THE EFFECT OF ARTIFICIAL PEPTIDES, COLLAGEN HYDROLYSATE, AND THICKENERS ON GELATINE FUNCTIONS IN APPLE JUICE CLARIFICATION

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The aim of the study was to enhance the efficiency of gelatine in the fruit juice clarification process by increasing its functional properties by three different methods (addition of artificially synthesized peptide, hydrolyzed gelatine, and thickeners). Firstly, peptides were synthesized using a solid-phase peptide synthesis method based on the amino acid structure of gelatine obtained from cow, pig, and fish. Following the peptide validation, the quality of each mixture was investigated and each mixture was used in the clarification process of apple juice. The addition of synthesized peptides, collagene hydrolysate, and some thickeners to gelatine caused a statistically significant difference ($p < 0.05$) on the Bloom, viscosity, pH, conductivity, and isoelectric point values. Arabic gum, carrageenan, and chitosan had no significant ($p > 0,05$) effect on the viscosity value of the thickener-gelatine mixture. The addition of synthesized peptides to the gelatine resulted in better ($p < 0,05$) color scores (94 %-$T_{440nm)}$ compared to gelatine used alone (90 %-T_{440nm}). Similar results ($p < 0,05$) were also obtained for mixtures with hydrolizated gelatine at 5 % and 10 % levels. Except for locust bean gum ($p > 0,05$), each thickener-gelatine mixture had better ($p < 0,05$) color scores compared to gelatine. For transmittance values, out of fish peptide at 2.5 % ratio, the use of synthesized peptides in the mixture gave better ($p < 0,05$) results compared to gelatine. The addition of gelatine hydrolysate at 10 % level to the mixture increased ($p < 0,05$) the transmittance value. The addition of each thickener gave better ($p < 0,05$) results for the same parameter at various ratios.
Keywords: synthetic peptides gelatine thickener apple juice clarification

Die Wirkung von künstlichen Peptiden, Kollagenhydrolysat und Verdickungsmitteln auf die Gelatinefunktion bei der Apfelsaftklärung. Das Ziel der Studie war es, die Effizienz von Gelatine im Fruchtsaftklärungsprozess zu verbessern, indem ihre funktionellen Eigenschaften durch drei verschiedene Methoden (Zugabe von künstlich synthetisiertem Peptid, hydrolysierter Gelatine und Verdickungsmitteln) verbessert wurden. Zunächst wurden Peptide unter Verwendung eines Festphasenpeptidsyntheseverfahrens synthetisiert, das auf der Aminosäurestruktur von Gelatine basierte, die aus Rindern, Schweinen und Fischen erhalten wurde. Danach wurden Mischungen hergestellt und folgende Qualitätsparameter untersucht. Jede Mischen wurde zur Klärung von Apfelsaft verwendet. Die Zugabe von synthetisierten Peptiden, Kollagenhydrolysat und einigen Verdickungsmitteln zur Gelatine verursachte einen statistisch signifikanten Unterschied ($p < 0,05$) bei Bloom-Zahl, Viskosität, pH-Wert, Leitfähigkeit und isoelektrischem Punkt. Der Zusatz von Gummi arabicum, Carrageen und Chitosan hatte keinen signifikanten ($p > 0,05$) Effekt auf die Viskosität der Gelatine-Mischung. Die Zugabe von synthetisierten Peptiden zur Gelatine führte zu besseren ($p < 0,05$) Farbwerten (94 %) im Vergleich zur alleinigen Verwendung von Gelatine (90 %). Ähnliche Ergebnisse ($p < 0,05$) wurden auch für Gemische mit hydrolysierter Gelatine in Mengen von 5 % und 10 % erhalten. Mit Ausnahme von Johannisbrotkernmehl ($p > 0,05$) hatte jede Mischung aus Gelatine und Verdickungsmitteln im Vergleich zu reiner Gelatine bessere ($p < 0,05$) Farbwerte. Für Transmissionswerte, mit Ausnahme von Fischpeptid mit einem Verhältnis von 2,5 %, ergab die Verwendung synthetisierter Peptide in der Mischung bessere ($p < 0,05$) Ergebnisse im Vergleich zu Gelatine. Die Zugabe von 10 % Gelatinehydrolysat zu der Mischung erhöhte den Transmissionswert ($p < 0,05$). Die Zugabe jedes Verdickungsmittels ergab bessere ($p < 0,05$) Ergebnisse für denselben Parameter bei verschiedenen Verhältnissen.
Schlagwörter: synthetische Peptide Gelatine, Verdickungsmittel, Apfelsaft, Klärung

Extraction is a significant process applied in juice production for separating the liquid phase from the fruit. During the fruit juice extraction process, all fruit are broken down into their smallest parts and insoluble plant particles and colloid macromolecules that cause turbidity in the juice. Depending on the type of the product, these compounds should be completely or partially removed from the juice medium (Horvath-Kerkai and Steger-Mate, 2012). The process for removing these clouding agents from the fruit juices is called clarification. Besides, during the clarification process, better transmittance values (T_{440nm} and T_{625}) can be obtained by reducing the browning degree which is directly related to polyphenol oxidase activity (Li et al., 2019). Enzymes, clarifying agents or membrane application can be used to obtain clear products (Severcan et al., 2019). Among these clarifying agents, thickeners and gelatine have importance in obtaining the clarified fruit juice.

Thickeners are commonly used in foods to perform a range of functions, such as contributing to the textural and sensory structure of food; thickening and gelling aqueous solutions; stabilizing foams, emulsions and dispersions; inhibiting the formation of ice and sugar crystals; controlling the release of flavors. The gelation process of thickening molecules is quite complex and varies according to factors such as type, pH, and food temperature (Gao et al., 2017; Williams and Phillips, 2009).

