A study on some functional properties of salep obtained from *Orchis sancta* and its use in ice cream mixture model systems

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Summary
In this study, tubers of a wild orchid *Orchis sancta* grown in the Mediterranean region of Turkey were collected, processed into powdered salep and various characteristics (chemical composition, water holding capacity (*WHC*), swelling capacity, solubility at 70 °C, 80 °C, 90 °C during 15 min) of salep were determined. In the second stage, model ice cream mixes were prepared with salep at various concentrations (5 $g \cdot l^{-1}$, 10 $g \cdot l^{-1}$, 15 $g \cdot l^{-1}$, 20 $g \cdot l^{-1}$) and heat-treated at various temperatures (70 °C, 80 °C and 90 °C) during 15 min). Solubility of salep in water was found to be affected by the applied temperature, resulting in an increase at 80 °C but a decrease at 90 °C. Increasing salep concentration resulted in a reduction in titratable acidity, total protein, ash contents but an increase in *WHC*, apparent viscosity, thixotropic behaviour in mixture models. As the temperature increased, titratable acidity, *WHC*, apparent viscosity, hysteresis areas increased and the flow behaviour index diverged from Newtonian behaviour. A more uniform structure with the increase in salep concentration and temperature from 70 °C to 80 °C, but cluster formation at 90 °C, were observed by microscopy, supporting the results on solubility of salep in water.

Keywords
ice cream; salep; glucomannan; starch; rheology

Salep is obtained from the tubers of wild orchids, which belong to the largest family of flowering plants, Orchidaceae [1]. It is mainly used in the production of ice cream and milk-based beverages. Wild orchids grow spontaneously in meadows and pine forests, mostly in tropical and sub-tropical regions [1, 2]. The tubers of the vast majority of these wild orchid species are dried and ground to obtain powdered salep. The tubers may differ in terms of colour, size and shape depending on the species [3].

The first use of salep was probably due to its therapeutic effects on some diseases [3]. Salep has been mentioned in medical books since ancient times and, in these works, it was explained that salep has aphrodisiac, appetizing, refreshing, and mind-opening effects [2]. Subsequent studies showed that salep has effects on weight control, reducing stress on the pancreas and balancing blood sugar [4, 5].

Salep consists mainly of glucomannan and starch, in different proportions depending on the wild orchid species from which it is obtained, and it is known that its composition has a great effect on quality parameters of the foods prepared from it [2]. It was reported that glucomannan as a hydrocolloid has gelling, thickening, film forming and emulsifying functions in foods [6]. It was stated that starch contributes to the formation of the desired structure with its swelling feature [7].

Turkey is one of the richest countries for wild orchid species compared to Europe and the Middle East countries. At least 10 species and 80 subspecies were reported [8]. Wild orchids grow almost all over Turkey, with the highest abundance in the Mediterranean and Aegean regions [2, 9]. A mixture of salep obtained from various species of wild orchids is used in a highly popular ice cream produced in Turkey. This unique product "Maras type ice cream" is a traditional ice cream registered as a PDO (Protected designation of origin) product. Goats´ milk, sugar and salep are used as raw materials in its production. The unique flavour of the Maras type ice cream, its chewy elastic form, hard structure and high resistance to melting are mainly due to the use of salep in its production [8].

Salep production mainly relies on the collec-

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tion of salep tubers from nature. Hence, many species of wild orchids from which salep is obtained are threatened with extinction [2]. Consequently, studies on expanding the spreading areas, as well as the reproduction of various species in culture media are of interest [7, 8]. For the sustainability of salep and Maras type ice cream production, it is crucial to determine the effects of salep from various wild orchid species on the final product. To date, only a limited number of studies [7, 8, 10] investigated the gross composition of salep of various species and their effects on Maras type ice cream. Overall, a significant species-dependent variation was found.

Orchis sancta is one of the most collected species from nature in Turkey and has come to the fore in the production of salep. Regarding this species, the chemical composition, solubility and viscosity of salep in various sugar solutions [7] as well as rheological properties and zeta potential of Maras type ice cream mix [10] were reported. The current study aimed to identify some characteristics of the powdered salep obtained from *O. sancta*. Furthermore, effects of various concentrations of salep and various temperatures used in the ice cream mixture model were evaluated. Results of this study may be useful in optimizing the production of high-quality Maras type ice cream.

MATERIALS AND METHODS

Collection of orchids and salep production

Wild orchids were collected with their tubers from Bucak District of Burdur Province of Turkey. The species of the plant individuals were identified according to the morphological key provided by Sezik [2]. The tubers of the plants were divided into two groups and powdered salep was obtained from these groups separately by following the traditional method. The tubers were cleaned of soil by washing with cold water. Then they were boiled in water for approximately 15 min and dried in the shade at room temperature (20 ± 2 °C) for 10 days until they hardened. After drying, the tubers were ground with a laboratory knife mill (Art Laborteknik, Ankara, Turkey) at 170 Hz and the powdered salep was passed through a 0.5 mm sieve.

