www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 66.941:637.344]:664.684
DOI: 10.5937/ffr0-53501

Original research paper

IMPACT OF WHEY BIOACTIVE HYDROLYSATES ON THE QUALITY OF FAT FILLINGS FOR CONFECTIONERY PRODUCTS

Milica V. Stožinić[*1], Danica B. Zarić[2], Marica B. Rakin[3], Ivana S. Lončarević[1], Biljana S. Pajin[1], Maja Lj. Bulatović[3]

[1] University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia
[2] University of Belgrade, Innovation Centre of the Faculty of Technology and Metallurgy, 11000 Belgrade, Karnegijeva 4, Serbia
[3]University of Belgrade, Faculty of Technology and Metallurgy, 11000 Belgrade, Karnegijeva 4, Serbia

Abstract: Whey protein hydrolysates can be used in a wide range of applications because they offer numerous advantages compared to non-hydrolysed whey proteins. They are more heat stable, with improved foaming and emulsifying properties due to the presence of bioactive peptides of lower viscosity. Whey hydrolysates have improved absorption, digestibility, excellent nutritional and functional properties, and the ability to extend the shelf life of food products. Due to the large differences in the technological and other physicochemical properties of hydrolysates, the addition of whey protein hydrolysates into confectionery products is much more complicated. This research aims to determine the possibilities of enriching filled confectionery products with whey peptides obtained in two ways: through enzymatic hydrolysis of whey protein concentrate and fermentation (using microorganism *Lb. rhamnosus* ATCC 7469). Peptides were added to a fatty milk cream at a 5% concentration. The study was focused on assessing antioxidant activity, physical, rheological, textural, and sensory properties of three fat fillings: C (control without whey peptides), EWP (5% peptides from enzymatic hydrolysis), and MWP (5% peptides from whey fermentation). The Enzymatic hydrolysates increased DPPH radical inhibition by 32%, and fermented hydrolysates by 19%. Enzymatic hydrolysates also demonstrated superior inhibition of lipid peroxidation (IC50 value of 811.54 mg mL-1) compared to fermented hydrolysates (IC50 value of 178.36 mg mL-1). EWP showed the highest antioxidant activity. The addition of enzymatic hydrolysates increased filling firmness by 2.5 times, while fermented hydrolysates had reduced firmness compared to the control. Both types of hydrolysates did not adversely affect the size or distribution of the particles in the fat cream. Thixotropic properties of the fat filling remained unchanged post-incorporation. MWP exhibited the most optimal rheological characteristics with the lowest yield stress. The best sensory characteristics (better than the control sample) were found in the EWP.

Key words: *enzymatic hydrolysis, microbial hydrolysis, rheology, whey protein, antioxidant activity*

INTRODUCTION

Whey protein, characterized by its high nutritional value (rich in branched-chain amino acids (BCAA) and glutathione), and excellent technological properties, is widely used in the

Corresponding author: Phone: +381214853788 Fax: +38121450413
E-mail address: milica.stozinic@uns.ac.rs

food industry. Recently, bioactive peptides derived from whey protein have been employed to develop functional food products (Saubenova et al., 2024). These peptides are generated through enzymatic hydrolysis of whey protein concentrate or isolates, utilizing proteolytic enzymes or the action of plant or microbial proteases (Korhonen & Pihlanto, 2006). The resulting hydrolysates are rich in dipeptides, tripeptides, and oligopeptides, which have been documented to offer various health benefits (anti-inflammatory, antioxidant, antimicrobial, antihypertensive, and antithrombotic activity).

Enzymatic hydrolysis is a controlled process that utilizes enzymes to break down proteins into smaller peptides and amino acids through enzymatic cleavage. This process occurs at moderate temperatures and pressure, where the enzyme activity is optimized. The gentle conditions result in better yields compared to chemical processes (Krunic, Rakin, Bulatovic & Zaric, 2018). The most commonly used enzymes for enzymatic hydrolysis are trypsin and chymotrypsin, which are naturally present in the digestive tract. In addition to enzymatic hydrolysis, microbial fermentation is also employed to produce high-quality bioactive peptides. *Lactobacillus rhamnosus* ATCC 7469 is a commonly used microorganism for this purpose (Embiriekah, Bulatović, Borić, Zarić & Rakin, 2017). The process typically concludes with the inactivation of the enzyme through thermal or pH manipulation, once a desired level of hydrolysis has been achieved.

