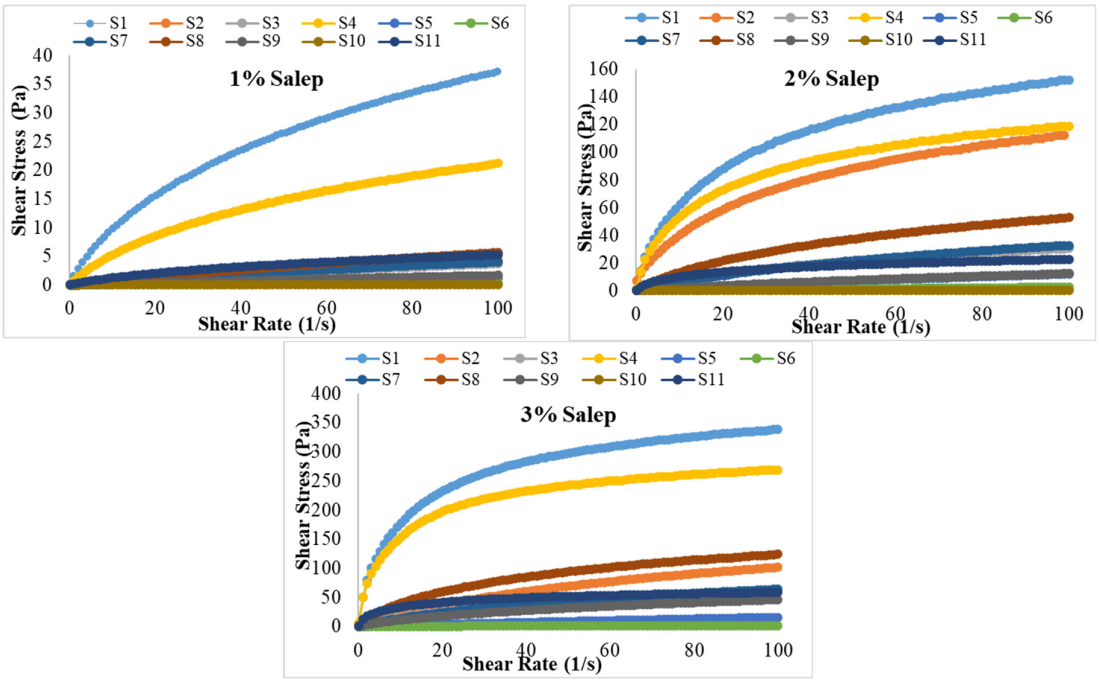

Figure 2. Flow behavior characteristics of 1-3 % salep solutions for different types of salep

The data obtained from flow behavior characteristic of the salep solutions were modeled using the Power Law model. It has been shown in many previous studies that the Power Law model is suitable for explaining the flow behavior of gum solutions (Hijazi *et al.*, 2022; Koocheki *et al.*, 2013; Marcotte *et al.*, 2001). Flow behavior parameters of salep solutions are shown in Table 4. According to the Power Law model, the coefficient of determination (R^2) was found to be $R^2>0.90$ for all salep solutions.

Table 4. Steady shear power-law parameters of the salep solutions contained a different type of salep at 1-3% concentration

	1% salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K (Pa.s^n)	2.910^a	0.050^f	0.076^e	1.526^b	0.015^h	0.006^i	0.088^e	0.121^d	0.025^g	0.003^i	0.344^c
n	0.558	0.920	0.847	0.576	0.938	0.845	0.833	0.838	0.914	0.801	0.596
R^2	0.998	0.999	0.999	0.998	0.999	0.999	0.999	0.999	0.999	0.996	0.999
	2% salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K (Pa.s^n)	28.810^a	14.500^c	2.151^e	25.001^b	0.238^g	0.042^h	1.490^e	3.928^d	0.391^f	0.003^i	4.655^d
n	0.380	0.450	0.582	0.347	0.857	0.898	0.677	0.570	0.753	0.837	0.349
R^2	0.990	0.990	0.998	0.991	0.999	0.999	0.999	0.998	0.999	0.996	0.995
	3% salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K (Pa.s^n)	94.143^a	5.718^e	4.752^f	87.059^b	0.256^h	0.051^i	3.764^g	14.272^d	3.023^g	0.017^i	18.593^c
n	0.287	0.631	0.558	0.255	0.897	0.871	0.621	0.475	0.596	0.636	0.257
R^2	0.984	0.998	0.998	0.978	0.999	0.999	0.998	0.996	0.999	0.993	0.987

