PAPER

EFFECTS OF PROBIOTIC FERMENTATION OF SELECTED MILK AND WHEY PROTEIN PREPARATIONS ON BIOACTIVE AND TECHNOLOGICAL PROPERTIES

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ABSTRACT

The objective of this study was to compare the effect of probiotic fermentation (conducted by *L. acidophilus* LA-5) of milk containing an addition of selected whey or milk protein preparations (whey protein concentrates, whole milk, whey protein isolate, casein glycomacropeptide or α-lactalbumin) on the technological and functional properties of obtained milk beverages. Determination of the antioxidative activities and identification sequences of biologically active peptides generated in selected protein preparations during probiotic fermentation also constituted the aim of the research. The results obtained indicate that the addition (1%) of α-lactalbumin into regenerated skimmed milk in the highest degree reduced the syneresis level in fermented products. Also, α-lactalbumin hydrolysates exhibited the strongest antioxidative properties (57.57±6.6%), while casein glycomacropeptide hydrolysates allowed us to obtain the highest amount of various biopeptides.

Keywords: biopeptides, lactic acid fermentation, probiotics

1. INTRODUCTION

The area of functional food that becomes a part of an everyday diet (NAGPAL *et al*., 2012) and the significance of many dairy products, especially fermented ones, is dynamically developing. Nowadays, it is observed that the consumers' awareness of the role of diet and beneficial health effects of some food compounds is on the increase, a fact also reflected in higher consumption of fermented milk products. Simultaneously, the requirements of and demand for various the health-promoting features of those kinds of products have been growing too. Therefore, to meet those expectations, scientists and the dairy industry are aiming at improving the technological and functional properties of dairy products as well as creating new ones.

Proteins are the most valuable constituent of whey protein isolates (WPI) and whey protein concentrates (WPC) that supply good nutritional quality and enhance the functionality of many product formulations (GUSTAW and MLEKO, 2007; JEEWANTHI *et al*., 2015). It is suggested that selected whey protein constituents (e.g.α-lactalbumin, lactoferrin, serum albumin lactoperoxidase, β-lactoglobulin, bovine) and peptides deriving from them exhibit, among others, an anticarcinogenic activity, may stimulate the immune system and influence metabolic processes (GOBBETTI *et al.,* 2002). Therefore, they constitute an important source of bioactive ingredients that might find an application, e.g. in the preparation of some of the functional food and therapeutic formulations (KABAŠINSKIENĖ *et al.*, 2015). Furthermore, apart from nutritional aspects, whey (as well as products deriving from it) is also a functional component that might influence some products' properties, including colour, flavour, and texture (LIUTKEVIČIUS *et al.*, 2016). Thus, incorporation of selected milk and whey protein preparation might not only enhance the technological properties of a product but might also positively influence the functional properties. This is largely associated with the fact that milk and whey proteins are precursors of biologically active peptide sequences (biopeptides), which exhibit a wide range of desired beneficial health effects affecting the regulation of the human body's system functions (MOHANTY *et al.*, 2016; LUCARINI, 2017). However, biopeptides enclosed within a native structure of milk and whey proteins are inactive, whereas enzymatic hydrolysis with digestive enzymes as well as fermentation process conducted by a starter culture with an efficient proteolytic enzyme system contributes to the release of various sequences of active peptides (KORHONEN and PIHLANTO, 2003; MICHAELIDOU, 2008). Moreover, bacteria involved in the fermentation processes not only contribute to the formation of bioactive substances that give food product a functional characteristic, but determined strains meeting certain requirements may also constitute a functional food component.

The microorganisms claimed as probiotics constitute another important functional food constituent that might be introduced to milk products. An activity of probiotics contributes to the modification of the microbiota composition in the host's intestines, which influences some of the physiological process leading to favourable health consequences.

The health-promoting effects caused by probiotics concern their desired influence on maintaining proper intestine micrflora (normalization of the bacteria composition, e.g. after antybioticoteraphy), hypocholesterolemic or anticarcinogenesis effects, inhibition growth of some pathogens, stimulating the immunity system and also alleviation of some of the allergy, including food allergies and lactose intolerance (SARKAR *et al.*, 2016).