The incorporation of whey protein hydrolysates into bakery products, snack products, and chocolate, can significantly enhance the composition and quality of confectionery products (Wronkowska et al., 2015). In this study, whey protein hydrolysates were first produced and then 5% of them were incorporated into fat fillings. These fillings consist of whole and skimmed milk, sugar, vegetable fat, lecithin as an emulsifier, and milk flavour. Confectionary fillings typically contain a significant amount of fat, ranging from 30 to 40%. Fat plays a crucial role in determining the physical properties of fillings and is a key factor in the sensory quality of the final products (Lidefelt, 2002). Fat, as the primary carrier of flavour and aroma, also influences the texture, consistency, and melting behavior of the fillings. Furthermore, it affects the rate of flavour release and can impact the overall mouthfeel. The physical properties of fat-based fillings can vary significantly, ranging from semi-solid to more spreadable and plastic, depending on their specific application. The compatibility and adhesion between the filling and shell are also dependent on the optimal choice of fat, which helps to prevent fat blooming and undesirable softening (Pedersen, 2001). Apart from the physical characteristics, the sensory attributes of fat fillings are also important. Whey protein hydrolysates can be bitter. These hydrolysates are composed of a large number of low molecular weight peptides containing predominantly hydro-phobic amino acids (arginine, leucine, glycine, phenyllanine, proline) that were identified as the predominant contributors to the bitter taste (Alim, Song, Raza & Hua, 2020). Also, bioactive whey peptides can slow down oxidative processes and food spoilage, which is very important when they are incorporated into fatty fillings with a high percentage of fat (more than 30%).

In this study we produced and then used whey hydrolysates obtained in two different ways: through enzymatic hydrolysis of whey protein concentrate (using trypsin) and by fermentation (using microorganism *Lb. rhamnosus* ATCC 7469). We added these hydrolysates to two identical confectionery fat fillings, one hydrolysate in each, at a concentration of 5% and compared the antioxidant and physical characteristics of enriched fat fillings.

MATERIALS AND METHODS

Production of whey protein hydrolysate

Whey

The whey powder (DMV International, Nederland), with a protein content of 12.1% (w/w) was used in this study. An 8% (w/w) aqueous solution of whey powder was used as a substrate for fermentation, which was mixed after preparation for 1 hour by a magnetic stirrer at room temperature for better hydration. After hydration, the suspension was pasteurized for 60 minutes at 60 °C in a water bath, and then cooled to the fermentation temperature.

Whey fermentation

The prepared whey was inoculated with the appropriate amount of inoculum (2%, w/v) of the applied microorganism *Lb. rhamnosus* ATCC

7469. Subsequently, the samples were placed in a water bath and incubated at 37 ºC for 24 hours. The fermentation process was halted by rapidly cooling the samples after incubation.

Whey protein concentrate

A whey protein concentrate with a high concentration of 80% (w/w) was used in the study. The concentrate, in powder form, was obtained from DMV International, Netherlands (WPC 80). As a substrate for hydrolysis, a 5% (w/v) solution of WPC 80 in water was prepared, which, after dissolution, was mixed for 1 hour by a magnetic stirrer at room temperature for better hydration. After hydration, the pH level of the suspension was tuned to 8 and the suspension was heated to the hydrolysis temperature in a water bath.

Enzymatic hydrolysis of whey protein concentrate

The conditions for the enzymatic hydrolysis were as follows: the used enzyme was Trypsin (EC 3.4.21.4, Sigma Aldrich, St. Louis, MO, USA), temperature of 37 °C and a pH of 8.0, at an enzyme/substrate ratio of 0.5% for 4 hours. During the hydrolysis process, the pH value of the samples was maintained constant (pH 8.0) by adding 1M NaOH. After the hydrolysis, the samples were boiled for 15 minutes at 90 °C to inactivate the enzyme and stop the hydrolysis.

Spray drying of whey protein hydrolysate

After the end of the fermentation or enzymatic hydrolysis process, the obtained hydrolysates were centrifuged at 6000 rpm for 15 minutes. The permeate was then dried by a spray drying process using a laboratory Mini spray dryer B-290 (BUCHI, Labortechnik AG, Switzerland) for the production of concentrated whey protein hydrolysate powder. The inlet temperature is 160 ˚C, and the outlet temperature is 75 ˚C.

Preparation of fat filling samples

Confectionery fat filling is manufactured at the Atlantic Stark factory in Belgrade using a Bühler ball mill. The formulation comprises a combination of sugar, skimmed milk powder, whole milk powder, vegetable fat, lecithin, and milk flavouring. The chemical composition is as follows: total carbohydrates 43.99 g, sucrose 24.19 g, lactose 19.8 g, total fat 37.18 g, milk fat 3.6 g, and proteins 13.91 g. Standard AOAC methods (AOAC, 2000) were used for the determination of the chemical profile of the samples. The produced whey protein hydrolysate powders were subsequently incorporated into the fat filling at a concentration of 5.0% (w/w) in laboratory conditions. The resulting samples were then homogenized at a rotational speed of 15000 rpm to achieve a uniform raw material base using high speed stand mixer (Bosch, MSM6M8X1, Milton Keynes, United Kingdom).