Gelatine, a hydrocolloid structure with many functional properties, is widely used in food, medicine, pharmacy, and cosmetic industries (Schrieber and Gareis, 2007; Ledward, 2000). Since gelatine is a biodegradable, bioactive, and non-toxic substance approved by the Food and Drug Administration (FDA), it is safe to use as a biomedical material (Kosmala et al., 2000; Bigi and Rubini, 2004; Lee and Lee, 2007; Yu and Xiao, 2008; Moraes et al., 2009; Kumari et al., 2010; Kabiri et al., 2011). In the food industry, gelatine is generally used as a gelling agent, stabilizer, emulsifier, thickener, and film former (Gudmundsson, 2002; Karim and Bahat, 2008; Yang et al., 2007; Zhou and Regenstein, 2004; Grundy et al., 2016). Gelatine is mainly used in juice and wine production for the removal of the phenolic compounds that cause undesired changes in the sensory characteristics of the product and is a significant agent used in clarification and stabilization stages (Marangon et al., 2019). Because of the low pH of fruit juice, proteins, used as clarifying agents, carry a positive charge and are able to form complexes with negatively charged colloids, which cause turbidity (Versari et al., 1999; Pinelo et al., 2010). In addition to protein molecules, the surfaces of carbohydrates are negatively charged. The partial hydrolysis of the negatively charged layer reveals a positively charged surface. The attraction between differently charged molecules causes a flock formation. Gelatine-ligand complexes are formed by the addition of gelatine (Yamasaki et al., 1964; Singh and Gupta, 2003). To enhance the efficiency of the gelatine in the clarification process of apple juice, obtaining functional peptide chains with the use of solid-phase peptide synthesis is promising.

Solid-phase peptide synthesis was discovered by Nobel Prize-winning researcher Bruce Merrifield. This method allows synthesis of long artificial peptides in a short period (Murray et al., 2014). The advantages of solid-phase peptide synthesis are that purer products can be obtained; carrier polymers can be reused for various synthesis sessions; multi-step syntheses are carried out faster; and the separation of reactants from the product obtained in the solid phase is quite easy (Kurt, 2010).

The research approaches that are developed to enhance the yield of the agents, which can be used individually or jointly, in the clarification process of the juice are important. In order to reach a better clarification, the use of different clarification agents at different ratios would provide significant information to reach the purpose. On the other hand, solid-phase peptide synthesis can also offer significant potential. The objective of this study was to enhance the efficiency of the clarification process in apple juice by adding functional properties to gelatine with three different peptides with increased ligand-binding capabilities, that were artificially synthesized using solid-phase peptide synthesis method. Besides, to understand the efficiency of the artificially synthesized functional peptides combined with the gelatine in the clarification process of the apple juice, collagen hydrolysate and thickeners at certain ratios were also used.

MATERIALS AND METHODS

PEPTIDE SYNTHESIS AND ITS CHARACTERIZATION

In this study, three different peptides with different amino acid sequences were synthesized using Bruce Merrifield's (Murray et al., 2014) solid-phase peptide synthesis method. The determination of the amino acid

structure of the synthesized peptides was carried out by basing the process on a Gly-X-Y amino acid structure, which constitutes the general structure of gelatine (Kurt, 2010; Eastoe, 1955). The ratios and sequences of the amino acids were determined by considering amino acid ratios and sequences of bovine, porcine, and fish gelatine. Lysine and arginine amino acid peptide sequences were increased to standard gelatine levels to increase the efficiency of the apple juice clarification process and to retain any negatively charged particles. In order to strengthen the structure specific to gelatine, proline and hydroxyproline ratios were also increased (Eastoe, 1956).

Prior to starting the synthesis, the system was washed with dimethyl formamide (DMF; Merck, Darmstadt, Germany) and calibrated. The amounts of chemicals required for the synthesis of peptide sequences were determined via PepDriver software (Ver 2.x CEM, Matthews, NC, USA).

Fmoc-Wang-Gly resin was used as the solid phase on which the peptide chain would grow; as a basic solution DMF was used as the main solvent and dichloromethane (DCM) was used for washing (Merck, Darmstadt, Germany). Fmoc protected amino acids were dissolved in DMF. Activators; (HBTU (N-[(1H-Benzotriazol-1-yl) (dimethylamino)methylene]-N-methylmet-hanaminium hexafluorophosphate N-oxide)/HOBt (1-Hydroxybenzotriazole), activator alkalines, Fmoc-protected amino acids, and de-protection solution (20 % piperidine) (Sigma, Steinheim, Germany) were transferred into the device and syntheses were performed. After the cleavage process was applied to the acquired solution, the peptides were separated from the solid phase and dried (Binder, Tuttlingen, Germany). Table 1 lists the amino acid structures of the synthesized peptides.

Peptides were used in their crude form. The purification process of the peptides was not carried out. The characterization of the peptides was done with LC-MS/MS (Agilent, Santa Clara, CA, USA). For LC procedure: The column used in the LC was Agilent AdvanceBio Peptide Plus 2.1 × 150 mm, 2.7 µm. The mobile phases were 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). Column temperature was 55 °C. For MS/MS procedure: Nitrogen drying gas temperature and flow rate were 325 °C and 13 l/min, respectively. Sheath gas temperature and sheath gas flow were 275 °C and 12 l/min, respectively. Nebulizer pressure was 35 psi. MS/MS mass range was 50 to 1,700 m/z. Capillary voltage, Fragmentor voltage, and Skimmer voltage were 4,000 V, 175 V and 65 V, respectively. Data analysis was performed with the Agilent BioConfirm software B.08.00. Mass measurements of the synthesized peptides were performed with LC-MS/MS. Thus, mass verification of the synthesized peptides was done with the theoretical mass obtained by summing the individual molecular size of amino acids.

Each of the synthesized crude peptides was mixed with gelatine at 2,5 %, 5 %, and 10 % by mass to form individual mixtures. Quality tests for the obtained mixtures were carried out. In these tests, the viscosity, Bloom, conductivity, pH, and isoelectric point parameters were examined in the three replicates (Gmia, 2018).

QUALITY TESTS FOR EDIBLE GELATINE

ANALYSES OF ISOELECTRIC POINT

A 100 ml volume of 1 % gelatine solution was prepared and allowed to swell at room temperature. Then it was put into a hot water bath (Nucleon HC650; Nükleon®, Ankara, Turkey) at 60⁰ for 30 minutes. Thereafter, solution of 32.5 ml was taken up in an erlenmeyer, placed onto 12.5 g (Analytical scale, Ohaus Explorer Pro., Nänikon, Switzerland) of resin (CAS Number: 150604-77-6, Sigma, St. Louis, MO, USA). Then, the erlenmeyer was closed and stored under room conditions for 12 to 16 hours. Finally, the non-resin top was removed and isoelectric point was measured with the pH meter (HI 221, Hanna, Smithfield, RI, USA) (Schrieber and Gareis, 2007).