The objective of this study was to compare the effect of probiotic fermentation (conducted by *L. acidophilus* LA-5) of milk containing the addition of chosen whey and milk protein preparations on technological and functional properties of obtained milk beverages. The study was also focused on evaluation of the antioxidative activities and determination of

the biologically active peptide sequences generated during probiotic fermentation of selected protein preparations.

2. MATERIAL AND METHODS

2.1. Microorganism and growth conditions

The probiotics strain *Lactobacillus acidophilus* LA-5 (Chr. Hansen, Poland) was stored at -80°C in Man- Rogosa- Sharpe broth (BTL, Łódź, Poland) containing 15% (v/v) glycerol stock. For further analysis, the strain culture was activated and routinely cultured (2%v/v of inoculum) in MRS broth and incubated at 37°C for 18h under aerobic conditions.

2.2. Fermentation of milk with the addition of protein preparations

The samples of 13% regenerated skim milk (OSM Krasnystaw, Poland) were enriched by the 1% (w/v) addition of one of the following protein preparations: whole milk powder (OSM Krasnystaw, Poland) - WMP; whey protein concentrates (Polsero, Poland) - WPC 30 and WPC40; whey protein isolate (Milei GmbH, Allgau, Germany) - WPI; Casein glycomacropeptide (Arla Food, Denmark) - CGMP and α-lactalbumin (Arla Food, Denmark) - α-la. Samples of milk without any protein addition (RSM) were used as a control variant.
All obtained sample variants were pasteurized (80°C/30min), then cooled down to ambient temperature and inoculated by 1% (v/v) of *Lactobacillus acidophilus* LA-5 cell suspension, which was previously prepared as follows: an overnight culture of analyzed probiotic strain (grown in MRS broth) was used to inoculate fresh medium (sterile MRS broth) to obtain an optical density at 600 nm (OD_{600}) of 0.05 (Helios Gamma; Thermo Fisher Scientific), subsequently the bacteria strain was cultivated at 37 °C until an OD_{600} of 0.7 (exponential phase). Afterwards, bacteria cells were harvested by centrifugation at 8000g for 10 min at 4 °C (MPW 350-R; MPW). The pellet was washed twice with sterile phosphate-buffered saline (PBS, pH 7.0) and resuspended (also in PBS) to obtain cell suspension of OD_{600} equal to 0.3 that was used for inoculation. Then, inoculated samples of all milk variants were transferred in equal volumes (40mL) into sterile packages and sealed. After the process of fermentation (42°C/12h, thermostatic method), all samples were cooled down up to 4°C and maintained in this temperature for another 12h before further analysis.

2.3. Texture profile analysis

To compare the textural properties of the fermented products received, the texture profile analysis (TPA) was performed, using a TA-XT2i (Stable Micro Systems, Godalming, UK), according to the procedure described by GUSTAW *et al.* (2016). The parameters hardness, fracturability springiness, cohesiveness, gumminess and chewiness were included in the analyses.

2.4. Spontaneous syneresis assay

The acidic milk gels (the fermented final products) obtained were analyzed also in terms of their ability for water binding. Therefore, the level of syneresis in received products was measured according to the method described by AMATAYAKUL *et al.* (2006).

2.5. Hydrolysis of milk and whey protein preparations

Aqueous solutions (1% w/v) of skim milk powder (SMP) and all above-mentioned protein preparations were pasteurized in a water bath (80 °C/30 min). After cooling down up to 35 °C, all variants of solutions were inoculated (1% v/v) by previously prepared inoculum (uninoculated samples were control variants) and incubated at 37 °C for 24 h. Subsequently, all samples were heated at 100 °C for 5 min in water bath to inhibit the hydrolysis process and inactivation of bacterial enzymes. Then, all samples were filtered (syringe filter Ø = 0.45 μm) and subjected to further analysis.

2.6. Antioxidant activity

An antioxidant activity in obtained filtrates (of fermented and nonfermented 1% (w/v) aqueous solutions of skim milk powder [SMP] and all analyzed protein preparations) were determined as an ability for free radical scavenging, using alcoholic solution of DPPH (1,1-Diphenyl-2-picrylhydrazyl, Sigma-Aldrich, Poland) in accordance with the method described by NAMDARI and NEJATI (2016). Briefly, the analyzed samples were diluted with phosphate buffer (0.1 M) in ratio 1:4. Afterwards, to 1 ml of each of the diluted samples 2.5 ml of 0.1 mM DPPH (in 60% methanol) was added and vigorously mixed. After 30 min of sample incubation (in darkness at ambient temperature), the absorbance was measured at λ=517 nm. The determination of the value of the analyzed activity for tested samples was carried out in a five-fold repetition (n=5). Antioxidant activity (radical-scavenging activity) was expressed as % inhibition of DPPH-absorbance that was calculated using the equation:

$$\text{Inhibition } [\%] = [(Ac-As)/Ac] \times 100$$

where, As - absorbance (λ=517 nm) of the test sample, and Ac - the absorbance (λ=517 nm) of the control sample consisting of 1 ml phosphate buffer mixed with 2.5 ml of DPPH (the methanolic solution of free radicals).