Chemical characterization of salep

For pH measurement, 100 ml of salep solution (10 $g \cdot l^{-1}$) was prepared with distilled water and stirred with a magnetic stirrer for 30 min at 12 Hz at 25 °C. The pH value of the solution was measured with a calibrated pH meter (Mettler Toledo, Zurich, Switzerland). A gravimetric method was used to determine total solids (AOAC 930.15) and ash (AOAC 923.03) contents of salep. The total solids content was subtracted from 100 in order to calculate the moisture content [11]. Total protein content was determined by the Kjeldahl method (AOAC 920.87) using multiplication of the total nitrogen content by the factor 5.7 [11].

In order to measure the glucomannan and starch contents, the kits coded as K-GLUM and AA/AMG (Megazyme, Wicklow, Ireland) were used, respectively. The procedures specified in the kits were performed using a spectrophotometer Lambda 25 UV-Vis (Perkin Elmer, Waltham, Massachusetts, USA) at 340 nm and 510 nm for glucomannan and total starch contents, respectively. Calculations were performed using the following equations as specified in the kits:

$$\Delta A_{GLM} = \Delta(A_3 - A_1)_S - = \Delta(A_3 - A_1)_0 \quad (1)$$

$$GLM = \frac{\Delta A_{GLM}}{6300} \times \frac{V}{1000} \times MW \times \frac{FEV}{v} \times \frac{100}{w} \times F \quad (2)$$

where ΔA_{GLM} is absorbance difference of sample and blank solutions, ΔA_S is absorbance values of the sample solutions, ΔA_0 is absorbance difference of the blank solutions, A_1 and A_3 are the absorbance values of the solutions at the begining and at the end of the reactions, respectively. *GLM* is total glucomannan (expressed in percent), number 6300 represents the extinction coefficient of NADPH at 340 nm, *V* is the final volume in assay cuvette (2.86 ml), *V*/1000 is the factor to convert molarity to moles of NADPH, *MW* is the molecular weight of anhydro-D-glucose/D-mannose (162.14) as occurs in glucomannan polysaccharide, *FEV* is the final extraction volume (250 ml), *v* is the volume of sample added to the cuvette (0.5 ml), *w* is the weight of sample extracted (0.1 g), 100/*w* is the factor to express glucomannan content as a percentage of the sample and *F* represents the dilution factor (1, i.e. no dilution).

$$TS = \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} = \Delta A \times \frac{F}{W} \times FV \times 0.9 \quad (3)$$

where *TS* is total starch (expressed in percent), ΔA is the absorbance read against the reactive blank (reaction), *F* is the conversion from absorbance to micrograms (88.57), *FV* is the total volume (10 ml), 0.1 is the analysed sample volume (in grams), 1/1000 is the conversation from micrograms to milligrams, 100/*W* is the "starch" expression factor, *W* is the weight of flour analysed in milligrams (100) and 162/180 is the adjustment

from free D-glucose to anhydrous D-glucose. Results in percent were converted to grams per litre by multiplying by 10.

Water holding capacity

Water holding capacity (*WHC*) of powdered salep was determined according to the method described by BCHIR et al. [12]. Briefly, 1 g of salep sample and 15 ml of distilled water were transferred into a centrifuge tube and left standing overnight (approximately 16 h) at room temperature (20 ± 2 °C). Then it was centrifuged at 15 000 ×*g* for 20 min. Immediately after centrifugation, the supernatant was removed and the sediment in the tube was weighed. *WHC* was given as grams of water per kilogram of powdered salep.

Swelling capacity

Swelling capacity of powdered salep was measured following the method described by BCHIR et al. [12]. Briefly, 1 g of salep sample and 15 ml of distilled water were weighed into a glass cylinder and the mixture was left overnight (approximately 16 h) at room temperature (20 ± 2 °C). The equilibrium swelling capacity (*ES*) was calculated using the following formula [13] and expressed as grams of swollen sample per kilogram of powdered salep.

$$ES = \frac{W_2 - W_1}{W_1} \tag{4}$$

where W_1 and W_2 are the weights of dry powder and swollen gel, respectively.

Solubility

Solubility of powdered salep was determined following the method described by KURT and KAHYAOGLU [14] with some modifications. An amount of 0.1 g of salep was dispersed in 24.90 ml distilled water. The mixtures were heat-treated in water bath SBD 50 (Heto-Holten, Allerød, Denmark) at 70 °C, 80 °C or 90 °C during 15 min. Then the mixtures were centrifuged at 4 500 ×*g* for 20 min at 20 °C and approximately 10 g of supernatant was weighed and dried to constant weight at 105 °C. Solubility (*S*) was calculated as follows and expressed as percent:

$$S = \frac{m \times 2.5}{w} \times 100 \tag{5}$$

where *m* is the dry matter content of dried supernatant and *w* is the total mass of the sample.