ANALYSES OF BLOOM

The bloom value of the samples was performed by GMIA standard testing for edible gelatine (GMIA, 2018) with a Texture Analyzer (Brookfield, Middleboro, MA, USA). In the bloom bottle, a solution of 6.67 % was prepared (7.5 g gelatine + 105 ml water) and waited to swell for a while (shaking occasionally). Then, the bottle was stored at 60 °C (shaking occasionally) for 20 min. It was waited for 16 to 18 hours in water of 9 to 10 °C. Finally, the Bloom value was measured.

ANALYSES OF PH

The pH value of the samples was measured using a Hanna HI 221 pH meter (Hanna, Smithfield, RI, USA) according to GMIA standard testing method for edible gelatine (Gmia, 2018).

ANALYSES OF CONDUCTIVITY

A solution, prepared from each mixture individually, of 6.67 % was prepared and waited to swell for a while (shaking occasionally). Afterwards, it was waited for 60 °C (shaking occasionally), then conductivity (µS/cm) was measured by conductivity meter (HI 221, Hanna, Smithfield, RI, USA), (Schrieber and Gareis, 2007).

ANALYSES OF VISCOSITY

Analyses were carried out based on the GMIA standard testing for edible gelatine (Gmia, 2018) method. A solution of 6.67 % was prepared and waited to swell for a while (shaking occasionally). When the solution became more viscose, 16 ml were taken from the solution and measured by a viscometer (Brookfield, Middleboro, MA, USA).

PREPARATION OF THE COLLAGEN HYDROLYSATE MIXTURE

The collagen hydrolysate (Halavet Co., İstanbul, Turkey) was mixed with standard bovine gelatine (Halavet Co., İstanbul, Turkey) at 2.5 %, 5 %, and 10 % by mass. A solution, individually prepared from each mixture, with a 6.67 % concentration was prepared. The quality parameters (viscosity, pH, and conductivity) of the hydrolysate-gelatine mixtures were analyzed in the three replicates (Gmia, 2018).

PREPARATION OF THE MIXTURES FORMED BY DIFFERENT THICKENERS

Each of the carrageenan, Arabic gum, carboxymethyl cellulose (CMC), pectin, modified potato starch, chitosan, locust bean gum, and xanthan gum (Smart Co., İstanbul, Turkey) which are used in the food industry for their thickening properties, was separately mixed with commercial gelatine at 2.5 %, 5 %, and 10 % level by mass. Deionised water (Millipore Simfilter, ABD) was used for the preparation of solutions (individually prepared from each mixture) with 6.7 % concentration. The quality parameters (viscosity, Bloom, pH, conductivity, and isoelectric point) of the obtained solutions were examined in the three replicates (Gmia, 2018).

THE CLARIFICATION PROCESS OF APPLE JUICE

Fresh apples were washed with water obtained via ultra-filtration and divided into small 10 mm diameter pieces. The apple juice was made via pressing method (Bump, 1989). The peptide-gelatine mixtures and the collagen hydrolysate-gelatine mixtures were added separately to the apple juices at 5 mg/10 ml concentrations (Blanck and Eykamp, 1986). After centrifuging (Heidolph Unimax 1010; Heidolph Instruments, Kelheim, Germany) the mixtures at 5000 rpm for one minute, the clarity tests were performed using the spectrophotometric method that measures the transmittance at different wavelengths. Turbidity was expressed as percentage of transmittance (T%) at 650 nm and color was expressed as percentage of transmittance at 440 nm (Gokmen et al., 2001), by using UV spectrophotometer (Agilent Carry Series UV-Vis, Santa Clara, CA, USA) (Zhao et al., 2014; Yilmaz, 2005) and T% values were calculated by Varian Calculation Program (Varian Agilent, Santa Clara, CA, USA). Each of the mixtures prepared with the thickeners was individually added to the apple juices in 5 mg/10 ml concentrations. The samples were then centrifuged at 5000 rpm for one minute. T% values of the obtained products were measured at 440 nm and 625 nm wavelengths.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 8.0 (StatSoft Inc., Minneapolis, MN, USA). ANOVA was carried out to determine the differences between the samples. Tukey and HSD multiple comparison tests were used to determine the differences between parameters with significance level of 0.05.

RESULTS AND DISCUSSION

VALIDATION OF ARTIFICIALLY SYNTHESIZED PEPTIDES

When the LC-MS/MS results of the synthesized peptide, which was designed according to bovine peptide profile, was obtained, the theoretical expected to be 2770 Da was confirmed by the mass spectrophotometer (Fig. 1).