2.7. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) and peptide sequencing

To analyze the biopeptides content, those protein preparations characterized by the highest antioxidative activities and the ones that allowed obtaining the fermented products with the most desirable textural properties were selected.
The analysis was conducted through the previously described procedure (SKRZYPCZAK *et al.*, 2017) using Agilent Mass Hunter acquisition B.05.01 software to acquire data. The data analysis and peptide mapping were performed using the Agilent Mass Hunter qualitative analysis B.07 with integrated Bioconfirm add-on software.

2.8. Determination of the biological activities of peptide sequences

To determine the profiles of potential biological activities, the obtained peptide sequences were subjected to further analysis that was performed according to the procedure included in the databases BIOPEP-UWMP (MINKIEWICZ *et al.*, 2008; DZIUBA *et al.*, 2009; www.uwm.edu.pl/biochemia/index.php/pl/biopep) and BioPepDB (bis.zju.edu.cn/biopepdbr).

2.9. Statistical analysis

Statistical analysis was performed using the Statistica 8.0 (StatSoft, Poland) program. To evaluate the differences between means values, the data were subjected to analysis of variance (ANOVA) using Tukey's test with a level of significance set at $p<0.05$.
The similarities of the textural properties analyzed between obtained fermented final products were determined on the basis of the results of bottom-up hierarchical cluster analysis using average linkage clustering as a linkage criterion (UPGMA). To avoid the effect of the differences in measurement units between the parameters on the values of Euclidean distances, the data were standardized prior to the analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of addition of selected protein preparation on the textural properties and syneresis level of fermented products

The fermented milk products obtained with the addition of analyzed protein preparations exhibited some variety in terms of texture properties as well as level of spontaneous syneresis (Table 1).
Analyzing hardness parameter, there were no statistically significant differences ($p<0.05$) between control variant and products containing the addition of WPC30, WMP or α-la additions. The highest values of measured parameter were exhibited by samples with addition of CGMP, but simultaneously these fermented products were characterized by the most intensified syneresis effect (Table 1). This might be caused by the formation of a strong bond between generated products of hydrolyzed CGMP that influenced the moulding a strong structural network increasing the strength (hardness) of the gels and enhancing water disposal from the structure of curds.
Regarding the values of fracturability as well as cohesiveness (Table 1), there were no differences ($p<0.05$) between analyzed products. However, comparative analysis of all textural parameters indicated that, in terms of similar of texture profiles, the obtained variants of fermented products might be divided into three clusters consisting of pairs of fermented products' variants containing one of the tested protein preparations' addition (WMP with α-la, control variant and WPC30, WPI and CGMP) (Fig. 1).
Moreover, the results of cluster analysis indicated that in terms of values of analyzed texture profile parameters (springiness hardness, fracturability, cohesiveness, gumminess and chewiness), fermented beverages containing a WPI or CGMP additive were distinguished from all tested products and constituted a separate and farthest cluster that represents textural properties (Fig. 1).
The addition of whey protein concentrate (WPC 30 and WPC40) or CGMP to milk increased the intensity of syneresis in fermented products, while acidic milky gels containing the α-la or WMP addition were characterized by the highest water-binding capacity exhibiting the lowest syneresis effect (Table 1). The obtained results are in accordance with JOVANOVIĆ *et al*. (2005), who suggested that α-la influence the increase in the hydrophilic properties of the coaggregates at pH 4.5. Moreover, the study of MATUMOTO-PINTRO *et al*. (2011) indicates that a higher proportion of α -la in the yoghurt ingredients or partial hydrolysis of whey protein polymers inhibit the rate of sedimentation in the fermented product.