K: the consistency coefficient (Pa.sn), n: the flow behavior index values. S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris*, S4: *Orchis morio*, S5: *Serapias vomeracea* subsp. *artemisiae*, S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial Salep

The consistency coefficient (K) and flow behavior index (n) values of the salep solutions were calculated using the Power Law model. The fact that the flow behavior index is in the range of $0<n<1$ indicates that the samples exhibit shear thinning (pseudoplastic) flow behavior, which is non-Newtonian flow behavior. A value of n close to 1 indicates that the flow behavior of the mixture approaches the Newtonian property, while a value close to 0 indicates that the flow behavior of the mixture approaches the pseudoplastic flow property (Aime *et al.*, 2001; Zhang *et al.*, 2018). The flow index value is less than 1 and exhibits non-Newtonian pseudoplastic flow behavior in all solutions. Table 4 showed that K and n values for 1% salep solutions vary between 0.003-2.910 Pa.s^n and 0.558-0.938, respectively. It is seen that the sample with the best consistency at 1% concentration is the S1-coded salep sample. The consistency coefficients of 2% salep solutions were in the range of 0.003-28.810 Pa.sn, and the flow indexes were found in the range of 0.380-0.857. There was a significant increase in the consistency coefficients in most of the 2% salep solutions compared to the 1% concentration solutions. Higher K values cause a higher apparent viscosity increase in the product (Cottrell *et al.*, 1980; Goff *et al.*, 1994). At 2% concentration, S1-coded sample 28,810 Pa.s^n and S4 coded sample had a consistency coefficient of 25,001 Pa.s^n, while n values were found to be 0.558 and 0.576, respectively. It is seen that the viscosity of S1-coded salep is higher compared to other salep types. The K and n values of 3% salep solutions were found in the range of 0.017-94.143 Pa.s^n and 0.287-0.897, respectively. As the salep concentration increased, the n value decreased and especially the n values of the S1 and S4 coded samples, including low concentrations, were low. The low flow index (n) value is effective in creating the desired viscosity and mouthfeel in gum solutions (Farhoosh Riazi, 2007; Marcotte *et al.*, 2001). The concentration increases of the S5, S6 and S10 coded samples did not cause a significant increase in the consistency coefficient, and the K values were reported as 0.256-0.051-0.017 Pa.s^n, respectively. In particular, the increase in consistency in the 3% solutions of the S1 and S4 coded samples compared to the 2% concentration (K values 28,810-25,001 Pa.s^n) draws attention, and the K values at 3% concentration were found to be 94.143-87.059 Pa.s^n, respectively. Bulut-Solak *et al.*, 2017) investigated the rheological properties of salep solutions prepared at different concentrations (0.5%, 1.5%, 2.0%, 2.5%) belonging to the *Orchis anatolica* species, and observed an increase in viscosity values as the concentration increased (Bulut-Solak *et al.*, 2017). Similarly, in our study, the concentration increases in other salep types, except S5, S6, and S10 salep types, caused an increase in viscosity.