The final products were packaged and labelled as follows: C - fat filling without hydrolysate; EWP - fat filling with 5% peptides from enzymatic hydrolysis; MWP - fat filling with 5% peptides from whey fermentation. The samples were stored at a temperature of 20 ± 2 °C.

The final products were packaged and labelled as follows: C - fat filling without hydrolysate; EWP - fat filling with 5% peptides from enzymatic hydrolysis; MWP - fat filling with 5% peptides from whey fermentation. The samples were stored at a temperature of 20 ± 2 °C.

Determination of antioxidant activity

The DPPH scavenging activity was evaluated according to the method obtained by McCue and Shetty (2005) with slightly modifications. The samples were diluted at five different concentrations (50, 100, 150, 200 and 250 mg/mL). Then, 250 µL of each dilution was mixed with 4 mL of 0.1 mM DPPH radical dissolved in ethanol. After vortexing, the mixtures were left in the dark for 60 minutes at room temperature to incubate. Following incubation, the samples were centrifuged for 15 minutes at 6000 rpm at room temperature, and the resulting supernatants were filtered.

The control sample contained deionized water instead of the sample. The absorbance of the supernatant and the control was determined by measuring the absorbance at 517 nm, with ethanol as a blank. The DPPH scavenging activity was expressed as a percentage of inhibition by using the following formula: where: Ac is the absorbance of the control sample, and As is the absorbance of the sample with hydrolysate. The antioxidant activity was expressed as half maximal inhibition concentration (IC50, mg mL-1) of the fat filling needed to scavenge of the DPPH free radicals.

$$\text{DPPH radical scavenging activity (\%)} = [(Ac-As)/Ac] \times 100$$

where: Ac is the absorbance of the control sample, and As is the absorbance of the sample with hydrolysate. The antioxidant activity was expressed as half maximal inhibition concentration (IC50, mg mL-1) of the fat filling needed to scavenge of the DPPH free radicals.

Particle size distribution and rheological characteristics in fat filling samples

Particle size distribution (PSD) was measured using the Mastersizer 2000 (Malvern Instruments, England), a laser diffraction particle size analyser. Confectionery fat filling samples were dispersed in sunflower oil at ambient temperature (20 ± 2 °C) and results were quantified as volume-based particle size distribution using a Mastersizer 2000 Software. The results were presented as the volume-based PSD and described by PSD parameters: volume mean diameter D[4,3] and parameters d(0,1), d(0,5), d(0,9) that represent the particle sizes where 10, 50 or 90% of the total particle volume include particles that are smaller than that size.

Rheological properties were determined using the RheoStress (600 HP, Haake, Germany) rotation viscometer, according to the IOCCC (2000) method. Measurements were taken at 40 ± 0.1°C using a concentric cylinder system (sensor Z20 DIN). Flow curves were obtained by generating a hysteresis loop over a range of shear rates from 1 to 60 s^{-1}. The shear rate was raised from 1 to 60 s^{-1} over a period of 240 seconds, held at 60 s^{-1} for 60 seconds, and then reduced from 60 to 1 s^{-1} over a period of 240 seconds.

Texture of fat filling samples

Textural properties of fat fillings, including hardness, were determined at ambient temperature (20 ± 2 °C) using the Texture Analyser TA.XT Plus (Stable Micro System, UK) according to method Chocolate Spread – SPRD2_SR_PRJ. The maximum force was expressed in grams (g) (Lončarević et al., 2017).

Determination of sensory quality of fat filling samples

The investigation was initiated 24 hours after sample preparation according to the guidelines of the ISO 8586:2012 standards (ISO 8586, 2012). All samples were maintained at ambient temperature (20 °C ± 1 °C) and presented in plastic containers labelled with three-digit numbers. Between each sample, the oral cavity was rinsed with water and pieces of bread to minimize any residual effects. A panel of ten trained assessors evaluated each sample using a 5-point rating scale. The following attributes were examined: external appearance (1-gray and damaged surface, separation of the fatty phase, 5-smooth, shiny surface, characteristic colour) structure (1-inhomogeneous structure, poor lubricity, inadequate strength, 5-homogeneous structure, adequate strength, soft, lubricating consistency), chewability (1-slow solubility, sandiness, stickiness, 5 -unique, solubility in the mouth), flavour (1-extremely bad, foreign, 5-extremely good, characteristic), taste (1-extremely bad, foreign, 5 - extremely good, peculiar). All evaluators participated in the sensory analysis procedure voluntarily, without any coercion and without revealing their identity. Conducted experiments comply with the Helsinki Declaration of 1975, which was revised in 2013.