Ice cream mixture model preparation

The composition of 200 g of ice cream mixture models are given in Tab. 1. The amount of granulated sugar beet (Bal Kupu, Ankara, Turkey) was 36 g (180 g·kg^{-1} of the mixture) for each sample. Skimmed goats' milk powder (Enka Sut, Konya, Turkey) concentration was adjusted considering the content of salep (5 g·l^{-1}, 10 g·l^{-1}, 15 g·l^{-1}, 20 g·l^{-1}) to be added in order to keep constant the total solids content (30 g·l^{-1}) of the mixture. Milk powder contained 955 g·kg^{-1} total dry matter, 357 g·kg^{-1} protein and 84 g·kg^{-1} ash.

For ice cream mixture preparation, the required amount of milk powder was reconstituted with distilled water (temperature approximately 55 °C) and stirred with a magnetic stirrer. Then, approximately 2/3 of the sugar was mixed with each reconstituted milk sample seperately. The rest of the sugar was blended with powdered salep before adding it to each mixture. The mixtures were homogenized for 5 min with an Ultraturrax DIAX 900 homogenizer (Heidolph Instruments, Schwabach, Germany) and then heat-treated in a water bath Heto SBD 50 at 70 °C (samples group A), 80 °C (samples group B) and 90 °C (samples group C) for 15 min (Tab. 1). The mixtures were immediately cooled to approximately 20 °C and incubated at 4 °C for 24 h.

Titratable acidity and chemical composition of the mixtures

Titratable acidity values were determined by titration and expressed as lactic acid (as grams per litre). The determination of total solids and ash contents were carried out by the gravimetric method [15]. Total protein content of the mixtures

Tab. 1. Composition of ice cream mixtures and applied heat treatment.

Samples group	Sample code	Raw material [g·kg^{-1}]			*t* [°C]
		Salep	Goats' milk powder	Sugar	
A	A5	5	115	180	70
	A10	10	110	180	70
	A15	15	105	180	70
	A20	20	100	180	70
B	B5	5	115	180	80
	B10	10	110	180	80
	B15	15	105	180	80
	B20	20	100	180	80
C	C5	5	115	180	90
	C10	10	110	180	90
	C15	15	105	180	90
	C20	20	100	180	90

t – heat treatment temperature (the heat treatment lasted 15 min).

was determined according to the Kjeldahl method AOAC 920.87 [11].

Rheological characteristics

Rheological evaluations were performed with a rheometer Kinexus Pro+ (Malvern Panalytical, Malvern, United Kingdom). The measurements were carried out at 4 °C using a cone and plate geometry. Mixture samples were allowed to equilibrate at 4 °C for approximately 10 min before measurement. Shear rate at 0.1–300 s^{-1} was determined. Herschel-Bulkley model was used to describe the rheological properties using the following equation:

$$\tau = \tau_0 + K\gamma^n \quad (6)$$

where τ is the shear stress (in pascals), τ_0 is the yield stress (in pascals), K is the consistency coefficient (in pascal seconds), γ^n is the shear rate (in reciprocal seconds) and n is the flow behaviour index.

In addition, the samples were sheared continuously at a shear rate increasing from 0 s^{-1} to 300 s^{-1} (forward) in 150 s, followed by a decrease from 300 s^{-1} to 0 s^{-1} (backward) for 150 s. The flow curves of ice cream mixtures were measured at 4 °C. Hysteresis (thixotropic) areas were calculated considering the areas under ascending and descending flow curves.

Water holding capacity test

WHC of ice cream mixtures was evaluated by the centrifugation method described by Acar and Kurt [16] with some modifications. Approximately 6 g of the sample was transferred to a centrifuge tube and centrifuged at 10 000 $\times g$ for 45 min at 4 °C. The supernatant was then poured off and the sediment was weighed. *WHC* was calculated according to the following equation:

$$WHC = \frac{W_p}{W_s} \times 100 \quad (7)$$

where W_s and W_p are the mass of the analysed sample and the mass of the sediment, respectively.

Light microscopy

The microstructure of ice cream mixes was visualized at 20× magnification with a light microscope DM2500 equipped with a digital camera DFC450 (both Leica, Wetzlar, Germany). Images were processed with Adobe Photoshop CS6 (Adobe Systems, San Jose, California, USA).

Statistical analysis

Powdered salep and ice cream production were carried out in 2 replications and all of the analysis were performed in duplicate for each parameter. The temperature factor had three levels and salep concentration factor had four levels in experiments. The results were evaluated using analysis of variance (ANOVA) in a factorial design. Duncan's multiple comparison test was applied to determine the significance of the differences among samples at a confidence level of 95 % ($p < 0.05$). Statistical analysis was performed using IBM SPSS Statistics 20.0 software (IBM, Armonk, New York, USA).