It can be said that the higher viscosity of S1 and S4 coded samples is due to their high glucomannan content and strong interactions in hydrogen bonds (Hijazi *et al.*, 2022). Farhoosh and Riazi reported that the viscosity of solutions prepared with salep with high glucomannan content is higher than the viscosity of solutions prepared with salep with low glucomannan content (Farhoosh Riazi, 2007). In the same study, it was observed that the consistency coefficients of two different types of salep increased significantly as the concentration of salep solutions increased at different concentrations (2%, 3%, 4%, 5%, 6% and 7%) (Farhoosh Riazi, 2007). (Kurt Kahyaoglu, 2017)concluded that the apparent viscosity of saleps increases in direct proportion to their glucomannan content. Liver et all. investigated the rheological properties of mixtures of salep and solutions of different gums (guar, xanthan and alginate). In the study where they mixed two different salep concentrations (0.05% and 0.1%) in addition to the samples without salep, it was reported that the consistency coefficients increased as the salep concentration and gum concentration increased (Kayacier Dogan, 2006).

Dynamic rheological properties of salep solutions

The dynamic flow properties of salep solutions are studied to give an idea about the viscoelastic behavior of salep solutions throughout their shelf life and to help with their sensory properties (Sun *et al.*, 2014). Dynamic flow rheological tests are a tool for measuring the viscoelastic properties of food systems. Dynamic rheological measurements are used to define the texture and structure of dairy emulsions (Dogan *et al.*, 2007; Wildmoser *et al.*, 2004).

The dynamic rheological test is performed to give an idea about the viscoelastic behavior of the salep solution during its shelf life. The important parameters used here are; denotes storage modulus (G', elastic character), loss modulus (G", viscous character). The storage modulus (G') of salep solutions at 1-2-3% ratios of the salep types in the study is shown in Figure 3 and the loss modules (G") are shown in Figure 4. The Power Law model was used to evaluate the dynamic rheological properties and the flow parameters of the model It is indicated in Table 5.

The increase in G' and G" values of salep solutions in response to increasing frequency shows that they exhibit a gel-like flow behavior (Kurt and Kahyaoglu, 2015). Looking at Figure 3 and Figure 4, it is seen that the G" values are higher at 2% and 3% concentrations. In 1% salep solutions, only S1 and S4 coded samples are G">G', which is the opposite in other salep solutions. Karaman *et al.*, 2013) reported that the G" values of the salep samples were higher than the G' values. They stated that the elastic property of the salep samples was dominant over the viscous property and they were prone to the liquid character (Karaman *et al.*, 2013). The G"> G' of the salep solutions at 2-3% concentration. It can be said that the dynamic flow properties of salep form a weak gel form with viscoelastic liquid character. In another study, in which the dynamic rheological properties of different salep concentrations and casein mixtures were evaluated, it was stated that the viscous modulus of the salep solutions was higher than the elastic modulus, and the structure was soft and weak gel form (Karaman, 2019). The salep solutions in this study also show flow behavior characteristics compatible with the literature.

K′, K″, n′, n″ and R^2 values were calculated using non-linear regression using the Power Law model of 1-2-3% salep solutions of different types of salep. These values are shown in Table 5. As seen in Table 6, R^2 values were found as R^2>0.89 for 1-2-3% solutions. The high value of R^2 shows that the model can accurately explain the dynamic rheological behavior of salep solutions. As can be seen from Table 5, K′ and K″ values for 1% solutions of the samples are 0.00-0.4589, 0.00-2.127 Pa.sn, respectively, K′ and K″ values for 2% solutions are 0.00-11.016, 0, respectively. For 00-18,368 Pa.sn, 3% solutions, K′ and K″ values were calculated as 0.003-40,953, 0.00-60.579 Pa.sn, respectively. As the concentration of salep solutions except S6 and S10 increased, the n values approached zero. The closeness of n' and n'' values to 0 indicates the solid character of salep. These values show that the samples exhibit a viscoelastic liquid character. As the consistency coefficient increases, a decrease in the n value is observed. It can be said that the n value of the samples with low consistency coefficient is larger, as a result, the n′ value decreases with the increase in the K′ value, as in the flow behavior characteristics

