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THE EFFECT OF HEAT TREATMENT TEMPERATURE AND ACTIVE ACIDITY ON TEXTURAL PROPERTIES OF WHEY PROTEIN-PECTIN GELS

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Summary. The aim of this study was to determine the effect of heat treatment of whey protein (50–90°C) and active acidity (pH 4.0–10.0) on the textural properties and appearance of whey protein-pectin gels. The following parameters were determined: hardness, adhesiveness, springiness index, gumminess and appearance of gels. It was found that the increase of heat treatment temperature of whey proteins leads to the increase of textural parameters. The highest values of hardness, springiness index and gumminess was observed for gels from whey proteins heated at 90°C. The highest adhesiveness was demonstrated by gels from whey proteins heated at 80°C. Gels at pH 5.5 and 6.0 exhibited the highest hardness, whereas gels at alkaline pH (above 8.0) – the highest adhesiveness. Additionally, the study showed a possibility to form protein-pectin gels by modifying the heat treatment temperature and acidity of the environment.

Key words: gel, whey proteins, pectin, pH, conjugates

INTRODUCTION

In food products the interactions between proteins and polysaccharides are subject of numerous studies, since the products obtained in consequence of such interactions vary considerably from the substrates with respect to structural and functional properties. Among the main functional properties of (WPs) the most important are stabilization

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of emulsions and foams, and formation of spatial structures (gels and edible coatings) [Foegeding et al. 2007, Gondek et al. 2013, Spotti et al. 2013].

Low methoxyl (LM) pectin gels are formed as a result of interaction in the presence of calcium within a wide pH range whey proteins [Wehr et al. 2004]. The mechanism of LM pectin gelation relies mainly on egg box-model, which is a result of the electrostatic interactions formed between the dissociated carboxylic groups of the pectin and the bivalent calcium ions [Vithanage et al. 2010]. Commercially used pectins are often amidated which improves gelling process of LM pectins.

Whey proteins are susceptible to higher heat treatment temperature. By the application of higher heat treatment temperature (above 75°C) the WPs can create gel (heat-induced gelation) [Brownlow et al. 1997, Havea et al. 2009, Sağlam et al. 2014]. Heat-induced gel formation occurs following partial unfolding of native proteins which results in exposure of non-polar residues, and this leads to clustering of aggregates to form a gel network [Clark et al. 2001, Munialo et al. 2016]. Another mechanism to form WPs gels is known as cold gelation and involves two steps: in the first step, a solution of aggregates is formed by heating the WPs and in the second step, the electrostatic repulsion between aggregates is reduced either by addition of salt (salt-induced gelation) or decrease of the pH towards the isoionic point (IP = 5.0) (acid-induced gelation) [Nicolai et al. 2011, Nicolai and Durand 2013, Kharlamova et al. 2016, 2018a, b].

The addition of polysaccharides to proteins during gel formation can alter the mechanical and textural properties of the resultant gels [Munialo et al. 2016]. Many researchers revealed that mixing native or denatured WPs with neutral or anionic polysaccharides results in the creation of new products or allows to design textural and sensory properties of existing products [Spotti et al. 2013, Krzeminski et al. 2014, Munialo et al. 2016]. Such complexes can have desirable hydration capacity, rheological and interfacial properties which depend mainly on factors like pH, ionic strength, temperature and pressure treatment, as well as the application of mechanical forces [Qi and Onwulata 2011].

The structure of whey protein-low methoxyl pectin is in similar size like milk fat globules which can lead to replace part of milk fat in fermented milk products [Krzeminski et al. 2014].

The aim of this study was to obtain whey protein-pectin gels and next determine the effect of heat treatment of WPs (50–90°C) and active acidity (pH 4.0–10.0) on the textural properties and appearance of whey protein-pectin gels. Low methoxyl amidated pectin was chosen due to its ability to form gels at wide range of pH and less prone to precipitate.

MATERIAL AND METHODS

Material

The following materials were used in the study: WPC 80 (whey protein concentrate) (Milkiland, Ostrów Mazowiecki, Poland), which contains 80% nitrogen substances (N 6.38) and Unipectine AYS 407C, which was low methoxyl amidated pectin (esterification degree 26–32%, amidation degree 17–22%) (Cargill, Minneapolis, MN, USA).