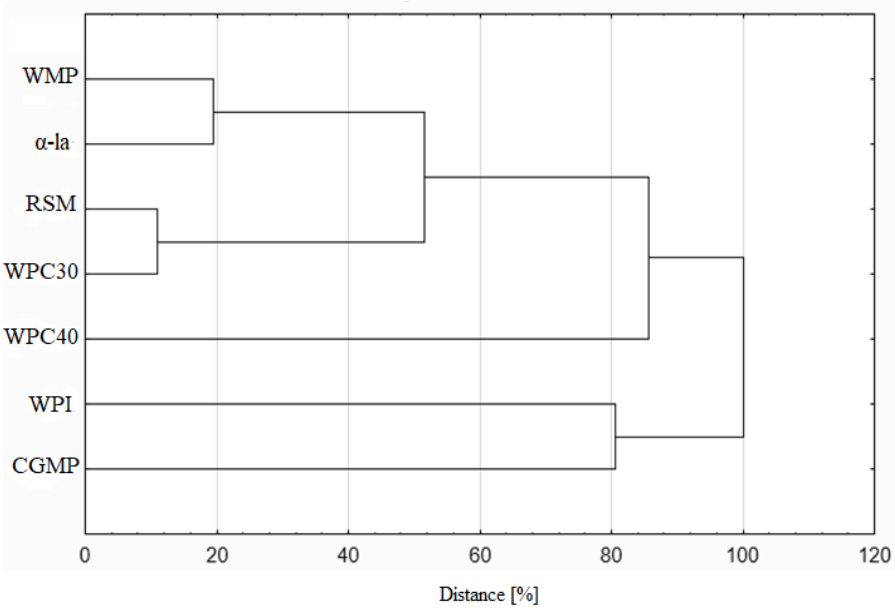

Figure 1. The result of UPGMA analyses.
Diagram expresses the similarity of texture characteristics between fermented products regarding all parameters measured in texture profile analysis; the similarity between particular groups of products was expressed as distance [%]; RSM is the control sample (fermented regenerated skim milk [13 %] without addition of any protein preparation).

It was claimed that the gelation properties of whey proteins are dependent on hydrolysis conditions and the degree of conducted enzymatic process (JEEWANTHI *et al.*, 2015). The process of gel formation is an effect accompanying the fermentation conducted by starter cultures. Therefore, the modification conditions of the hydrolysis process enable the creation of gels with various rheological properties (CREUSOT and GRUPPEN 2007; POULIOT *et al.*, 2009; JEEWANTHI *et al.*, 2015). Moreover, the results obtained might suggest that preferences of the probiotic strain to composition of fermenting medium (RSM samples containing selected protein preparations) and the specificity of bacterial enzymes toward selected milk and whey proteins might also influence the degree of hydrolysis and various characteristics of received gels (fermented products). It was implied that some of the generated peptide sequences might also initiate the aggregation process of whey protein hydrolyzates that influence the textural and rheological characteristic of fermented milk products. In WPI hydrolysates obtained using bacterial enzymes, CREUSOT and GRUPPEN (2007) identified such peptides and determined their sequences: β-Lg [f1-45], β-Lg AB[f(90-108)]-S-S-α-La [f(50-113)], α-La[f(12-49)]-S-S-α-La [f(50-113)], β-Lg AB[f(90-108)]-S-S-α-Lg AB[f(90-108)], β-Lg A[f(90-157)] and β-Lg AB[f(135-157/158)].
It was reported that in the production of yoghurt, the addition of WPI to milk enhances the formation of disulfide bonds during fermentation, which influences the increase in the mechanical resistance of received acidic gel (MATUMOTO-PINTRO *et al.*, 2011). However, in the presented study the results indicate that the 1% addition of WPI (compared with the control sample) has no significant effect on the improvement of textural property in terms of gel hardness.
Study results presented by DĄBROWSKA *et al.* (2017) revealed that the addition of whey protein hydrolysates (instead of other protein preparations like SMP or WPC80) to milk in yoghurt production might contribute to the increase in counts of starter culture bacteria at the initial stage of fermentation. Moreover, the addition of some whey protein

hydrolysates into milk improves the viability of probiotic bacteria in the final products (ZHAO *et al.*, 2006; DĄBROWSKA *et al.*, 2017). Also, the prebiotic-like properties of some whey proteins (including WPC) have been confirmed (GUSTAW *et al.*, 2016). This positive effect of selected milk and whey protein preparations, as well as their hydrolysates on the growth of the desired strains of probiotic bacteria, may strengthen the potential of the health-promoting properties of various fermented products.