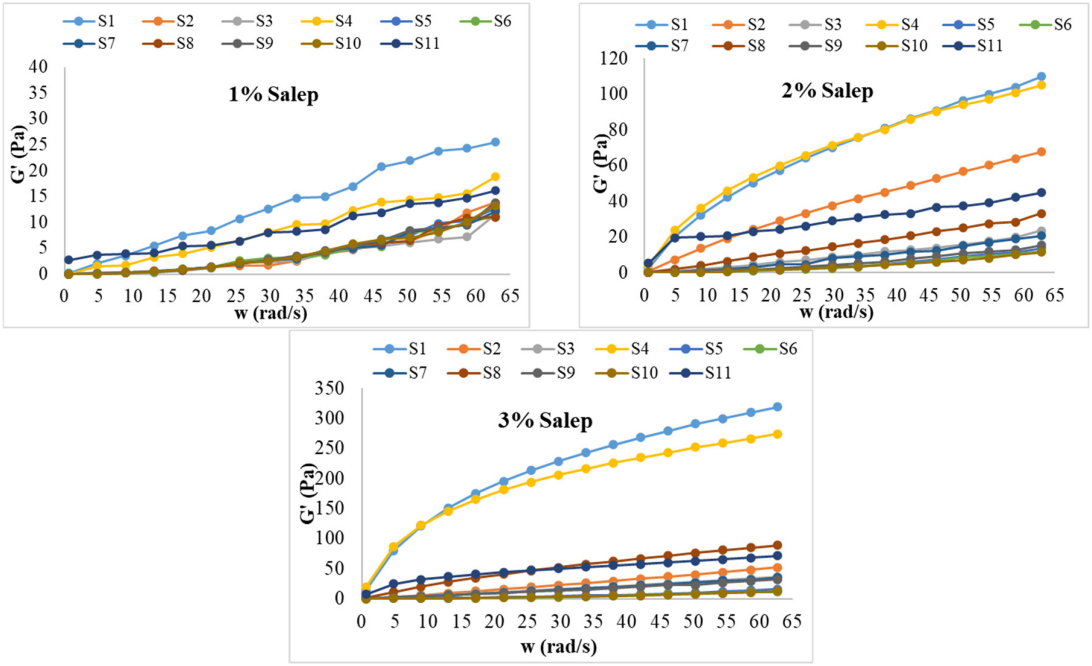

Figure 3. Different types of salep G' (Pa): Storage modulus versus angular velocity values (0.63-62.80 rad/s) of 1-3 % salep solution