RESULTS AND DISCUSSION

Properties of powdered salep

Data on composition and physico-chemical properties of powdered salep are presented in Tab. 2. In terms of general composition, the composition of powdered salep was within the range reported in previous works [7, 17–20]. It is well established that compositional variation in powered salep arises from genetic factors [19, 20], environmental factors [7, 17] and process parameters [8]. Incidentally, the moisture content of powdered salep was low enough (< 10 %) to provide a prolonged shelf-life [2].

Among the other compositional properties, the total glucomannan and starch composition have particular importance for Maras type ice cream technology as they affect the texture and rheology. Bearing in mind that salep with high glucomannan and high starch contents is preferred for Maras type ice cream production [8], the glucomannan and starch content of powdered salep were found to be 107.60 ± 1.99 g·kg^{-1} and 85.10 ± 0.12 g·kg^{-1}, respectively. These values are within the range of 78.40–602.00 g·kg^{-1} for glucomannan and 28.00–439.80 g·kg-1 for starch, respectively, as reported in previous studies [6, 7, 17, 18, 20, 21]. The variation in the glucomannan and starch contents of the powdered salep could be due to the plant species, soil composition and climatic conditions of the region where the orchids were grown [2, 7, 17].

WHC and swelling capacity of the powdered salep were 135.80 g·kg^{-1} and 129.50 g·kg^{-1}, respectively (Tab. 2). Numerous factors could have affected them, namely, chemical composition and physical properties of the sample [22], parameters of heat treatment, grinding and drying [23], as well as the environmental factors such as pH and osmotic pressure of the soil and weather temperature [13, 22]. Hydration is known to increase in parallel to the decrease in the particle size and the increase in the surface area of materials [13]. It is thought that *WHC* and swelling properties of powdered

salep are mainly due to the starch in its composition. Starch has the feature of swelling reversibly at room temperature by imbibing water [24].

Heat treatment yielded fluctuating and statistically significant results on the solubility of powdered salep ($p < 0.05$). The solubility increased by 27 % when the temperature rose from 70 °C to 80 °C (Tab. 2). Further increase in temperature, however, resulted in approximately 40% reduction in solubility. The positive effect of temperature up to a certain point could be explained by the OH groups, which are released when the H bonds between the polysaccharide chains break at high temperatures and interact with more water molecules [25]. KURT and KAHYAOĞLU [14] reported that solubility of the salep solution increased with increasing the applied temperature to 85 °C. Increasing solubility provides a higher viscosity and a firmer gel structure in food products [26]. The detrimental effect of the heat treatment observed at 90 °C per 15 min, however, could be explained by the high gelling effect of the powdered salep. The detrimental high gelling effect at a high temperature of 90 °C was also observed for solubility of xanthan [25].

Tab. 2. Properties of powdered salep obtained from *Orchis sancta*.

Characteristic	Value
pH	6.165 ± 0.003
Moisture [g·kg^{-1}]	76.48 ± 1.01
Total protein [g·kg^{-1}]	48.49 ± 1.72
Ash [g·kg^{-1}]	28.11 ± 1.39
Glucomannan [g·kg^{-1}]	107.56 ± 1.99
Total starch [g·kg^{-1}]	85.05 ± 0.12
Water holding capacity [g·kg^{-1}]	135.75 ± 0.97
Swelling capacity [g·kg^{-1}]	129.50 ± 0.86
Solubility at 70 °C per 15 min [%]	44.3 ± 1.1 ^{b}
Solubility at 80 °C per 15 min [%]	56.6 ± 1.9 ^{a}
Solubility at 90 °C per 15 min [%]	34.6 ± 0.6 ^{c}

Values represent mean ± standard error ($n = 2$). Values with different letter in superscript are significantly different ($p < 0.05$).

Ice cream mixture properties

As expected, the total solids content of the samples was not statistically different from each other due to the standardization during preparation of the mixture models ($p > 0.05$; Tab. 3). However, increasing the salep concentration in the mixture resulted in a decrease in both total protein and ash contents of the mixtures ($p < 0.05$). This was because the amount of milk powder used had to be reduced to keep the total dry matter constant. Since the total protein and ash content of the milk powder (356.70 g·kg^{-1} and 83.80 g·kg^{-1}) used in the preparation of the samples were higher than those of salep (48.50 g·kg^{-1} and 28.10 g·kg^{-1}), both the total protein and ash content of the samples gradually decreased with the increase of salep and the decrease of milk powder. Unlike protein and ash, the titratable acidity values of the mixture samples increased with increasing the salep concentration ($p < 0.05$). This was most likely due to the higher use of salep, which had higher acidity compared to milk (Tab. 2). The titratable acidity values of the mixture samples was also affected by the heat treatment ($p < 0.05$). When temperature was increased from 70 °C to 90 °C, the titratable acidity values increased. Thermal degradation of lactose into acids such as formic acid and lactic acid in high-temperature applications could contribute to this increase [27].