Preparation of whey protein-pectin gels

Gels were obtained by mixing a solution from WPC 80 with low methoxyl amidated pectin (LMAP) solution to the concentration of the final solution of 6.0% WPC and 1.0% LMAP. Before mixing/heat treatment solutions were left for hydration in 4°C for 24 h.

Plan of experiments

The study was divided into two parts in which heating and acidity factors were tested independently. In the first part, the WPC solution was heat treated in a water bath at the temperature range 50–90°C for 15 min. Solution of LMAP was prepared by carefully dissolving pectin in distilled water and heating in a water bath at 70°C for 30 min. Solutions of WPC and LMAP obtained in that way were mixed and after cooling they were left to solidify. A control test in which WPC solution was not heated was also performed. Active acidity of the obtained protein-pectin gels was 6.0 ±0.1.

In the second part of the study, WPC solutions with pH 4.0–10.0 were heated in water bath at 70°C for 15 min. The active acidity of the solutions was adjusted using 1M HCl or 1M NaOH. Next, both solutions were mixed and left to set after cooling. Obtained in the two stages gels were stored at 4°C. After 24 h measurements of texture parameters of the gels were carried out. Before testing gels were left at room temperature to reach temperature 20°C.

Measurement of textural properties

Texture profile analysis of the gels was performed using Brookfield CT3 texture analyser (Brookfield Engineering Laboratories, USA) by modified method proposed by Bourne [2002]. The gel samples having cylindrical shape of 70 mm diameter and 30 mm height were subjected to a test of double compression (to 40% of the initial height of the sample). A cylindrical analytical probe of 10 mm diameter was forced down into each sample at a rate of 0.5 $mm \cdot s^{-1}$. The gel samples were analysed with respect to the following parameters: hardness, adhesiveness, gumminess and springiness index. All variants of gels in the tested temperature and pH range were obtained in 10 replicates.

Statistical analysis

The obtained results were statistically analyzed with Statistica 10 software. The Tuckey test was applied to identify statistically significant differences between the mean values at a confidence level of 95% ($\alpha = 0.05$).

Results and discussion

The effect of heat treatment of WPs on the textural properties of protein-pectin gels was illustrated in Table 1. The active acidity of formed protein-pectin gels was 6.0 ±0.1.

The hardness of gels increased with increasing temperature. The highest hardness (0.53 N) values were obtained when WPC 80 solution were heated at 90°C. Whereas, gels with unheated WPC or those with WPC heated at low temperatures up to 60°C had

Table 1. The effect of heat treatment of whey proteins (WPC 80) on selected textural parameters of whey protein-pectin gels

Tabela 1. Wpływ obróbki termicznej białek serwatkowych (WPC 80) na wybrane właściwości teksturalne żeli białkowo-pektynowych

Temperature of heat treatment of WPC 80 Temperatura obróbki termicznej WPC 80 [°C]	Adhesiveness Przylepność [mJ]	Springiness index Wskaźnik sprężystości	Gumminess Gumowatość [N]	Hardness Twardość [N]
20	0.16[a]	0.75[a]	0.50[a]	0.08[a]
50	0.18[a]	0.71[a]	0.58[ab]	0.09[a]
60	0.38[a]	0.81[ab]	0.65[ab]	0.10[a]
70	0.64[ab]	0.84[ab]	0.73[b]	0.41[b]
80	1.36[bc]	0.92[b]	1.18[c]	0.43[b]
90	1.16[c]	0.94[b]	1.75[d]	0.53[c]

The values in columns with varied letters are statistically different at $p < 0.05$ ($n = 10$).

Wartości w kolumnach oznaczone niejednorodnymi literami są statystycznie różne przy $p < 0,05$ ($n = 10$).

the lowest hardness from 0.08 to 0.1 N and the hardness of such gels did not vary significantly.