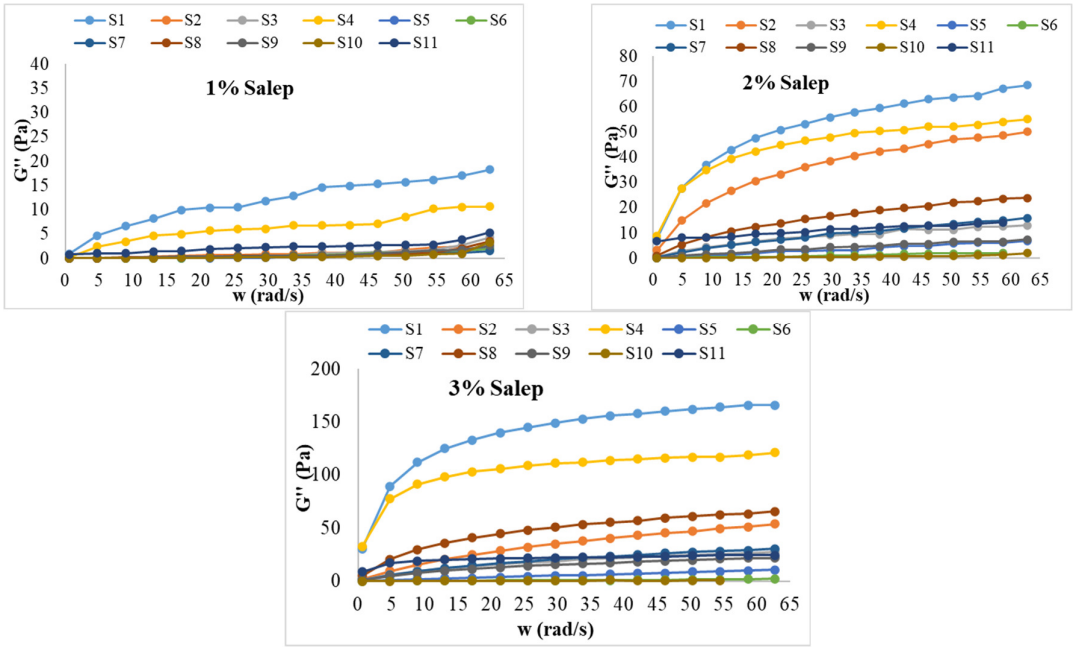

Figure 4. Different types of salep G'' (Pa): Loss modulus versus angular velocity values (0.63-62.80 rad/s) of 1-3% salep solutions

Table 5. Parameters of Power Law dynamic rheological properties of 1-3% salep solutions of different types of salep

	1% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K′	0.4138	0.0002	0.0023	0.1826	0.0023	0.0036	0.0026	0.0031	0.0019	0.0022	0.4589
n′	1.004	2.682	2.016	1.110	2.080	1.941	2.032	1.983	2.126	2.081	0.849
R2	0.997	0.994	0.982	0.995	0.997	0.994	0.995	0.994	0.992	0.993	0.972
K″	2.1277	0.0044	2×10^{-6}	0.8618	3×10^{-5}	0.0005	0.0004	2×10^{-6}	0.0001	4×10^{-7}	0.2004
n″	0.514	1.535	3.448	0.594	2.692	2.007	1.988	3.452	2.421	4.282	0.714
R2	0.996	0.985	0.976	0.972	0.997	0.996	0.987	0.929	0.974	0.885	0.891
	2% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K′	8.487	2.404	0.105	11.016	0.004	0.003	0.053	0.406	0.018	0.004	8.401
n′	0.619	0.807	1.289	0.547	1.933	2.002	1.438	1.054	1.628	1.942	0.385
R2	0.999	1.000	0.996	0.999	0.997	0.998	0.997	0.999	0.998	0.998	0.980
K″	17.198	8.470	1.354	18.368	0.120	0.015	0.818	2.595	0.270	0.001	5.376
n″	0.339	0.437	0.552	0.274	0.976	1.203	0.716	0.543	0.801	1.806	0.225
R2	0.989	0.994	0.993	0.978	0.993	0.985	0.999	0.998	0.997	0.955	0.950
	3% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
K′	40.953	0.513	0.396	47.247	0.007	0.004	0.265	4.122	0.332	0.003	11.781
n′	0.501	1.113	1.087	0.428	1.872	1.931	1.178	0.744	1.096	1.977	0.429
R2	0.997	0.999	0.999	0.996	0.999	0.997	0.999	0.999	0.998	0.999	0.998
K″	60.579	4.182	2.921	54.899	0.174	0.002	2.632	11.929	2.470	4×10^{-5}	12.247
n″	0.257	0.620	0.543	0.200	0.989	1.641	0.596	0.418	0.531	2.982	0.173
R2	0.974	0.998	0.997	0.961	0.999	0.988	0.998	0.994	0.997	0.980	0.974

G' (Pa): Storage modulus, G" (Pa): Loss modulus, K' & K": Consistency coefficient values, n' & n": Flow behavior index values, ω (1/s): Angular shows the speed value. S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris,* S4: *Orchis morio,* S5: *Serapias vomeracea* subsp. *artemisiae*, S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial Salep