Statistical analysis

Triplicate runs were carried out for each experiment and the data were subjected to statistical analysis. Statistical analysis was done using One-way ANOVA. The Tukey post hoc test was performed for means comparison using OriginPro 8 (Origin Lab Co., Northampton, USA). Statistical significance was determined at a p-value of less than 0.05.

RESULTS AND DISCUSSION

Antioxidative activity of fat filling samples

The antioxidant activity of confectionery fat filling was evaluated by assessing the ability of the sample to inhibit 50% of DPPH radical activity, as expressed by the IC_{50} value (mg mL^{-1}). The results, presented in Fig. 1, indicate that all samples of fat filling enriched with bioactive hydrolysate powder exhibited significantly ($p < 0.05$) enhanced antioxidant activity compared to the control fat filling sample (C). Sample with the strongest DPPH scavenging activity was the sample containing 5% EWP, with an IC_{50} value of 116.52 mg mL^{-1}.

This sample demonstrated a 32% increase in antioxidant potency compared to the control sample (171.37 mg mL^{-1}). The fat filling sample enhanced with 5% bioactive hydrolysate powder produced by fermentation of whey proteins (MWP) exhibited an IC_{50} value of 137.78 mg mL^{-1} and displayed a 20% im-

provement in antioxidant activity relative to the control fat filling sample.

Particle size distribution and rheological properties in confectionery fat filling

It is crucial to properly grind the ingredients to achieve a consistent particle size distribution in the fat filling, as this will directly impact the overall quality of the end product (Lončarević et al., 2017). The impact of bioactive whey protein hydrolysate powders on particle size distribution in produced samples of confectionery fat filling is presented in Table 1. The results (Table 1) show that 50% of the analysed fat fillings contain particles smaller than 16.48 μm. The control sample without added hydrolysate powder had the highest value in this regard. The MWP sample had the smallest d(0.1) and d(0.5) parameters at 3.46 μm and 14.75 μm, respectively, resulting in a specific surface of 0.75 m^2g^{-1}, the highest among the samples.

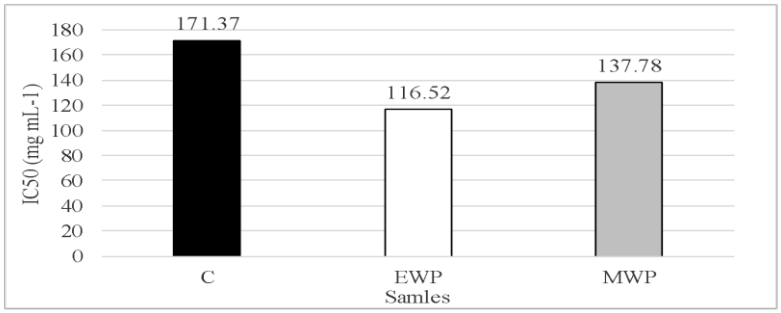

Figure 1. DPPH radical scavenging activity of fat filling samples

Table 1.
The influence of whey protein hydrolysate on the rheological characteristics and particle size distribution of confectionary fat filling

Sample	Parameters of particle size distribution				
	d (0.1)	d (0.5)	d (0.9)	D [4,3]	Specific surface (m^2g^{-1})
C	3.91 ± 0.02[a]	16.48 ± 0.02[a]	46.97 ± 0.32[a]	21.68 ± 0.41[a]	0.67 ± 0.01[a]
EWP	3.92 ± 0.04[a]	15.15± 0.02[b]	44.93 ± 0.27[a]	20.40 ± 0.15[b]	0.68 ± 0.02[a]
MWP	3.46 ± 0.04[b]	14.75 ± 0.03[c]	53.12 ± 0.10[b]	22.73 ± 0.42[c]	0.75 ± 0.01[a]
Sample	**Rheological parameters (40°C)**				
	Yield Stress –Casson (Pa)		**Viscosity - Casson (Pa·s)**		**Thixotropic curve area ($Pa·s^{-1}$)**
C	5.010 ± 0.09[a]		3.307 ± 0.02[a]		1210 ± 0.68[a]
EWP	5.461 ± 0.06[b]		6.066 ± 0.12[b]		2455 ± 0.89[b]
MWP	3.441 ± 0.02[c]		4.952 ± 0.15[c]		1628 ± 0.76[c]

Values followed by the same letter within the same column are not significantly different (p>0.05) according to Tukey post-hoc test