3.2. Comparing the antioxidant activity of milk and whey protein preparations' hydrolyzates

The fermentation process conducted using the probiotic strain (*L. acidophilus* LA-5) influenced the increase in the ability of analyzed protein preparation solutions to free radical scavenging (Fig. 2).
Unfermented samples of SMP, WMP, WPC 30 and WPC40 exhibited a similar level of DPPH radical scavenging activity (differences, not statistically significant, $p > 0.05$). Moreover, it was noted that the hydrolysis process carried by tested bacteria improved the antioxidative properties of all analyzed protein preparations (Fig. 2).

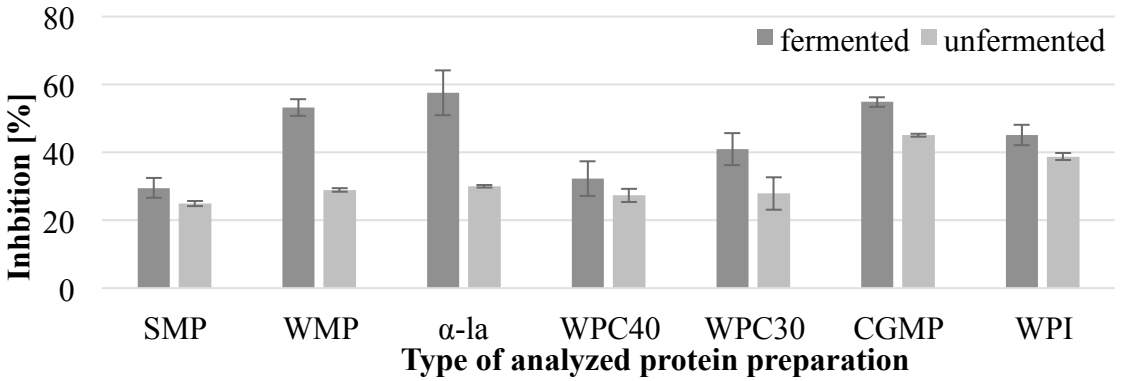

Figure 2. Antioxidant activity of analyzed protein preparations in DPPH assay.
Differences between mean values ($\bar{x}$; n=5) of antioxidant activity in obtained filtrates of tested samples (fermented and unfermented aqueous solutions (1% w/v) of skim milk powder [SMP] as control variant of samples and all analyzed protein preparations) denoted by different letters are statistically significant ($p<0.05$); error bars express the standard deviation (± SD).

The samples of α-la and CGMP fermented by probiotic strain exhibited the strongest hydrogen-donating capacity among analyzed solutions of protein preparations (57.57±6.6% and 54.87±1.3%, respectively). Differences in the value of antioxidative activity recorded for both types of samples were not statistically significant ($p > 0.05$), while the lowest values were noted for samples of SMP before (24.90±0.81%) as well as after hydrolysis (29.53±3.0%). These results are in accordance with RAHMAWATI and SUNTORNSUK (2016), who suggested that increased antioxidant activities in yoghurt (compared to raw milk material) might be associated with the released peptides (antioxidative) by bacterial proteolitic enzymes from protein molecules during the fermentation process. The peptides generated through protein hydrolysis as well as some products of the bacteria metabolism exhibit properties of electron donors and react with free radicals (DPPH) to achieve more stable molecule (KULLISAAR *et al.*, 2002).