Increasing the salep concentration and the applied temperature yielded higher *WHC* values in the mixture samples ($p < 0.05$; Tab. 3). Such behaviour could be attributed to the enhanced interaction between glucomannan and milk proteins [7, 28] and/or serum protein-casein interaction [29]. These interactions may result in retaining greater amounts of water. Increasing number of hydrophilic sites due to swelling of starch at high temperature may also have contributed to increasing *WHC* [24].

Rheological characteristics of the samples are presented in Tab. 4, Fig. 1 and Fig. 2. Both the concentration of salep and the temperature applied to the mixtures appeared to increase the consistency coefficient (K) values of the samples ($p < 0.05$). K values and apparent viscosity increased gradually with the increase in the concentration of salep and in the applied temperature. The flow behaviour index values (n) of all the mixture samples were below 1 and the Herschel-Bulkley model well described the rheological behaviour of the mix models ($r^2 > 0.99$), indicating a pseudoplastic behaviour of all samples. Expectedly, a higher concentration of salep or higher temperature resulted in higher viscosity of the samples. The n values gradually diverged from 1, which is the Newtonian behaviour, agreeing with the results of previous studies [28, 30].

Glucomannan content of salep is the most important factor responsible for the rheological properties of Maras type ice cream mixtures [7, 8, 17]. As previously mentioned, its interaction with milk proteins increases the amount of water retained in this structure. In accordance with this, as given in Tab. 3, increased *WHC* values of the

Tab. 3. Chemical composition, titratable acidity and water holding capacity values of aged ice cream mixtures.

Characteristic	Samples group	Salep concentration [g·l^{-1}]				
		5	10	15	20	Mean
Total solids [g·kg^{-1}]	A	320.15 ± 0.45	317.75 ± 0.95	318.85 ± 1.75	315.25 ± 0.65	318.00 ± 0.79
	B	317.30 ± 1.10	316.05 ± 0.25	315.55 ± 0.55	316.40 ± 1.40	316.33 ± 0.43
	C	316.95 ± 0.95	318.05 ± 2.15	316.55 ± 0.45	317.00 ± 1.20	317.14 ± 0.55
	Mean	318.13 ± 0.75	317.28 ± 0.73	316.98 ± 0.79	316.22 ± 0.60	
Total protein [g·kg^{-1}]	A	40.95 ± 0.65	40.15 ± 0.45	38.65 ± 0.65	36.70 ± 0.20	39.11 ± 0.64
	B	40.75 ± 0.25	39.80 ± 0.30	38.65 ± 0.35	36.65 ± 0.35	38.96 ± 0.59
	C	41.15 ± 0.35	40.05 ± 0.25	38.80 ± 0.30	36.75 ± 0.25	39.19 ± 0.63
	Mean	40.95 ± 0.21 ^{A}	40.00 ± 0.17 ^{B}	38.70 ± 0.21 ^{C}	36.70 ± 0.12 ^{D}	
Ash [g·kg^{-1}]	A	8.65 ± 0.65	8.20 ± 0.70	7.50 ± 0.40	6.90 ± 0.10	7.81 ± 0.32
	B	8.80 ± 0.20	7.85 ± 0.45	7.60 ± 0.40	7.10 ± 0.20	7.84 ± 0.27
	C	8.65 ± 0.45	7.90 ± 0.60	7.45 ± 0.45	7.10 ± 0.30	7.78 ± 0.28
	Mean	8.70 ± 0.21 ^{A}	7.98 ± 0.27 ^{AB}	7.52 ± 0.19 ^{BC}	7.03 ± 0.11 ^{C}	
Titratable acidity [%]	A	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0 ^{c}
	B	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0 ^{b}
	C	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0 ^{a}
	Mean	0.3 ± 0.0 ^{B}	0.3 ± 0.0 ^{A}	0.3 ± 0.0 ^{A}	0.3 ± 0.0 ^{A}	
Water holding capacity [%]	A	28.7 ± 1.1 ^{Dc}	83.7 ± 1.4 ^{Cc}	95.7 ± 0.5 ^{Bb}	98.4 ± 0.3 ^{Ab}	76.6 ± 10.7
	B	79.3 ± 0.9 ^{Cb}	94.7 ± 0.6 ^{Bb}	97.8 ± 0.5 ^{Ab}	99.2 ± 0.2 ^{Aa}	92.7 ± 3.0
	C	99.0 ± 0.7 ^{Ba}	99.2 ± 0.6 ^{Ba}	99.2 ± 0.6 ^{Ba}	99.5 ± 0.1 ^{Aa}	99.2 ± 0.0
	Mean	69.0 ± 6.2	92.5 ± 0.8	97.6 ± 0.2	99.0 ± 0.1	

Values represent mean ± standard error (n = 2). Values with the different upper case letter in superscript within the same row and lower case letter within the same column are significantly different ($p < 0.05$).
Titratable acidity is expressed as lactic acid.
Samples groups: A – heat-treated at 70 °C for 15 min, B – heat-treated at 80 °C for 15 min, C – heat-treated at 90 °C for 15 min.