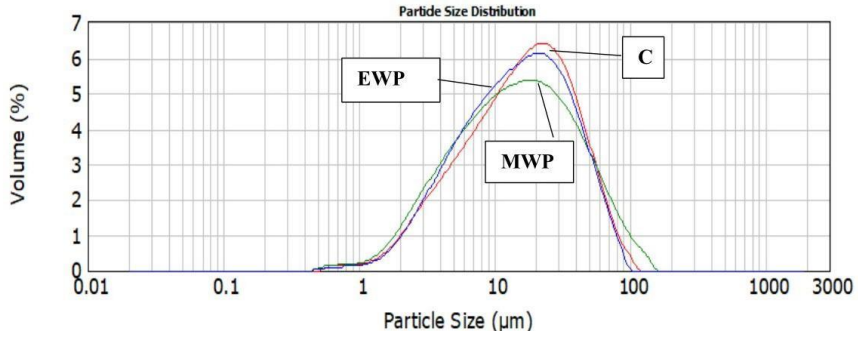

Figure 2. The influence of whey protein hydrolysate on the particle size distribution of confectionary fat filling (Legend: C- fat filling without hydrolysate; EWP- fat filling with 5% peptides from enzymatic hydrolysis; MWP- fat filling with 5% peptides from whey fermentation)

The average volume particle diameter D [4,3] and the parameter d (0.9) are notably reduced in the fat filling with the addition of hydrolysate powder produced through enzymatic whey hydrolysis (EWP).

This is associated with alterations in rheological properties and hardness. In Fig. 2, it is evident that the control sample (C), lacking added peptides, exhibits the narrowest span, indicating the simplest structure.

The confectionery fat filling consists of a solid phase (comprised of sucrose, cocoa powder, and powdered milk particles) dispersed in the fat phase (Pajin et al., 2012). Table 1 illustrates the effects of varying concentrations of bioactive whey protein hydrolysate powders on the rheological properties of the confectionery fat filling. The control sample (C) demonstrates good homogeneity, as evidenced by the surface value of the thixotropic loop (1210 $Pa \cdot s^{-1}$) and the Casson viscosity value (3.307 Pa·s) (Table 1). All rheological parameters of the three samples are statistically different ($p<0.05$). The differences in rheological attributes of samples are due to the existence of different bioactive hydrolysate powders, which represent an additional solid phase in the composition of confectionary fat filling (Zarić et al., 2016). The inclusion of hydrolysate powder impacts the arrangement of particles. It is noteworthy that the introduction of peptides primarily influences Casson viscosity rather than Casson yield, and the rise in viscosity can be mitigated by choosing a more suitable emulsifier. In comparison to the control sample, the MWP sample exhibits a 31.3% lower Casson yield, a 49.7% higher Casson viscosity, and a 34.5% higher thixotropic loop. In the confectionary fat filling, the EWP sample exhibits the highest values for Casson's viscosity (6.066 Pa·s), Casson's yield stress (5.461 Pa), and the surface area of the thixotropic loop (2455 $Pa \cdot s^{-1}$). This is attributed to the parameters of particle size distribution, specifically the mean volume particle diameter D [4.3], and the parameter d(0.9), being the lowest compared to other samples (Afoakwa, Paterson & Fowler, 2008; Afoakwa, Paterson, Fowler & Vieira, 2009).

The hardness of confectionery fat filling

The firmness of the confectionery fat filling is primarily determined by the type and quantity of fat, along with the emulsifier used. In addition to the fat content, the size distribution of particles also plays a significant role. The sample with the smallest particles is expected to have the highest firmness, as the distance between the particles is minimal and the attractive intermolecular forces are at their strongest. The hardness results are shown in Fig. 3. All three samples have the same amount and type of fat as the emulsifier, so the difference in hardness is solely due to the particle size.

The hardness of confectionery fat filling increases by 2.57 times when enzymatic hydrolysates are added, whereas the addition of hydrolysates obtained through whey protein fermentation decreases its hardness compared to the control sample. The EWH sample has the smallest particles (parameters d(0.9) and D [4,3]), resulting in the highest hardness (Do, Hargreaves, Wolf, Hort & Mitchell, 2009).

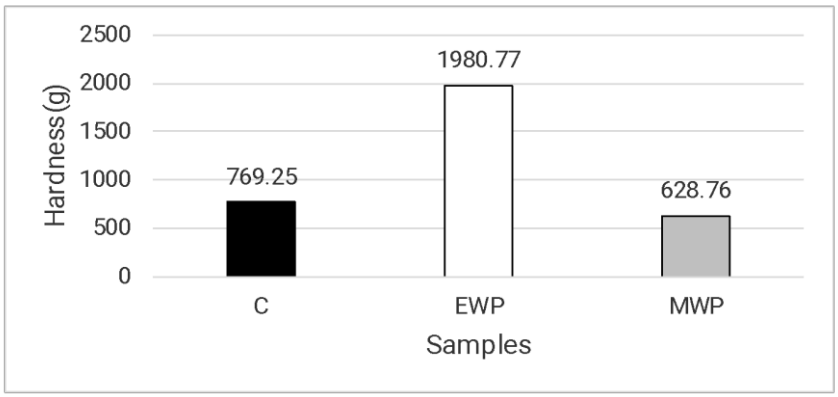

Figure 3. The influence of whey protein hydrolysate on the particle size distribution of confectionary fat filling