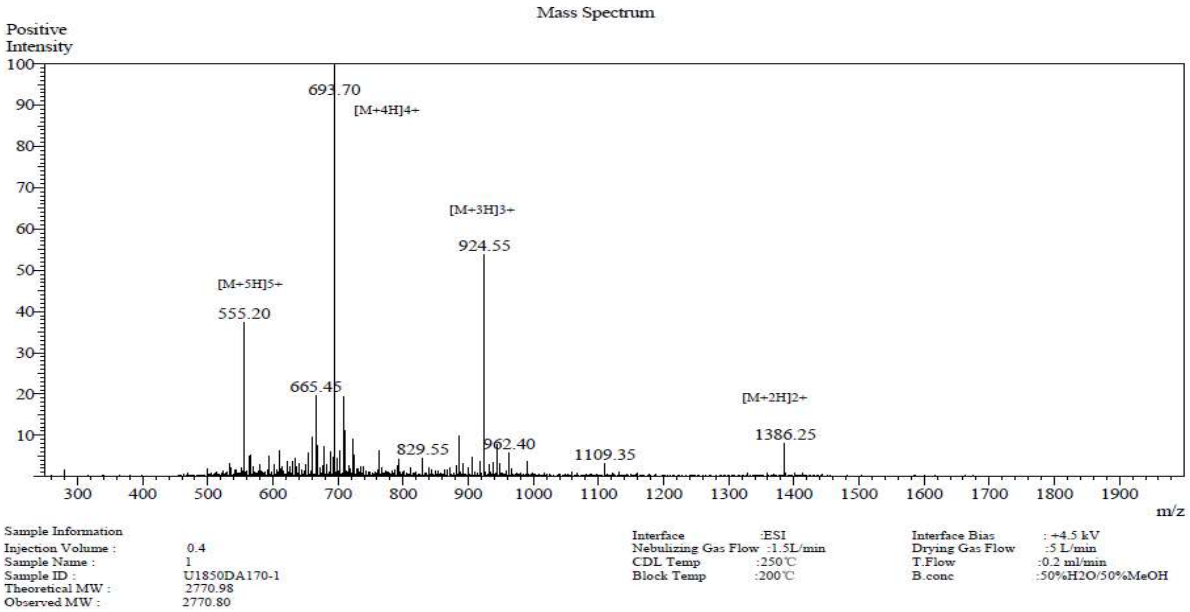

Fig. 1: LC-MS/MS Mass Spectrum of peptide A (the peptide prepared based on the amino acid structure of the gelatine from cow)

LC-MS/MS results of the peptide synthesized by the gelatine obtained from fish verified the mass calculated as 2981 Da (Fig. 2).

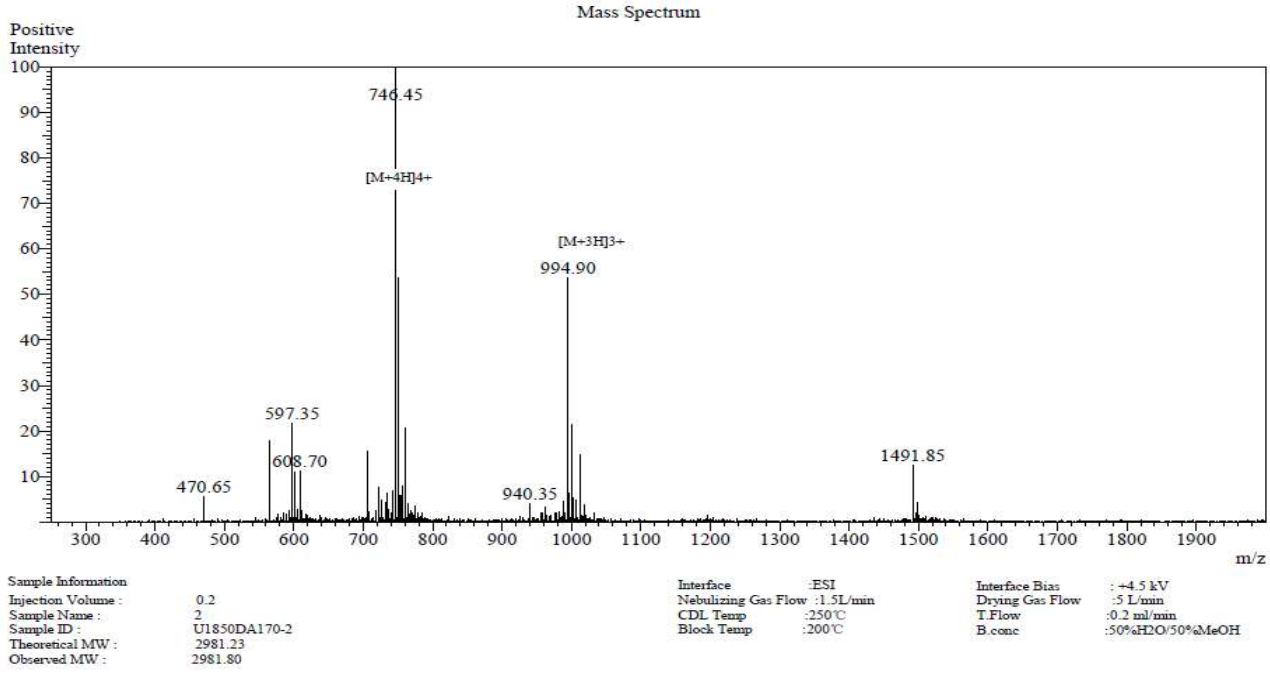

Fig. 2: LC- MS/MS Mass Spectrum of peptide B (the peptide prepared based on the amino acid structure of the gelatine from fish)

When the results of the synthesized peptide were taken as a sample of the gelatine obtained from pig, it was observed that the mass calculated as 2871 Da was confirmed by LC-MS/MS (Fig. 3).