3.3. Biological activities of peptide sequences identified in hydrolyzes of selected protein preparations

The analysis of identified biopeptides indicates that diversity of amino acid sequences as well as the quantity of the peptides in tested hydrolyzates depended on the protein matrix, namely the type of protein preparation (Table 2).
The results of liquid chromatography-high-resolution mass spectrometry (LC-HRMS)-and peptide sequencing revealed that most of the identified biopeptides sequences in analyzed hydrolyzates of protein preparations possess potential antihypertensive activities (Table 2) while in only one hydrolyzate (WPI) the presence of the sequence (RELEELNVPGEIVESLSSSEESITR) with potential of mineral-binding was confirmed. Furthermore, some of the biopeptide sequences possess more than one biologically active function, for instance, TTMPLW detected in hydrolyzate of CGMP or AYPS presented in hydrolyzed α-la (Table 2).
The analysis of peptide sequences revealed the presence of different biopeptides with antioxidant properties (IKH, IPNPIGSE, NEN, AYPS, LLR) in all analyzed hydrolyzate samples (Table 2). However, fermented CGMP was characterized by the largest number of different peptides with antioxidative properties. Moreover, within 21 of various biopeptides identified in this hydrolyzate samples, the potential of their biologically active properties also involved the following activities: antihypertensive, antithrombotic, antibacterial, immuno- and cytomodulatory peptide as well as the function of opioid and ACE inhibitor. Furthermore, the antioxidant peptide IPNPIGSE [αS1 -CN, f(182-189)], previously identified by GÚTIEZ *et al.* (2013) as a major peptide fragment in supernatants of *E. faecalis* strains grown in bovine skim milk has also been detected in CGMP hydrolized by the analyzed probiotic *L. acidophilus* strain. These results are of particular importance in the creation of functional food products.
It is claimed that peptide abilities to scavenge free radicals is connected with the presence of hydrophobic amino acid residues like: Met (M), Ile (I), Val (V), Leu (L), Phe (F), Trp (W), Ala (A), Tyr (Y) and Pro (P) (PENA- RAMOS *et al.*, 2004; CHEISON *et al.*, 2007; REN *et al.*, 2008). An example of such peptide sequence is AYPS that was identified in hydrolyzate of α-la. It was also proved that the presence of amino acids with aromatic residues enhances the ability for radical scavenging (RAJAPAKSE *et al.*, 2005).
The findings of the investigations indicate that the probiotic strain *L. acidophilus* LA-5 is also capable of degrading whey and milk proteins and generating peptide sequences exhibited (among others) the ACE-inhibitory activities (Table 2). Obtained results are in accordance with GÚTIEZ *et al.* (2013), who noticed that proline residue is present in most amino acid sequences of ACE-inhibitory peptides. It was also suggested that this residue is favourable for peptide binding to the active site of ACE. Moreover, GÚTIEZ *et al.* (2013) identified the peptide LHLPLP that is a competitive inhibitor of ACE exhibiting resistance toward gastrointestinal enzymes (QUIRÓS *et al.*, 2009). Interestingly, another sequence LPYPYY (identified as angiotensin-I-converting enzyme inhibitory peptide) identified in analyzed CGMP hydrolyzates was detected through ESI-MS/MS in samples of yak milk casein hydrolysates exhibiting a high level of ACE inhibition (83.16±1.37 %) (JIANG *et al.*, 2007). Moreover, HERNÁNDEZ-LEDESMA *et al.* (2007) described the β-lg-derived dipeptide, WY [f(19–20)] as a sequence with ACE-inhibitory bioactivity and radical-scavenging capacity. The same biopeptide was detected in the samples of WPI hydrolyzates obtained using *L. acidophilus* LA-5 (Table 2).

Table 1. Texture profiles and spontaneous syneresis levels exhibited by obtained fermented milk products.

Added protein preparation	Texture parameter						Syneresis [%]
	Hardness [N]	Fracturability [N]	Springiness	Cohesiveness	Gumminess	Chewiness	
Control*	0.153±2.59^{ab}	4.59±0.84^{a}	0.82±0.05^{c}	0.45±0.10^{a}	5.32±0.21^{ab}	4.70±0.44^{bc}	19.79±1.86^{ab}
CGMP	0.192±0.35^{a}	4.23±1.08^{a}	0.92±0.00^{ab}	0.44±0.09^{a}	7.69±0.45^{ab}	7.32±0.07^{a}	31.79±3.17^{a}
WPI	0.128±0.30^{b}	4.25±0.51^{a}	0.99±0.01^{a}	0.64±0.07^{a}	8.26±0.48^{a}	7.59±0.50^{a}	11.27±1.29^{bc}
WMP	0.133±1.02^{ab}	5.66±1.04^{a}	0.94±0.03^{ab}	0.55±0.05^{a}	6.43±0.44^{ab}	6.76±0.69^{ab}	7.33±0.08^{c}
α-la	0.136±2.37^{ab}	4.11±0.52^{a}	0.94±0.01^{ab}	0.51±0.02^{a}	6.95±1.00^{ab}	6.52±0.96^{ab}	6.95±0.66^{c}
WPC30	0.147±2.39^{ab}	3.94±0.40^{a}	0.89±0.01^{b}	0.50±0.07^{a}	5.03±0.86^{ab}	5.91±0.82^{abc}	26.07±3.8^{a}
WPC40	0.096±1.00^{b}	5.14±0.49^{a}	0.92±0.01^{abc}	0.49±0.06^{a}	3.97±0.77^{b}	3.73±0.37^{c}	27.63±6.03^{a}