Tab. 4. Rheological characteristics of aged ice cream mixtures.

Characteristic	Samples group	Salep concentration [g·l^{-1}]				
		5	10	15	20	Mean
Consistency coefficient *K* [Pa·s]	A	0.81 ± 0.07 ^{Db}	13.48 ± 0.32 ^{Cc}	55.56 ± 4.60 ^{Bc}	264.60 ± 14.50 ^{Ac}	83.60 ± 40.30
	B	3.98 ± 0.45 ^{Db}	50.86 ± 2.61 ^{Cb}	90.87 ± 6.49 ^{Bb}	353.60 ± 10.90 ^{Ab}	124.80 ± 51.30
	C	22.59 ± 0.92 ^{Da}	129.10 ± 2.70 ^{Ca}	270.40 ± 28.40 ^{Ba}	1123.00 ± 83.00 ^{Aa}	386.00 ± 165.00
	Mean	9.13 ± 4.30	64.50 ± 21.60	138.90 ± 42.70	580.00 ± 174.00	
Flow behaviour index *n*	A	0.59 ± 0.00 ^{Aa}	0.31 ± 0.01 ^{Ba}	0.21 ± 0.01 ^{Ca}	0.08 ± 0.00 ^{Da}	0.30 ± 0.07
	B	0.42 ± 0.02 ^{Ab}	0.19 ± 0.02 ^{Bb}	0.14 ± 0.01 ^{Cb}	0.05 ± 0.00 ^{Db}	0.20 ± 0.05
	C	0.25 ± 0.00 ^{Ac}	0.12 ± 0.00 ^{Bc}	0.11 ± 0.01 ^{Bc}	0.03 ± 0.00 ^{Cb}	0.13 ± 0.03
	Mean	0.42 ± 0.06	0.21 ± 0.04	0.15 ± 0.02	0.06 ± 0.01	
Correlation coefficient r^2	A	1.000	1.000	1.000	0.999	
	B	1.000	0.999	0.998	0.996	
	C	0.999	0.997	0.996	0.993	
Hysteresis area [Pa·s^{-1}]	A	145 ± 25 ^{Ca}	355 ± 45 ^{BCa}	576 ± 16 ^{ABb}	965 ± 145 ^{Ac}	510 ± 118
	B	270 ± 20 ^{Ca}	552 ± 28 ^{BCa}	685 ± 55 ^{Bab}	2035 ± 165 ^{Ab}	886 ± 259
	C	550 ± 40 ^{Ca}	746 ± 34 ^{BCa}	1055 ± 135 ^{Ba}	3850 ± 350 ^{Aa}	1550 ± 511
	Mean	322 ± 77	551 ± 73	772 ± 99	2283 ± 543	

Values represent mean ± standard error (n = 2). Values with different upper case letters in superscript within the same row and lower case letter within the same column are significantly different ($p < 0.05$).
Samples groups: A – heat-treated at 70 °C for 15 min, B – heat-treated at 80 °C for 15 min, C – heat-treated at 90 °C for 15 min.

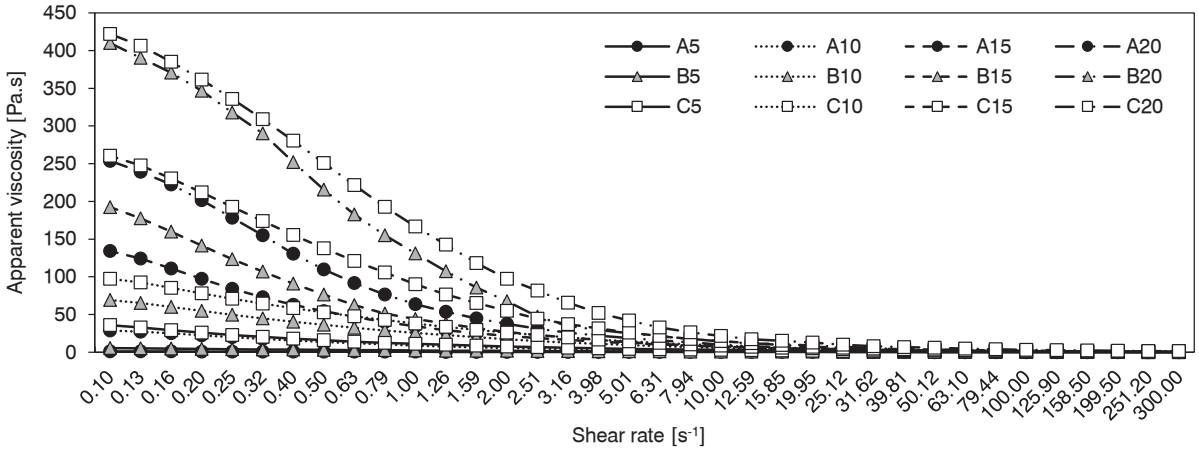

Fig. 1. Apparent viscosity of aged ice cream mixtures at 0.1-300 s-1 shear rate.