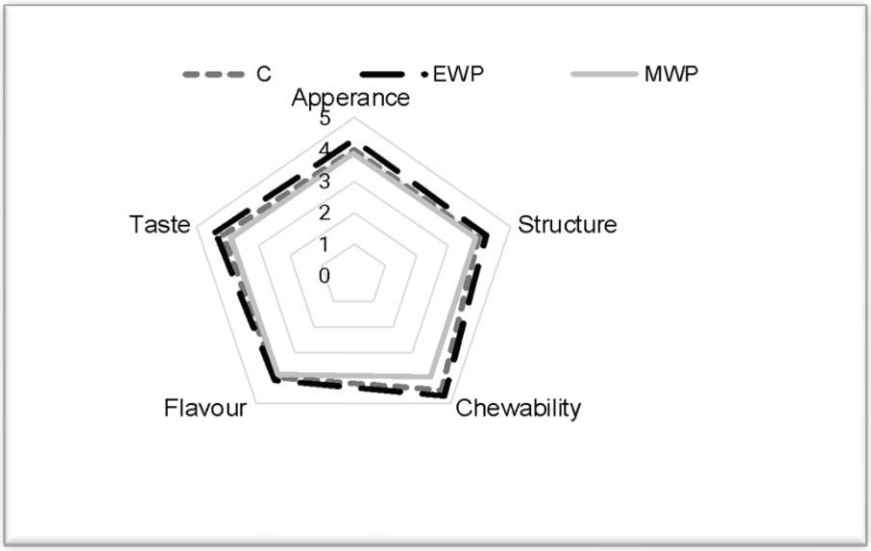

Figure 4. The influence of whey protein hydrolysate on the sensory characteristics of confectionary fat filling (Legend: C- fat filling without hydrolysate; EWP- fat filling with 5% peptides from enzymatic hydrolysis; MWP- fat filling with 5% peptides from whey fermentation)

The addition of fermented hydrolysates decreases the hardness of the confectionery fat filling by 18% compared to the control sample without hydrolysates. These findings align with the results of particle size distribution in the samples.

We cannot say that any confectionery fat filling is good or bad from the point of view of hardness, because it depends on the application. Both sample EWP and MWP can be used in filled chocolates, while when filling biscuits, a better choice is fat filling with a higher strength, which in our case is sample EWP.

Sensory properties of confectionery fat filling

After analysing the data presented in Fig. 4, it is clear that incorporating whey protein hydrolysate did not impact the texture and taste of the products. This is evident from the close similarity between the EWP and MWR samples and the control sample. Therefore, adding bioactive whey protein hydrolysate powder to confectionery fat filling does not alter the sensory characteristics of the fat filling.

The strong resemblance between the EWP, MWR samples and the control sample is apparent. It's worth noting that the EWP sample displayed superior texture, chewiness, taste, and appearance compared to the other samples, including the control sample (C). All ten tasters-technologists noted the significant firmness of the EWP sample, which resulted in improved chewability, and this is where the most significant rating variances are observed. Apart from the EWP sample, the control sample is better than the MWP in the most important sensory characteristic - chewability. The same marks are also in terms of taste.

CONCLUSIONS

The study investigated the effects of introducing whey protein hydrolysates (enzymatic hydrolysis and fermentation) into the formulation of confectionery fat filling samples. The samples were supplemented with 5% peptides from enzymatic hydrolysis (EWP) and 5% peptides from whey fermentation (MWP).

The results showed that:

- EWP increased the inhibition of DPPH radicals by 32%, the hardness by 2.57 times, and slightly reduced the particle size distribution parameters in contrast to the control sample. However, the viscosity was increased but could be adjusted by selecting other emulsifiers. The sensory characteristics of this sample were better than the control one. EWP is suitable for filling biscuit products.
- MWP increased the inhibition of DPPH radicals by 20%, slightly increased the particle size distribution parameters (d (0.9)

and D [4,3]) compared to the control sample, which resulted in optimal rheological parameters. Due to the sample's reduced hardness compared to the control, MWP is suitable for filling both biscuits and chocolate products.

- In summary, the enrichment of confectionery fat-filling samples with whey protein hydrolysates can improve their physical properties, antioxidative activity, and sensory characteristics. Additionally, enhancement of fat fillings with whey protein hydrolysates improves the nutritional values of these products. The choice of certain whey protein hydrolysate and its concentration depends on the kind of specific application and required properties of confectionery fat filling.