3-ITT rheological properties of salep solutions

Foods go through different processes such as mixing, transporting, and pumping for a certain period of time during production or as a final product. The effect of processes in foods, viscosity change can be simulated by different rheological techniques, one of which is the observation of the change in viscosity at a constant shear rate. However, in this method, it cannot give precise information about whether the deformation in the structure of the food is permanent or temporary in the sudden applied forces. The test, which expresses the recovery in the structure of the food as a result of a deformation outside of the immediate and linear viscoelastic region, has been studied as 3-ITT (Toker *et al.*, 2015). The flow behavior of all salep solutions in the third range is shown in Figure 5. The recovery tendency of all salep solutions changed with different salep types and concentrations. According to 3-ITT findings, all samples showed recoverable character at their all concentration. Parameters obtained by the second-order structural kinetic model (G_0, G_e, k×1000, G_e/G_0) are shown in Table 6. G_0, G_e, k×1000, G_e/G_0 values for aqueous solutions containing 2% salep were found to be in the range of 0.068-37.226, 0.045-2.470, 0.049-95.010, 0.077-61.587, respectively. The k value shows thixotropic constant and varied according to their concentration and species. S1 samples showed full recovery at its all concentration, indicating that it exhibited well recovery.

Table 6. 3-ITT rheological properties of the salep solutions contained a different type of salep at 1-3% concentration

Model Parameters	1% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
G_0	2.361	0.053	0.073	0.521	0.008	0.029	0.166	0.190	0.140	0.016	0.527
G_e	2.470	0.120	2.348	1.124	0.523	0.045	0.207	0.732	0.543	0.082	0.612
k×1000	11.506	4.341	0.049	0.147	0.346	23.335	22.418	0.075	0.168	10.688	6.020
G_e/G_0	1.046	2.250	32.171	2.160	61.587	1.558	1.246	3.855	3.873	4.974	1.160
R^2	0.952	0.996	0.223	0.418	0.162	0.972	0.927	0.387	0.969	0.950	0.830
	2% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
G_0	37.226	5.598	0.313	26.001	0.209	0.068	0.390	0.869	0.417	0.096	5.127
G_e	39.273	6.199	0.337	27.901	0.221	0.347	0.439	2.258	0.510	0.152	5.573
k×1000	17.426	3.081	21.322	13.691	16.189	0.231	35.137	0.145	9.267	3.636	8.851
G_e/G_0	1.055	1.107	1.078	1.073	1.059	5.088	1.125	2.597	1.225	1.577	1.087
R^2	0.995	0.989	0.970	0.988	0.922	0.799	0.982	0.977	0.972	0.946	0.995
	3% Salep										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
G_0	116.30	4.406	3.108	114.73	0.212	0.006	2.512	14.793	4.303	0.164	22.830
G_e	126.77	4.322	3.911	123.42	0.914	0.000	2.608	15.422	4.379	0.498	24.108
k×1000	9.415	6.558	0.101	17.499	0.106	17.111	9.224	5.973	95.010	8.167	27.671
G_e/G_0	1.090	0.981	1.258	1.076	4.312	0.077	1.038	1.042	1.018	3.040	1.056
R^2	0.988	0.914	0.500	0.982	0.454	0.365	0.935	0.927	0.799	0.997	0.994

k: Thixotropic rate constant, G_0 (Pa): First elastic modulus in the third time interval, G_e: Equilibrium elastic modulus. S1: *Anacamptis pyramidalis*, S2: *Orchis isaura*, S3: *Anacamptis palustris* subsp. *palustris*, S4: *Orchis morio,* S5: *Serapias vomeracea* subsp. *artemisiae,* S6: *Orchis italica*, S7: *Ophrys mammosa*, S8: *Orchis sancta*, S9: *Dactylorhiza euxina*, S10: *Ranunculus ficaria* subsp. *calthifolius*, S11: Commercial salep