*Control - samples consisting of 13% regenerated skim milk without any addition of protein preparation.
Differences between mean values (n = 8) ± standard deviation in the same column with the same letter designation are not statistically significant ($p < 0.05$).

Table 2. The sequences of biopeptides identified in analyzed hydrolysates obtained using *Lactobacillus acidophilus* LA-5.

Source of peptides (analyzed hydrolysate)	Identified peptide sequence	Mass (Da)	ID of the bioactive peptide in the data base	Activity/function reported in the data base
CGMP	LPYPYY	814.30	biopep00859/BioPepDB[b]	antihypertensive
CGMP	SPPEIN	655.30	biopep01254/BioPepDB	antihypertensive
CGMP WPI	ERF	450.22	biopep00189/BioPepDB	antihypertensive
CGMP	VRSP	457.26	biopep01460/BioPepDB	antihypertensive
α-la	SRY	424.21	biopep01260/BioPepDB	antihypertensive
CGMP α-la	RPKHPIKHQGLPQEVLNEN	2233.22	biopep01215/BioPepDB	antihypertensive
CGMP	LEQL	501.28	biopep00755/BioPepDB	antihypertensive
WPI	WY	367.15	biopep01540/BioPepDB	antihypertensive
WPI	GKEKV	559.33	biopep00360/BioPepDB	antihypertensive
WPI	VAPFPEVFGKE	1218.63	biopep01336/BioPepDB	antihypertensive
WPI	FVAPFPEV	904.47	biopep00301/BioPepDB	antihypertensive
CGMP	RPK	399.26	biopep01206/BioPepDB	antihypertensive
WPI	VLNENL	700.37	biopep01413/BioPepDB	antihypertensive
WPI	VAPFPEVFGKE	1218.63	biopep01336/BioPepDB	antihypertensive
WPI	LQPEVMGVSK	1086.57	biopep00867/BioPepDB	antihypertensive
WPI	LVYPFPGPI	1001.56	biopep00927/BioPepDB	antihypertensive

CGMP	HKEMPFPKYPVEPF	1744.86	biopep00457/BioPepDB	antihypertensive
CGMP WPI	SQSKVLPVPQ	1081.61	biopep01258/BioPepDB	antihypertensive
WPI	LQSW	532.26	biopep00875/BioPepDB	antihypertensive
CGMP	TQSLVYP	806.42	biopep01306/BioPepDB	antihypertensive
WPI	TEDELQDKIHP	1323.62	biopep01279/BioPepDB	antihypertensive
CGMP WPI	MAIPPKK	783.46	biopep00946/BioPepDB 3294/BIOPEP-UWM[c]	antihypertensive, antithrombotic
CGMP	IPNPIGSE	825.42	biopep00561/BioPepDB	antioxidative
WPI	IKH	396.25	biopep00532/BioPepDB	antioxidative
CGMP	NEN	375.32	9363/BIOPEP-UWM	antioxidative
α-la	AYPS	436.20	8472/BIOPEP-UWM 8380/BIOPEP-UWM	antioxidative, ACE inhibitor
CGMP WPI	LLR	400.28	8484/BIOPEP-UWM biopep00827/BioPepDB	antioxidative, antihypertensive
CGMP	LKTVYQHQKAMKPWIQPKTKVIPYVRYL	3455.96	8337/BIOPEP-UWM	antibacterial
WPI	RELEELNVPGEIVESLSSSEESITR	2801.39	biopep04772/BioPepDB	mineral-binding
CGMP α-la	YQEPVLGPVRGPFPIIV	1880.06	biopep04801/BioPepDB biopep04091/BioPepDB biopep01621/BioPepDB	immuno- and cyto-modulatory peptides antimicrobial antihypertensive
CGMP	NLHLPLP	802.47	2669/BIOPEP-UWM, biopep01010 /BioPepDB	ACE inhibitor antihypertensive
CGMP WPI	VTSTAV	576.30	7481/BIOPEP-UWM biopep01475/BioPepDB	ACE inhibitor, antihypertensive
α-la	LLYQEPVLGPVRGPFPIIV	2106.22	8174/BIOPEP-UWM	immunomodulating
CGMP	TTMPLW	747.30	3530/BIOPEP-UWM, 3127/BIOPEP-UWM, 8172/BIOPEP-UWM, biopep0479 6/ BioPepDB, biopep01313/BioPepDB	ACE inhibitor, opioid, immunomodulating immuno- and cytomodulatory peptide, antihypertensive
CGMP α-la	MAIPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVATL EDSPEVIESPPEINTVQVTSTAV	6703.37	biopep03480/BioPepDB	antibacterial
α-la	YYQQKP	825.40	8383/BIOPEP-UWM	ACE inhibitor
CGMP WPI	VQVTSTAV	803.40	biopep01445/BioPepDB, 8264/BIOPEP-UWM	antihypertensive, antibacterial