Between gels contained WPC heated at 60 and 70°C hardness increased 4 times which might indicate that in this range of heat treatment temperatures high WP denaturation occurred. No significant variation in the hardness was observed for gels with WP treated at temperature of pasteurization (i.e. 70 and 80°C). It was observed that the adhesiveness, springiness index and gumminess of the examined gels increased with the increasing heat treatment temperature of WPs solution (Table 1). The highest values of these texture parameters, just like hardness, were found in gels containing WPs heated at 90°C. Gels formed at low heat treatment temperatures (i.e. 50 and 60°C) of WPC solutions and the unheated gel (20°C) were weak, irregular and slightly more translucent. Gels formed at higher temperatures 70–90°C demonstrated higher cohesiveness and were whiter in colour (Fig. 1). Whey proteins denature at temperature above 70°C for 20–30 s and then form sulphur bridges, which could increase both hardness and adhesiveness [Anema 2009, Spotti et al. 2013].

Brownlow et al. [1997] demonstrated that WPs gels obtained by heating at temperature above 70°C had better textural properties when compared with their unheated counterparts. This situation is explained by the increase in reactivity of SH groups in ß-lactoglobulin when heated. The concentration of free SH groups in heat treated WPs solutions was almost 3 times higher than the one in unheated solutions [Havea et al. 2004, Giroux et al. 2010].

In our study, the gels formed at 80 or 90°C were hard and white-coloured. It should be noted that used in study the time of heat treatment was enough for WPs to denature at 90°C to such an extent that the gels obtained at this temperature had lumpy structure. According to Zhang et al. [2012], when concentration of pectin is relative low (pectin to

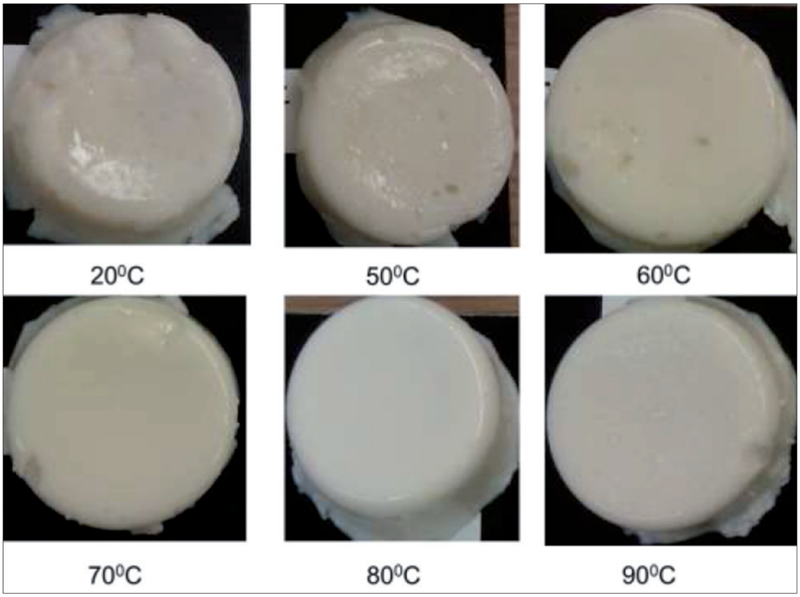

Fig. 1. Appearance of gels depending on the heat treatment temperature of whey proteins

Rys. 1. Wygląd żeli w zależności od obróbki termicznej białek serwatkowych

protein ratio above 0.2) lumpy structures of gels after heating are obtained due to formation of large protein-protein aggregates. However, when pectin concentration is higher (pectin to protein ratio above 0.2), turbidity and particle size decreases due to formation of WP-LMAP aggregates, which limits the protein-protein interactions. Taking above into consideration, using higher concentration of pectin may lead to obtaining smooth, homogeneous WP-LMAP gels.

The time of heat treatment at lower temperature (70 and 80°C) was enough to form smooth gels having firm structure (Table 1). The control gel (unheated) was weak, melting and too loose. The obtained results indicated that heat treatment is an indispensable condition for protein-pectin gels to be formed. To obtain tight and flexible gels, WPs should be heating at temperature more equal 70°C. During such heat treatment homo- and heteropolymers are formed as a result of activation of cysteine residues [van Vliet et al. 2004]. According to Setiowati et al. [2017], whey protein-pectin conjugates are significantly more heat stable that pure WPs solutions. After heating WP-pectin conjugates at 80°C during 20 min there was no change in particle size distribution towards no heated conjugates.