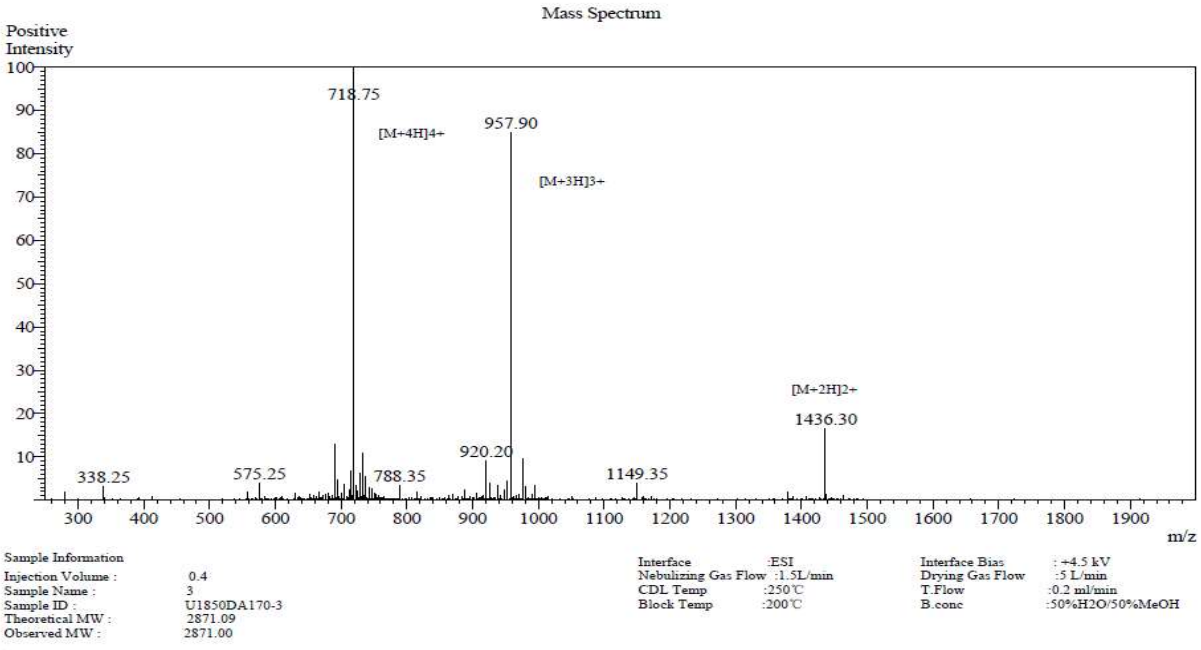

Fig. 3: LC- MS/MS Mass Spectrum of peptide C (the peptide prepared based on the amino acid structure of the gelatine from pig)

QUALITY TESTS FOR MIXTURES OBTAINED VIA SYNTHESIZED PEPTIDES

The amino acid sequences of gelatine obtained from cow, pig, and fish were used for peptide synthesis. Arginine and lysine amino acids were added to enhance the flocculation of gelatine to the synthesized peptide sequences.

The quality test results of each synthesized peptide-gelatine mixture on Bloom, viscosity, pH, conductivity, and isoelectric point are presented in Table 2. The addition of synthesized peptides to the gelatine caused a decrease in the viscosity values of the mixtures. The most drastic decreases were observed at 10 % synthesized peptides in each group. Similar to viscosity, the Bloom values changed inversely proportional to the ratio of the peptide in the mixture. The use of synthesized peptides at the level of 2.5 % in the mixture decreased the pH values in each mixture. However, the rise in the rate (5 %, 10 %) significantly ($p < 0,05$) increased the pH values. Other parameters that were affected by the addition of synthesized peptides in gelatine were conductivity and isoelectric point. These parameters showed a similar and statistically significant upward trend due to the increase of synthesized peptides in the ratio. These changes were probably due to the proportions and types of amino acid residues present in the structure of the synthesized peptides which were added to the mixtures (Zhang et al., 2006).

Table 1: The amino acid sequences of the synthesized peptides

BP*	ALA PRO HYP GLY ARG ILE GLY PRO HIS GLU SER GLY TYR VAL PRO GLY ASP MET GLY THR PHE GLY GLU HYP GLY LYS ALA HYP PRO GLY
FP**	GLY PRO GLU HYP GLY ALA ARG GLY LYS VAL GLY ALA HYL GLY LYS HIS GLY GLU ALA GLY ASP SER GLY PRO THR LEU GLY PRO HYP GLY
PP***	GLY PRO HYP GLY ARG ALA GLY PRO HIS GLU SER LEU VAL ILE PRO GLY ASP ALA GLY THR PHE GLY GLU HYP GLY LYS ALA HYP PRO GLY

BP*: the peptide prepared based on the amino acid structure of the gelatine from bovine
FP**: the peptide prepared based on the amino acid structure of the gelatine from fish
PP***: the peptide prepared based on the amino acid structure of the gelatine from porcine

Table 2: Results for the quality parameters of gelatine and synthesized peptide-gelatine

Peptide	%	Viscosity (mPa/s)	Bloom	Conductivity (µS/cm)	pH	Isoelectric Point
Gelatine	100	4,21±1,26f	243±1,24d	2530±1,27b	5,18±0,02de	4,86±1,12d
BP*	2,5	4,09±1,32a	238±1,21c	2687±1,87d	5,17±0,01cd	4,94±1,10c
BP	5	4,03±1,27a	227±1,56b	2693±2,01a	5,2±0,02ab	5,02±1,14b
BP	10	3,49±1,52c	214±1,84a	2737±1,98g	5,21±0,01ab	5,11±1,21a
PP**	2,5	4,07±1,28a	238±1,74c	2691±1,58a	5,17±0,00c	4,94±1,17c
PP	5	3,97±1,86d	227±0,98b	2704±1,62f	5,2±0,00ab	5,02±1,20b
PP	10	3,43±1,45b	214±1,12a	2753±1,65h	5,21±0,01a	5,11±1,19a
FP***	2,5	4,06±1,22a	238±0,95c	2678±1,24a	5,17±0,02cd	4,94±1,13c
FP	5	3,99±1,24de	227±0,76b	2699±1,32e	5,2±0,01ab	5,02±1,12b
FP	10	3,49±1,31bc	214±1,21a	2790±1,44i	5,21±0,00ab	5,11±1,25a

BP*: the peptide prepared based on the amino acid structure of the gelatine from cow

PP**: the peptide prepared based on the amino acid structure of the gelatine from pig

FP***: the peptide prepared based on the amino acid structure of the gelatine from fish

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).

QUALITY TESTS OF THE COLLAGEN HYDROLYSATE-GELATINE MIXTURE

The results for the quality parameters of the collagen hydrolysate-gelatine are presented in Table 3. The addition of collagen hydrolysate to the gelatine decreased the viscosity and Bloom parameters significantly ($p < 0,05$). On the other hand, increases ($p < 0,05$) in conductivity, pH, and isoelectric point were also observed with the rise of the collagen hydrolysate level. The isoelectric point of a protein depends on its base amino residues and amino acid residues. During the hydrolyzation of collagen, new polypeptides with different molecular weights arise, and these new compositions determine the isoelectric point (Zhang et al., 2006). The differences in measured isoelectric points of the collagen and collagen hydrolysate-collagen mixtures in this study were probably due to the newly formed components that arose during the hydrolyzation step. Compared to the results obtained for the mixtures containing synthesized peptides (Table 2), the use of collagen hydrolysate in the mixture revealed similar outcomes for similar quality parameters (Table 3). The similarities in the results of these different groups could be due to the peptide chains that come out during the production of collagen hydrolysate.