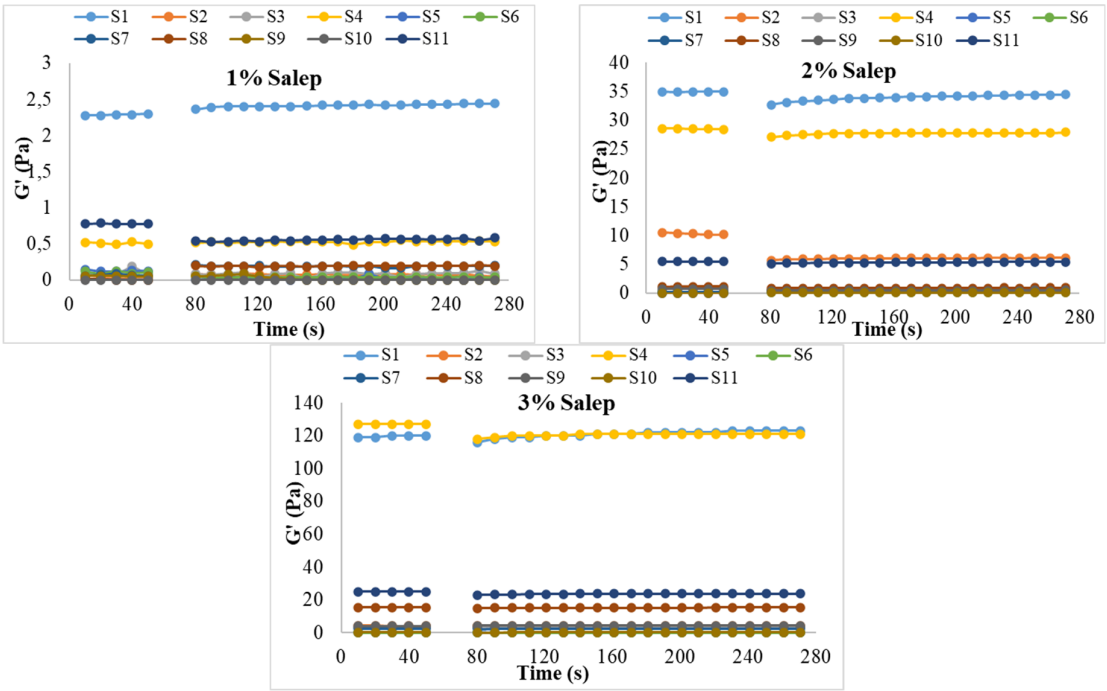

Figure 5. 3-ITT flow values of 1-3% salep solutions for different types of salep

Conclusions

In this study, ten different types of salep were obtained from wild orchids growing in different regions of Turkey: *Anacamptis pyramidalis, Orchis isaura, Anacamptis palustris* subsp. *palustris, Orchis morio, Serapias vomeracea* subsp. *artemisiae, Orchis italica, Ophrys mammosa, Orchis sancta, Dactylorhiza euxina, Ranunculus ficaria* subsp. *calthifolius* physicochemical, bioactive and rheological properties of orchids species and commercial salep samples were examined. TPC and antioxidant activity values of salep species were investigated, and it was concluded that the sample with the highest bioactive properties was *Orchis sancta*. In the study, the flow behavior, dynamic flow properties and 3-ITT rheological properties of 1%, 2% and 3% aqueous solutions of salep samples were examined in detail. It has been stated that the dynamic and flow behavior rheological properties of salep species vary according to salep concentration and salep type. In addition, in the study, the 3-ITT rheological behavior of salep, which is the recovery test of the product after deformation, was determined for the first time on a species basis. Salep is a preferable stabilizer due to its functional properties, bioactive components and polysaccharide structure, and its rheological properties indicate that especially *Anacamptis pyramidalis, Orchis morio, Orchis sancta* species can be used as stabilizers in the food industry. In addition, gum-salep mix trials can be made with different gums to improve the stabilizer's effectiveness.

Authors' Contributions

Data curation: A. A., Z. H. T.-C. & S. K.-C.; Formal analysis: A. A. & Z. H. T.-C.; Investigation: A. A., Z. H. T.-C., S. K.-C, M. Y. & S. K.; Methodology: S. K. & O. S.; Project administration: O. S.; Resources: O. S.; Software: A. A. & Z. H. T.-C.; Supervision: O. S.; Visualization: O. S.; Writing - original draft: A. A., Z. H. T.-C., S. K.-C. & S. K.; Writing - review and editing: O. S.. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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