[b]data base: bis.zju.edu.cn/biopepdbr/

[c]data base: www.uwm.edu.pl/biochemia/index.php/pl/biopep

Hydrolysis of CGMP conducted with the application of the probiotic *L. acidophilus* strain allowed to receive the sequence TTMPLW exhibited multi-directional biological activities (opioid, ACE inhibitor, antihypertensive, immuno- and cytomodulatory peptide) (Table 2). The results of analysis of ACE-inhibitory and antihypertensive activity in spontaneously hypertensive rats of biopeptides (generated through tripsine hydrolysis of milk proteins) claimed that this sequence was effective in decreasing the level of systolic blood pressure (KARAKI *et al.*, 1990). The concentration of peptide (TTMPLW) needed to inhibit 50% ACE activity (IC50) achieved the value of 16 μM, whereas for sequences LQSW [β-CN, f(155−158)], YQEPVLGPVRGPFPIIV [β-CN, f(208−224)] and MAIPPKK [κ-CN f(106-112)], the IC50 values were 500, 101 and 4785 μM, respectively (KARAKI *et al.*, 1990; MAENO *et al.*, 1996; MIGUEL *et al.*, 2007; HERNÁNDEZ-LEDESMA *et al.*, 2011). All the sequences mentioned above were also identified in whey and milk protein preparations hydrolyzed by *L. acidophilus* LA-5 (LQSW in samples of hydrolyzed WPI; MAIPPKK in CGMP and WPI hydrolyzates; YQEPVLGPVRGPFPIIV in CGMP and α-la hydrolyzates).
The presented results of the research have practical relevance because health-promoting properties provided in food by biopeptides may find an application in personalized nutrition as well as individual dietary practices (KORHONEN and PIHLANTO, 2006). Biologically active peptides are important constituents of food and allow the design of novel foods, including functional food products, supplements, nutraceuticals or even pharmaceuticals (MEISEL, 2005; LIUTKEVIČIUS *et al.*, 2016; LUCARINI *et al.*, 2017).

4. CONCLUSIONS AND FUTURE WORK

The obtained study findings demonstrate that analyzed protein preparations are an important source of dietary antioxidants. Furthermore, due to their influence on fermented product, texture properties may find a wider application in the formulation of new dairy products, especially functional food.
The use of 1% α-la additive into regenerated skimmed milk is conducive to the reduction of the level of syneresis, with 1% addition of CGMP leaded to obtain the strong, hard gels in fermented milk products, but with a weaker capacity to water-binding. In addition, the hydrolysis process of protein preparations carried out by the probiotic strain improved their antioxidative properties. Furthermore, using the *L. acidophilus* LA-5 to hydrolysis of CGMP and α-la seems to be an effective method of obtaining products with high antioxidant properties as well as some various biopeptide sequences. Among all analyzed hydrolysates, the sequences with potential antihypertensive activity were the most numerous biopeptides, whereby fermented (by the probiotic *L. acidophilus* strain) CGMP allowed acquiring the largest amount of different biologically active peptides. However, despite the promising research results, undoubtedly, further investigations are necessary to verify *in vivo* the biological activity of biopeptide sequences identified in presented study. Also, further analyses might yield new biologically active substances or contribute to a more efficient use of *L. acidophilus* LA-5 in the production of functional foods, nutraceuticals or pharmaceuticals.

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