Active acidity is a very important factor affecting the gelation process and properties of formed gels. At pH values above *IP*, WPs are negatively charged, and at values below this point they are positively charged [Turgeon and Beaulieu 2001]. When both WPs and pectin is negatively charged (pH is above *IP* of proteins) they create co-soluble aggregates, when pH is lowering below *IP* – the complex coacervates occurs [de Kruif et al. 2004]. No particular tendency was observed when comparing the hardness of gels formed from WPs at pH in the range of 4.0–10.0 (Table 2). In the pH range of 5.5–6.0 the gels exhibited the highest hardness, although at pH 7.5 the formed gels were found to have equally high hardness (1.25 N). The effect of these pH values on the gel hardness parameter was not statistically relevant.

Strongly negative charged pectin could absorb on unfolded proteins before protein aggregation which lead to create protein-pectin aggregates by electrostatic interactions. When pH is below *IP*, absorption of polysaccharide could lead to flocculation and phase separation. In pH above *IP* when protein have positive charge absorption of strongly negative pectin lead to obtain large whey protein-pectin aggregates [Tuinier et al. 2002]. The lowest hardness (0.3 N) was observed for gels at pH 4.0 which is in line with above researchers.

Table 2. Effect of pH of the whey protein solution on selected textural parameters of protein-pectin gels

Tabela 2. Wpływ pH roztworu białka serwatkowego na wybrane właściowości teksturalne żeli białkowo-pektynowych

Active acidity (pH) of WPC 80 Kwasowość czynna (pH) WPC 80	Adhesiveness Adhesiveness [mJ]	Springiness index Springiness index	Gumminess Gumowatość [N]	Hardness Twardość [N]
4.0	0.32^{ab}	0.66^{ab}	0.14^{a}	0.30^{a}
4.5	0.72^{bcd}	0.75^{ab}	0.34^{bc}	0.69^{b}
5.0	0.40^{abc}	0.59^{a}	0.27^{abc}	0.86^{bc}
5.5	0.12^{a}	0.86^{b}	0.91^{e}	1.38^{d}
6.0	0.30^{ab}	0.69^{ab}	0.62^{d}	1.41^{d}
6.5	0.64^{bcd}	0.72^{ab}	0.33^{bc}	0.90^{bc}
7.0	0.40^{abc}	0.64^{a}	0.40^{c}	1.06^{ce}
7.5	0.74^{bcd}	0.77^{ab}	0.39^{c}	1.25^{de}
8.5	0.86^{cd}	0.78^{ab}	0.23^{ab}	0.82^{bc}
10.0	1.14^{d}	0.73^{ab}	0.25^{abc}	0.78^{bc}

The values in columns with varied letters are statistically different at $p < 0.05$ ($n = 10$).

Wartości w kolumnach oznaczone niejednorodnymi literami są statystycznie różne przy $p < 0,05$ ($n = 10$).

The examined gels exhibited the highest adhesiveness (1.14 mJ) for solutions at pH 10.0 (Table 2). The highest values of springiness index (above 0.73) were found for gels formed from WPs solutions at pH 5.5 and above 7.5. All the examined gels in the whole pH range applied in the study exhibited properties of elastic substances (value of springiness index above 0.5). A lot of values of the springiness index did not show statistically significant differences depending on the pH of the WPs solution. Gels formed from WPs solutions at pH 5.5 showed the highest gumminess (0.91 N).

Protein-pectin gels formed from WPs solutions at pH 4.0 were the weakest and not firm after removal from the container. Whereas, gels formed from WPs solutions at pH 8.46 and 10.00 demonstrated the highest elasticity (Fig. 2).

Li and Zhong [2016] observed that it is possible to obtain preheated whey protein--pectin gels at pH below 3.0 but in this conditions gels were weak and gelation process were dependent on biopolymer ratio and composition. Wijaya et al. [2017] observed that whey protein-pectin gels show pH-responsive behaviour especially in higher pH values and stability in wide range of pH. Our results confirmed these observations which can make whey protein-pectin gels a very attractive for food applications. To obtain hard, rubbery and elastic gels pH of WPs should be adjust to 5.5, while pH at 10.0 will lead to obtain the most sticky gels.