Designation of samples is given in Tab. 1.

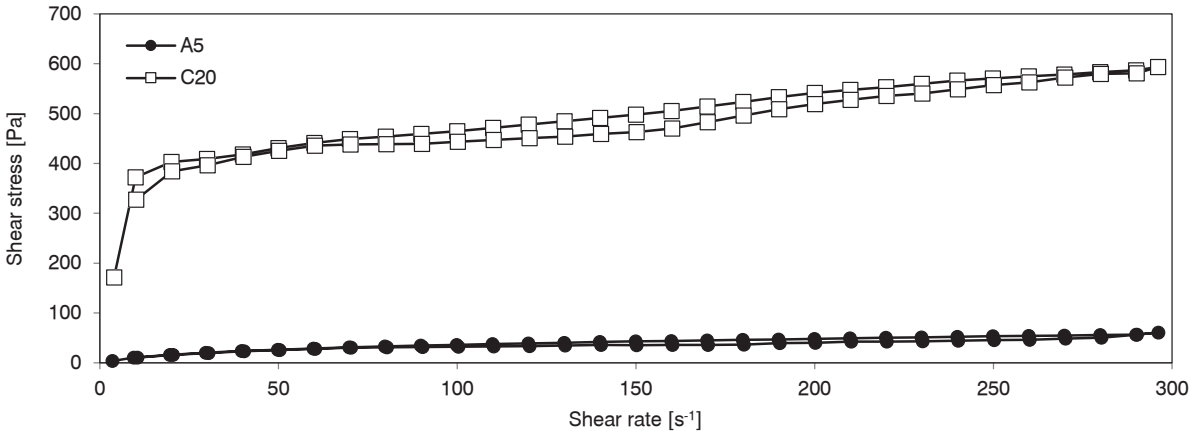

Fig. 2. Hysteresis loops of the flow curves of aged ice cream mixtures.

Designation of samples is given in Tab. 1. A5 and C20 represent the samples with minimum and maximum hysteresis areas, respectively.

samples also contributed to viscosity, in line with data of Develi Işıkli et al. [30].

Starch, as an auxiliary component to glucomannan, contributes to viscosity, especially due to its swelling ability [2]. It is well established that starch granules have a very low water solubility at room temperature with no contribution to viscosity [31]. However, this situation changes when the temperature is increased to 65–70 °C, which is the swelling temperature of starch, and viscosity starts to increase with this increase continuing up to 95 °C [32]. Along with the heat treatment, separation of starch molecules from each other and more water binding increases the randomness in the structure and reduces the number and size of the crystallic regions [24].

In this study, increasing and decreasing shear rates for ice cream mixture models showed a hysteresis loop, indicating a thixotropic behavior, which is a time-dependent fluid behaviour [33]. As shown in Tab. 4, a significant difference was determined between the hysteresis areas of the ice cream mixture samples ($p < 0.05$). The hysteresis area increased with the increasing concentration of salep and temperature, similar to the findings of Kuş et al. [28]. Those authors explained the thixotropic effect with the interaction between salep components, primarily glucomannan, and milk proteins.

A larger hysteresis area of the samples indicated more resistance to flow. As a matter of fact, the *K* values and apparent viscosity values (Tab. 4,

Fig. 1) were found to be compatible with the hysteresis areas. Thixotropic behaviour is also associated with the ability to recover the structure of the sample during the decrease in shear rate [34]. Therefore, as shown in Tab. 4, sample A5 was the sample with the highest recovery ability. This situation was probably associated with the interaction between milk proteins and polysaccharides, as stated by KURT et al. [19]. The increase in stability was attributed to the co-existence of starch and protein in the system [35]. Due to the stronger interaction with the increase in the concentration of salep and the applied temperature, a more resistant structure is obtained against shear rate as a function of time [19]. In Fig. 2, the hysteresis loops of the samples with the highest (C20) and the lowest (A5) hysteresis areas among all ice cream mixture models are shown.

Microscopic images of aged mixture samples revealed a more uniform structure when salep concentration was increased. Likewise, the homogenity increased by increasing the applied temperature from 70 °C to 80 °C (samples A and B). This was in agreement with the *WHC* values of the mix samples (Tab. 3). As shown in Fig. 3, however, higher temperatures (90 °C) caused formation of clusters in group C samples. This behaviour could be explained by the increase in serum protein-casein and protein-glucomannan interaction due to the increase in the denaturation rate of serum proteins occurring at high temperatures. The fact that *WHC* values of group C samples were over 99 % (Tab. 3) and that solubility of salep in water at 90 °C decreased due to the gelling effect (Tab. 2) support this situation [25]. It should also be noted that the ice cream mixtures contain a high amount of sugar (180 $g \cdot kg^{-1}$), which decreases solubility of salep [7].