AUTHOR CONTRIBUTIONS

Conceptualization, B.M; Methodology, R.M. and Z.D.; Production of hydrolysates B.M.; Production fat filling samples L.I. and S.M.; Validation, writing-original draft preparation, S.M. and Z.D.; Writing-review and editing S.M.; Supervision, P.B. and R.M.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No.451-03-66/2024-03/200287).

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Afoakwa, E.O., Paterson, A., & Fowler, M. (2008). Relationship between rheological, textural and melting properties of dark chocolate as influenced by particle size distribution and composition. *European Food Research & Technology*, *227*(4), 1215–1223. https://doi.org/10.1007/s00217-008-0839-5

Afoakwa, E.O., Paterson, A., Fowler, M., & Vieira, J. (2009) Comparison of rheological models for determining dark chocolate viscosity. *International Journal of Food Science & Technology, 44,* 162 - 167. https://doi.org/10.1111/j.1365-2621.2008.01710.x

Alim, A., Song, H., Raza, A., & Hua, J. (2020). Identification of bitter constituents in milk-based infant formula with hydrolyzed milk protein through a sensory-guided technique. *International Dairy Journal, 110,* 104803. https://doi.org/10.1016/j.idairyj.2020.104803 .

AOAC. (2000). Official Methods of Analysis. Washington, DC: Association of Official Agricultural Chemists.

Do, T. A., Hargreaves, J. M., Wolf, B., Hort, J., & Mitchell, J. R. (2007). Impact of particle size distribution on rheological and textural properties of chocolate models with reduced fat content. *Journal of Food Science*, *72*(9), E541-E552. https://doi.org/10.1111/j.1750-3841.2007.00572.x

Embiriekah, S., Bulatović, M., Borić, M., Zarić, D., & Rakin, M. (2017). Antioxidant activity, functional properties and bioaccessibility of whey protein hydrolysates. *International Journal of Dairy Technology*, *71,* 243-252. https://doi.org/10.1111/1471-0307.12428

IOCCC. (2000). Analytical Method 46-2000. Viscosity of cocoa and chocolate products. Bruxelles, Belgium: International Office of Cocoa, Chocolate and Sugar Confectionery.

ISO 8586. (2023). Sensory analysis — Selection and training of sensory assessors. Geneva, Switzerland: ISO.

Korhonen, H. & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, *16,* 945-960. https://doi.org/10.1016/j.idairyj.2005.10.012

Krunic, T., Rakin, M., Bulatovic, M., & Zaric, D. (2018). The contribution of bioactive peptides of whey to quality of food products. In A. M. Grumezescu, & A. M. Holban (Eds.), *Food processing for increased quality and consumption*, *Handbook of Food Bioengineering* (pp. 251–285). Cambridge, MA, USA: Academic Press. https://doi.org/10.1016/B978-0-12-811447-6.00009-6

Lidefelt, J.O. (2002). *Handbook: Vegetable Oils and Fats*. Karlshamns AB. Sweden: Alfaprint.

Lončarević, I., Fišteš, A., Rakić, D., Pajin, B., Petrović, J., Torbica, A., & Zarić, D. (2017). Optimization of the ball mill processing parameters in the fat filling production. *Chemical Industry & Chemical Engineering Quarterly, 23*,197-206. http://oa.fins.uns.ac.rs/handle/123456789/100

McCue, P. P., & Shetty, K. (2005). Phenolic antioxidant mobilization during yogurt production from soymilk using kefir cultures. *Process Biochemistry*, *40,* 1791–1797. https://doi.org/10.1016/j.procbio.2004.06.067

Pajin, B., Radujko, I., Šereš, Z., Šoronja Simović, D., Gyura, J., & Sakač, M. (2012). Influence of low-melting milk fat fraction on crystallization and physical properties of chocolate. *British Food Journal*, *114*(6), 868-879. https://doi.org/10.1108/00070701211234381

Pedersen, A. (2001). Special fats for fillings can solve production problems. *Zucker-und Süßwarenwirtschaft, 54* (7–8), 27-28.

Properties of chocolate models with reduced fat content. *Food Engineering and Physical Properties, 72,* 541-552.