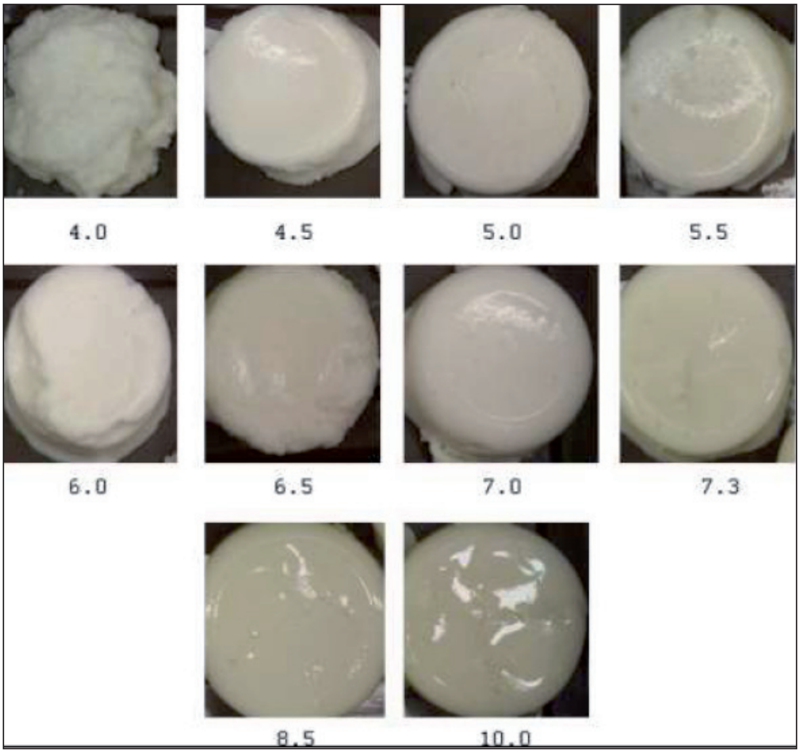

Fig. 2. Appearance of gels depending on the pH of the whey protein solution

Rys. 2. Wygląd żelów w zależności od pH roztworu białka serwatkowego

CONCLUSIONS

Increase of heat treatment temperature of WPs resulted in an increase of hardness, adhesiveness, springiness index and gumminess of pectin-whey proteins gels. Increasing of pH value resulted in ambiguous increase in the value of tested textural parameters. Both, temperature of heat treatment of WPs and pH value, have influence on appearance of obtained pectin-whey proteins gels. In the context of future research it is necessary to examine the combination of temperature and pH together in the range used in food industry.

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WPŁYW TEMPERATURY OBRÓBKI TERMICZNEJ ORAZ PH NA WŁAŚCIWOŚCI TEKSTURALNE ŻELI BIAŁKOWO-PEKTYNOWYCH

Streszczenie. Celem badań było określenie wpływu obróbki termicznej białek serwatkowych (w temperaturze 50–90°C) i pH (4,0–10,0) na właściwości teksturalne i wygląd żeli białkowo-pektynowych. Określono następujące parametry otrzymanych żeli: twardość, przylepność, wskaźnik sprężystości, gumowatość i wygląd ogólny. Stwierdzono, że wzrost temperatury obróbki termicznej białek serwatkowych prowadzi do wzrostu parametrów teksturalnych otrzymanych żeli. Największe wartości twardości, wskaźnika sprężystości i gumowatości zaobserwowano dla żeli z białek ogrzewanych w temperaturze 90°C. Największą lepkość wykazywały żele z białek serwatkowych poddanych ogrzewaniu w temperaturze 80°C. Żele o pH 5,5 i 6,0 wykazywały największą twardość, a żele o pH zasadowym (powyżej 8,0) największą przyczepność. Wykazano możliwość tworzenia żeli białkowo-pektynowych poprzez modyfikację temperatury obróbki cieplnej i kwasowości środowiska.

Słowa kluczowe: żel, białka serwatkowe, pektyny, pH, koniugaty