QUALITY TESTS OF THE THICKENER-GELATINE MIXTURE

Quality tests (viscosity, Bloom, conductivity, pH, and isoelectric point) of the mixtures were obtained by individually mixing the carrageenan, Arabic gum, carboxymethyl cellulose (CMC), pectin, modified potato starch, chitosan, locust bean gum, and xanthan gum with the commercial gelatine at 2,5 %, 5 %, and 10 % by mass in the three replicates.

The results for the quality parameters of the thickener-gelatine mixtures are presented in Table 4. The addition of xanthan gum, locust bean gum, and CMC had a statistically significant effect on viscosity value ($p < 0,05$), and the most significant increase (approx. 6-fold) in viscosity value was observed in mixtures containing CMC. CMC solutions have strong thixotropic behavior (Benchabane and Bekkour, 2008), and are known to increase the viscosity of the mixture with the addition of various ingredients (Yang and Zhu, 2007). Arabic gum, carrageenan and chitosan had no statistically significant ($p > ,0,05$) effect on viscosity values of the thickener-gelatine mixtures. Pectin and modified potato starch showed a significant effect at the levels of 5 %, 10 % and 2.5 %, respectively. Except for carrageenan at 10 % level ($p < 0,05$), the addition of thickeners at different ratios decreased the Bloom values of each thickener-gelatine mixtures ($p < 0,05$). The use of thickeners in mixtures at each level except for carrageenan at 5 % and 10 % levels ($p < 0,05$) decreased the conductivity values of all thickener-gelatine mixtures ($p < 0,05$). The addition of dif-

Table 3: Results for the quality parameters of the collagen hydrolysate-gelatine mixtures

	%	Viscosity (mPa/s)	Bloom	Conductivity (µS/cm)	pH	Isoelectric Point
Gelatine	100	4.21±0.01d	243±1.00d	2531±1.16a	5.18±0.01a	4.86±0.00a
Collagen hydrolysate	2.5	4.15±0.87c	238±1.12c	2620±0.89c	5.17±0.00a	4.94±1.21b
	5	4.07±0.95b	227±1.25b	2610±0.87b	5.20±0.01b	5.02±1.23c
	10	3.61±0.98a	214±0.98a	2690±0.94d	5.21±0.01b	5.11±1.36d

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).

Table 4: Results for the quality parameteres of the thickener-gelatine mixtures

Component	%	Viscosity (mPa/s)	Bloom	Conductivity (µS/cm)	pH	Isoelectric Point
Gelatine	100	4.21±0.85abc	243.00±1.03ghi	2530.00±7.00r	5.18±0.05a	4.86±0.01abc
CMC*	2.5	24.00±0.83j	206.00±1.11cde	2363.00±5.00o	5.46±0.05bcd	4.88±0.01abcd
	5	24.00+0.00j	202.00±1.19cd	2223.00±8.00n	5.48±0.04bcd	4.81±0.02ab
	10	24.00+0.00j	172.30±0.98b	2199.00±22.00m	5.51±0.02bcd	4.78±0.01a
Xantham Gum	2.5	9.70±0.97g	246.60±0.96hi	1860.00±3.00a	5.49±0.03bcd	5.39±0.98abcde
	5	10.80±0.78h	195.80±0.92c	1914.00±3.00b	5.46±0.02bcd	5.54±0.03e
	10	10.80±0.73h	195.80±0.88c	1936.00±2.00c	5.45±0.03bc	5.57±0.03e
Locust Bean Gum	2.5	7.75±0.86e	228.70±0.89fgh	2172.00±2.00l	5.49±0.03bcd	5.47±0.05cde
	5	5.90±0.85d	199.80±1.01cd	2140.00±4.00jk	5.53±0.02bcd	5.51±0.02de
	10	7.60±0.96e	132.70±0.56a	2125.00±4.00hi	5.53±0.04bcd	5.32±0.02abcde
Arabic Gum	2.5	4.08±0.75ab	223.90±0.62efg	2150.00±3.00k	5.48±0.02bcd	5.42±0.01bcde
	5	3.88±1.10ab	216.90±0.69def	2225.00±2.00n	5.45±0.02bc	5.52±0.01e
	10	4.08±1.12ab	194.50±0.53c	2236.00±6.00n	5.49±0.02bcd	5.41±0.06abcde
Pectin	2.5	4.50±0.97bc	225.80±0.82efg	2131.00±5.00hij	5.48±0.03bcd	5.43±0.04bcde
	5	7.28±0.92e	220.40±0.72def	2103.00±4.00fg	5.48±0.03bcd	5.50±0.09de
	10	11.80±0.83i	201.40±0.63cd	2116.00±5.00gh	5.40±0.02b	5.30±0.07abcde
Carrageenan	2.5	4.24±0.59abc	233.30±0.59fghi	2390.00±3.00p	5.56±0.03cd	5.35±0.06abcde
	5	4.08±0.61abc	250.80±0.601i	2582.00±3.00s	5.56±0.03cd	5.41±0.03abcde
	10	4.93±0.73c	283.30±0.68j	2576.00±6.00s	5.57±0.04cd	5.70±0.07e
M.P. Starch**	2.5	8.69±0.88f	228.90±0.63fgh	2092.00±1.00f	5.59±0.03d	5.46±0.08cde
	5	4.22±0.95abc	223.30±0.72efg	2041.00±4.00e	5.47±0.05bcd	5.49±0.03cde
	10	4.36±1.01abc	214.70±0.69cdef	2024.00±5.00e	5.49±0.06bcd	5.35±0.02abcde
Chitosan	2.5	4.51±0.66bc	219.60±0.59def	1841.00±3.00a	5.56±0.12cd	5.42±0.03def
	5	3.60±0.63a	214.70±0.72cdef	1973.00±4.00d	5.49±0.08bcd	5.55±0.07cdef
	10	3.65±0.84a	217.50±0.76def	1958.00±1.00d	5.80±0.03e	5.54±0.07def

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).
*carboxy methyl cellulose
**modified potato starch

ferent thickeners to gelatine showed a similar increasing behavior ($p < 0,05$) on pH values of all thickener-gelatine mixtures. Except for CMC, all thickeners used in the study increased the isoelectric points of all thickener-gelatine mixtures, and statistically significant effects ($p < 0,05$) at different levels were observed for locust bean gum (5 %), Arabic gum (5 %), pectin (5 %), xanthan gum (5 %, 10 %), carrageenan (10 %), and chitosan (2.5 % to 10 %). It has been understood that the use of these products will impart a functional property to the gelatine blends to be formed. It has been found that very low amounts of blends of gelatine with thickeners such as xanthan gum and CMC increase the viscosity by 100 to 600 %. As observed in the current study, the viscosity of food proteins like collagene dispersions could vary greatly depending on different factors such as concentration, pH etc. (Hermansson, 1975). In this regard, it is anticipated that gelatine will find a wide range of applications in areas other than apple juice, to suit the process requirements. It is thought that very important results will be obtained if the benefit of viscosity increase is considered in the production of food products such as pastry, confectionery, jam, and marmalade (Kristensen and Jensen, 2011).