CONCLUSIONS

In this study, chemical and technological properties of powdered salep obtained from wild orchid *Orchis sancta* were determined in aqueous and model ice cream mixture systems. In aqueous system, increasing the temperature increased the solubility up to a certain point (80 °C), then decreased it when this point was exceeded. As for ice cream mixture model, *WHC*, apparent viscos-

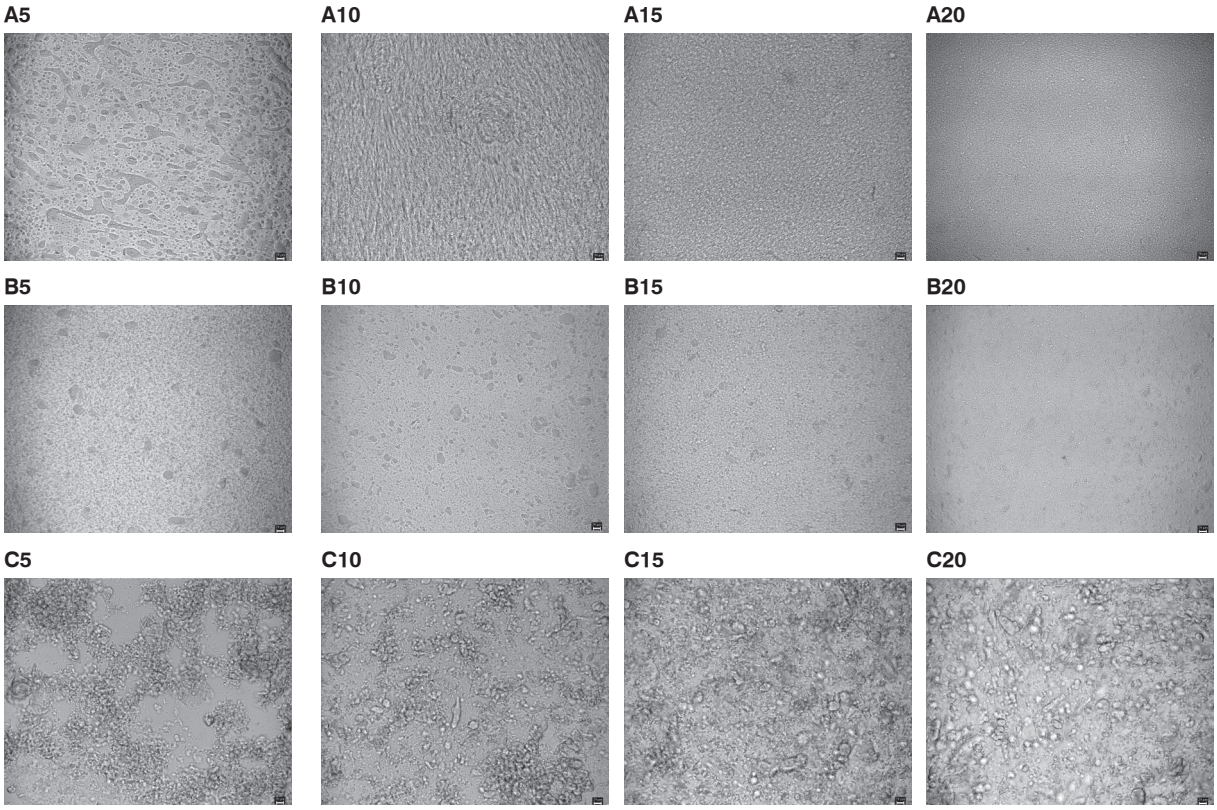

Fig. 3. Light micrographs of aged ice cream mixtures.

Magnification 20× (scale bar 20 μm). Designation of samples is given in Tab. 1.

ity and thixotropic behaviour increased in parallel to the concentration of the powdered salep, which could be attributed to the increased glucomannan and starch concentration. In accordance with the increase in viscosity, the flow behaviour index (*n*) of the samples gradually diverged from 1, indicating the non-Newtonian behaviour. With increasing the process temperature, titratable acidity, *WHC*, apparent viscosity values and hysteresis areas of the samples increased, and *n* values gradually diverged from 1. Similar behaviour was observed when salep concentration was increased. The light microscopic images of the aged mixtures revealed a more uniform structure with increasing the salep concentration or the applied temperature from 70 °C to 80 °C. Beyond this point (at 90 °C), however, cluster formation was observed most likely due to the decrease in water bound in the system. This situation observed in the mixture model was compatible with the results obtained in aqueous solutions of powdered salep. The results suggest that the concentration of salep obtained from *O. sancta* in the ice cream mixture and the temperature applied to the mixture significantly affect the quality of Maras type ice cream.

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