Saubenova, M., Oleinikova, Y., Rapoport, A., Maksimovich, S., Yermekbay, Z., & Khamedova, E. (2024). Bioactive peptides derived from whey proteins for health and functional beverages. *Fermentation*, *10*(7), 359. https://doi.org/10.3390/fermentation10070359

Vavrusova, M., Pindstrup, H., Johansen, L.B., Andersen, M.L., Andersen, H.J. & Skibsted L.H. (2015). Characterisation of a whey protein hydrolysate as antioxidant. *International Dairy Journal, 47,* 86-93. https://doi.org/10.1016/j.idairyj.2015.02.012

Wronkowska, M., Jadacka, M., Soral-Smietana, M., Zander, L., Dejnowiec, F., Banaszczyk, P., Jelinski, T., & Szmatowicz, B. (2015). ACID whey concentrated by ultrafiltration a tool for modeling bread properties. *LWT-Food Science and Technology, 61*, 172–176. https://doi.org/10.1016/j.lwt.2014.11.019

Zarić, D., Bulatović, M., Rakin, M., Krunić, T., Lončarević, I., & Pajin, B. (2016). Functional, rheological and sensory properties of probiotic milk chocolate produced in a ball mill. *RSC Advances*, *6*, 13934–13941. ttps://doi.org/10.1039/C5RA21363K

UTICAJ BIOAKTIVNIH HIDROLIZATA SURUTKE NA KVALITET MASNOG PUNJENJA ZA KONDITORSKE PROIZVODE

Milica V. Stožinić*[1], Danica B. Zarić[2], Marica B. Rakin[3], Ivana S. Lončarević[1], Biljana S. Pajin[1], Maja Lj. Bulatović[3]

[1]Univerzitet u Novom Sadu, Tehnološki fakultet, 21000 Novi Sad, Bulevar cara Lazara 1, Srbija
[2]Univerzitet u Beogradu, Inovacioni centar Tehnološko-metalurškog fakulteta, 11000 Beograd, Karnegijeva 4, Srbija
[3]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, 11000 Beograd, Karnegijeva 4, Srbija

Sažetak: Hidrolizati proteina surutke mogu se koristiti u širokom spektru aplikacija jer nude brojne prednosti u poređenju sa nehidrolizovanim proteinima surutke. Oni su stabilniji na toplotu, sa poboljšanim svojstvima pene i emulgovanja zbog prisustva bioaktivnih peptida nižeg viskoziteta. Hidrolizati surutke imaju poboljšanu apsorpciju, svarljivost, odlične nutritivne i funkcionalne osobine i sposobnost da produže rok trajanja prehrambenih proizvoda. Zbog velikih razlika u tehnološkim i drugim fizičko-hemijskim svojstvima hidrolizata, dodavanje hidrolizata proteina surutke u konditorske proizvode je mnogo komplikovanije. Cilj ovog istraživanja je utvrditi mogućnosti obogaćivanja punjenih konditorskih proizvoda peptidima surutke dobijenih na dva načina: enzimskom hidrolizom koncentrata surutkinih proteina i fermentacijom surutke (hidroliza mikroorganizmom: *Lb. rhamnosus* ATCC 7469). Peptidi su dodati u masni mlečni krem u koncentraciji od 5%. Studija je bila fokusirana na procenu antioksidativne aktivnosti, fizičkih, reoloških, teksturalnih i senzornih svojstava tri masna punjenja (C -kontrolni uzorak bez peptide surutke; EWP – masno punjenje sa 5% peptide dobijenih enzimskom hidrolizom surutke i MWP – masno punjenje sa 5% peptide dobijenih fermentacijom surutke). Enzimski hidrolizati povećavaju inhibiciju DPPH radikala za 32% a fermentisani za 19%. Veću sposobnost inhibicije lipidne peroksidacije pokazali su enzimski hidrolizati (IC_{50} vrednost od 811,54 mg mL^{-1}) u odnosu na fermentisane hidrolizate (IC_{50} vrednost od 178,36 mg mL^{-1}). Najveću antioksidativnu aktivnost postigao je uzorak EWP. Dodatak enzimskih hidrolizata povećava čvrstoću punjenja 2,5 puta, dok su fermentisani hidrolizati smanjili čvrstoću u odnosu na kontrolni uzorak. Obe vrste hidrolizata, nisu nepovoljno uticali na veličinu i distribuciju čestica u masnom kremu. Tiksotropna svojstva masnog punjenja su se zadržala i kada su u njega inkorporirani hidrolizati. Optimalni uzorak je što se tiče reologije je MWP, jer ima najmanji prinosni napon. Najbolje senzorne karakteristike (bolje i od kontrolnog uzorka) ima uzorak EWP.

Ključne reči: *enzimska hidroliza, mikrobna hidroliza, reologija, surutkini proteini, antioksidativna aktivnost*

Received: *17 September 2024/* ***Received in revised form:*** *04 October 2024/* ***Accepted:*** *08 October 2024*

Available online: *November 2024*

This work was reported at the 5th International Congress "Food Technology, Quality and Safety (FoodTech 2024)" held in Novi Sad, Serbia, October 16-18, 2024 organized by the Institute of Food Technology in Novi Sad.