USE OF SYNTHESIZED PEPTIDE-GELATINE MIXTURES IN THE CLARIFICATION PROCESS OF APPLE JUICE

Table 5 lists the results of the clarity tests for apple juice after the applied clarification process.

Transmittance value at 440 nm is an indicator of browning degree of apple juice and reflects the yellowness and brownness. The browning degree is directly related to polyphenol oxidase activity in the juice. Increase in transmittance values at 440 nm shows a lower browning degree as a color score (Li et al., 2019). In order to meet the market demands, color value ($T_{440\ nm}$) of clarified apple juice should not be lower than 40 % (Tülek and Yilmaz, 2006). Both the use of gelatine and synthesized peptides-gelatine mixtures in the clarification process of apple juice revealed desirable results for the color parameter. On the other hand, the use of synthesized peptides in the mixtures resulted in higher

Table 5: Clarity results of the clarification process of the apple juice in which synthesized peptides and gelatine mixtures were used

Peptide	Rate %	Color Value $T_{(440nm)}$ %	Transmittance Value $T_{(625nm)}$ %
Gelatine	100	90,52±1,01b	94,95±1,02a
BP*	2,5	92,71±0,98d	96,76±0,95d
	5	93,66±0,96a	96,55±0,98d
	10	94,05±0,98f	96,87±0,85g
PP**	2,5	93,68±1,12a	95,11±1,24b
	5	93,76±1,02a	95,78±1,13c
	10	94,33±1,15g	96,22±1,17f
FP***	2,5	92,44±1,25c	94,79±1,25ab
	5	93,11±1,29e	95,46±1,21e
	10	93,69±1,15a	95,98±1,28c

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).

BP*: the peptide prepared based on the amino acid structure of the gelatine from cow

PP**: the peptide prepared based on the amino acid structure of the gelatine from pig

FP***: the peptide prepared based on the amino acid structure of the gelatine from fish

color scores ($p < 0,05$) compared to gelatine use alone. Transmittance at 625 nm is a significant parameter for determining the effectiveness of the clarification process. The rise in the transmittance values reflects the degree of clarification (Li et al., 2019). The industrially acceptable minimum transmittance ($T_{625\ nm}$) value for apple juice is 80 % (Vladisavljević et al., 2013). The transmittance values at 625 nm for each group were higher than 90 % as preferred. Besides, except for fish peptide at 2.5 % ($p > 0,05$), each mixture containing synthesized peptides had higher ($p < 0,05$) transmittance scores compared to gelatine use alone. These results suggest that the addition of functional peptides led to an increase in the yield of the clarification process both for its color and transmittance parameters. The effects of the mixtures prepared with bovine peptide and porcine peptide were very close to each other. More effective clarification values were obtained in this study compared to the study of Araya-Farias et. al. (2008) in which the highest T% value was found as 63 % ± 0,5 % at 200 mg/l gelatine concentration.

USE OF COLLAGEN HYDROLYSATE-GELATINE MIXTURES IN THE CLARIFICATION PROCESS OF APPLE JUICE

The efficiency of the process was measured via the clarity test (Table 6).

Although much difference was not observed in numerical terms, the statistical difference ($p < 0,05$) was determined for the 10 % collagen hydrolysate mixture compared to gelatine use alone. In contrast, the use of collagen hydrolysate in the mixture at the level of 5 % decreased ($p < 0,05$) the color value. For transmittance value at 625 nm, only the mixtures with 10% collagen hydrolysate had a statistically ($p < 0,05$) higher score, and values for other mixtures were similar ($p > 0,05$) to the control group with gelatine. According to the results, the use of gelatine with the addition of collagen hydrolysate in the clarification process of apple juice increased the efficiency of the process at some ratios. From this point of view, the addition of a small amount of collagen hydrolysate to standard gelatine by mass would be enough to increase the yield of the clarification process. This may be due to differences in the molecular size of gelatine and hydrolysate (Ersch et al., 2016), because the hydrolysate has a smaller molecular structure than gelatine (Ersch et al., 2016; Rousselot Company, 2018), and hydrolysate does not cause coagulation of the globular protein gel compared to the addition of gelatine alone (Ersch et al., 2016). Collagene hydrolysate's efficiency on the transmittance values strongly depends on its concentration used in the clarification process. Increasing amounts of collagen hydrolysate in the clarification process reveal better transmittance results, and this phenomenon was also observed in the clarification process of chrysanthemum (a flowering plant; *Dendranthema morifolium* Ramat. Tzvel.) beverage by Zhang et al. (2018) in which polyphenols, flavonoids, proteins, and sugars were slightly reduced.

Table 6: Clarity results of the clarification process of the apple juice in which collagen hydrolysate-gelatine mixtures were used

Product	Rate %	Color Value $T_{(440nm)}$ %	Transmittance Value $T_{(625nm)}$ %
Gelatine	100	90,52±0,98a	94,95±0,78a
Collagen hydrolysate	2,5	90,55±0,95a	94,67±0,82a
	5	90,48±0,85b	94,96±0,79a
	10	90,61±0,84c	95,35±0,93b

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).

USE OF A THICKENER-GELATINE MIXTURE IN THE CLARIFICATION PROCESS OF APPLE JUICE

Table 7 summarizes the thickener-gelatine results.
The addition of thickener to the gelatine for clarification of apple juice mostly increased the efficiency of the process for the color parameter. The color (T_{440nm}) values of apple juices clarified with different thickener-gelatine mixtures were higher than 90 % except for chitosan at 10 % level and xanthan gum. The lowest values were detected in mixtures with xanthan gum, but all mean values for color were higher than the acceptable level (Vladisavljević et al., 2013). The highest mean values were measured in samples clarified with locust bean gum-gelatine mixture. Statistically significant differences ($p <$,0,05) were observed in each mixture for color value. As Table 7 shows, the use of CMC-gelatine (5 %, 10 %) mixtures in the apple juice increased the T440nm values and had almost no positive effect on the T_{625nm} except for 2,5 % level compared to gelatine-only use; furthermore, the efficiency of the clarification process decreased with the increasing of xanthan gum ratio in mixtures. In conformity with Genovese and Lozano (2001) and Liang et al. (2006), increase in xanthan gum ratio in the clarification process of apple juice revealed a decrease in the transmittance values and xanthan gum was more effective at low doses. The increase of locust bean gum in the locust bean gum-gelatine mixtures positively affected the color values compared to gelatine, while the efficiency of clarification was higher in Arabic gum-gelatine mixtures with 2,5 % and 5 % Arabic gum compared to the gelatine-only use. Similar to Arabic gum, same results were obtained in mixtures containing 5 % and 10 % pectin for color and transmittance values. The values measured for T440nm and T625nm were higher in samples clarified with carrageenan-gelatine mixtures at each carrageenan ratio compared to gelatine-only use. The use of modified potato starch-gelatine mixtures in clarification caused a slight increase in the efficiency of the process compared to gelatine-only use.
The last thickener added to the mixture was chitosan. An increase in the efficiency of the process was observed when 2,5 % and 5 % chitosan were added to the mixture, whereas a higher ratio (10 %) decreased the efficiency. The mass ratio is generally an important parameter for the gelatine-thickening mixture. The protein and polysaccharide ratio in the mixture is a critical rate and affects the load balance, interactions, density and bulk degree of the complex (Sarika et al., 2015). Furthermore, there is no direct change between the proportion of gravity and the degree of clarification in some thickener-protein mixtures. This may be due to the fact that the complex is affected by many parameters (thermodynamic properties, pH, charge density, molecular weight, etc.) as well as the mass ratio (Aberkane et al., 2010; Kayitmazer, 2017; Devi et al., 2017; Duhoranimana et al., 2017; Lim et al., 2014; Xiong et al., 2017). According to Chatterjee et al. (2004), bentonite, chitosan, and gelatine, which were used as clarifying agent, were not effective in the clarification process of apple, orange, grape, and lemon juice. As a result, the effect of chitosan on clarification was found to be greater than gelatine. In this study, it was found that the effect of gelatine on clarification was increased with the addition of chitosan, the results are consistent with the study. Similar to Tastan and Baysal (2017), it was found, that chitosan is more effective than gelatine in apple juice clarification. Moreover, in conformity with the studies (Benitez and Lozano, 2007), it was determined that gelatine clarification performance on fruit juice increased with the components added.

CONCLUSIONS

Clarification is a significant process in juice production for completely or partially removing the insoluble plant particles and colloid macromolecules. Enzymes, clarifying agents or membrane application can be used in clarification process. Among these agents, thickeners and gelatine have importance in obtaining the clarified fruit juice. As these agents can be used individually or jointly, giving functional properties by adding artificial peptide and collagen hydrolysate to gelatin also offers a significant potential in juice clarification. In this study, the effect of gelatine on the efficiency of the clarification process of apple juice was evaluated by adding functional properties. The results indicate that synthesized peptides like bovine peptide, porcine peptide, and fish peptide could be used by the food industry to enhance the functional properties of commercial gelatine. Collagene hydrolysate can also contribute to the clarification properties of gelatine to obtain clearer apple juice. The thickeners used in the study except for xhantham gum were also found to have improving effects on the functions of commercial gelatine in apple juice clarification. The use of functionalized gelatine could provide enhanced efficiency in industrial applications and can be used widely in other areas by combining the processing requirements and apple juice clarification. Besides, xanthan gum and CMC were found to increase the viscosity of the gelatine mixtures by 100 to 400 % even in very

Table 7: Clarity results of the clarification process of the apple juice in which thickener-gelatine mixtures were used

Component	Rate %	Color Value $T_{(440)}$ %	Transmittance Value $T_{(625)}$ %
Gelatine	100	90,52±0,78f	94,95±0,72f
CMC*	2,5	90,43±0,89f	95,64±0,85hi
	5	91,15±0,87gh	94,76±0,72d
	10	91,77±0,98j	94,45±0,73c
Xantham Gum	2,5	87,41±0,85b	95,34±0,83g
	5	88,71±0,75c	94,89±0,95ef
	10	84,55±0,65a	92,32±0,83a
Locust Bean Gum	2,5	93,31±0,81m	96,05±0,86kl
	5	94,14±0,79o	95,88±0,82j
	10	94,56±0,92p	96,45±0,83m
Arabic Gum	2,5	91,31±0,59hi	96,02±0,75hi
	5	91,15±0,91hi	95,65±0,73hi
	10	90,26±0,71ef	96,43±0,77ef
Pectin	2,5	90,01±0,69e	94,92±0,69f
	5	93,43±0,78m	96,24±0,67e
	10	91,85±0,73k	95,55±0,82hi
Carrageenan	2,5	93,57±0,81m	97,15±0,76o
	5	92,66±0,92l	95,44±0,72h
	10	93,86±0,92n	96,81±0,96n
M.P. Starch**	2,5	91,42±0,82ij	95,60±0,89hi
	5	91,62±0,72j	94,77±0,86de
	10	90,84±0,68g	94,83±0,89ef
Chitosan	2,5	92,67±0,53e	96,50±0,78m
	5	92,46±0,56e	96,12±0,82l
	10	89,30±0,74d	93,26±0,78b

Values with different superscript lowercase letters in the same column are significantly different ($p < 0.05$).
*carboxy methyl celulose; **modified potato starch

low quantities. Based on this finding, new researches can be conducted for the use of CMC-gelatine and xantham gum-gelatine mixtures in other food and non-food areas.

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