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PHYSICO–CHEMICAL PROPERTIES OF PROTEIN PREPARATIONS

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Abstract:
The analysis of cohesiveness and flowing properties is based on the determination of the physicochemical properties of powdered food. The research material of the study consisted of 7 types of protein preparations. The protein supplements were analysed in terms of: water activity and water content, bulk density, static angle of repose (cone angle) wettability and kinetic angle of repose in order to determine initially their suitability for transport on the production line. The products were differentiated by water content and activity. The highest level of these parameters was observed in organic pumpkin protein what resulted in reduction of bulk density of the product. The performed determinations indicated mostly intermediate cohesiveness and at most fair flowability of the majority of the tested powdered protein supplements. All preparations, with the exception of pumpkin and brown rice products, had poor wettability. Some of the preparations especially beef protein isolate may cause flow obstructions.
Keywords: bulk density, powdered food, water activity, wetta

Introduction

Important part of product management is Supply Chain Management (SCM) (Schuh et al., 2007). Primary added value of its counterpart in a food industry – Food Supply Chain Management is to ensure, first of all: safety, freshness and high-quality of products, but also to reduce energy losses and amount of wasted food (Zhong et al., 2017). The significance of this managerial perspective is emphasized by the highest energy consumption of production operations in manufacturing industry and huge food losses in the whole supply chain (Brundage et al., 2014; Zhong et al., 2017). Such losses seem unacceptable in an environmentally oriented society and for this reason there is a need to determine the optimal treatment of food products. What's more one of the effects of globalisation and digitalisation are consumer expectations concerning increase in production with a simultaneous decrease in the amount of generated waste. Providing such a balance is possible through high level of automation and the right size of product and process flow (Nåfors and Johansson, 2021).

Important share of agricultural trade are processed foods (Gopinath and Carver, 2002). Moreover popular form of food products, difficult in manipulation are powdered goods. The main advantages of them are: convenience of dosing, lower susceptibility to spoilage and reduced storage area (Jedlińska et al., 2012). However to ensure optimal handling operations on the production line, the flowability of powdered food is particularly important (Schulze, 2008). Assessment of this parameter is often based on bulk density or dynamic and static angle of repose. From a practical point of view static and kinetic angle of repose are the properties of the powder that determine the size of the storage area, storage capacity and silo unloading speed (Leśmian-Kordas, 2001; Ruszkowska i Palich, 2013; Tomporowski i Opielak, 2014). Bulk density is a parameter influencing the degree of filling packages or devices, determining the efficiency of transport devices and is of particular importance in estimating the size of the pressure forces acting on devices and tanks (Ruszkowska i Palich, 2013; Schulze, 2008; Tomporowski i Opielak, 2014).

Establishing whether the powdered product is characterized by good flow properties, despite factors discussed above, is also connected with dust generation, which depends more on a material properties, especially its cohesion (Plinke et al., 1995). What is more, taking into account intrinsic factors: physicochemical properties and hygroscopicity of powder and others, may result in preventing quality loss because of caking phenomena (Zafar et al., 2017).

Quality assessment of powdered food, besides handling or transport aspects, often includes the determination of its dispersion in liquid (Domian, 2005). All these parame-ters are most often assessed for products popular with final consumers, such as, pow-dered milk, freeze¬-dried coffee, medicines and less frequently, protein preparations.

According to definition, protein preparations are an example of powdered products characterized by high nutritional value resulting from the increased protein content and the reduced quantity of anti-nutritional factors (Gawęcki et al., 2017). Depending on the applied technological process and protein content, this group of products includes protein meals, grits, concentrates, hydrolysates and isolates (Gawęcki et al., 2017). Currently, there is a wide range of products of this type on the market, intended to support the performance of numerous groups of athletes. Moreover these products also differ in composition, protein content and plant or animal-origin. This variety of protein preparations is not without significance for their physicochemical properties.

Therefore the aim of the study was to evaluate selected physicochemical properties of powdered protein preparations, including the characteristics of: the angle of repose, untapped and tapped density, and the wettability of the tested protein preparations of plant and animal-origin. Argumentation for assessing these parameters is based on need to use test methods that provide empirical understanding, because how the powders react is often hard to predict (Zafar et al., 2017). It should be emphasized that the innovativeness of the research was based on the use of one of the most common sources of plant and animal-origin proteins – soy and whey, but also other less popular products: brown rice and pumpkin proteins. The work focuses on finding differences between plant and animal proteins, which is a novel approach to the subject.

This paper comprises three parts. Presentation of material as well as physico-chemical methods follows introduction. In next section authors discussed the results of the study. This section includes also statistical analysis of the results. Finally, at the end of the article summary is presented.

Material and methods

The research material consisted of seven selected protein Bulk Powders purchased in an organic food store in Gdynia (Table 1). The products were identified by codes from I to VII. Unflavoured products of animal and plant-origin, mostly consisting of one main ingredient, were selected for quality assessment. Thenamesandtheiringredientsarepresentedin

Table. 1. Product ingredients

Number		Product Names	Product Ingredients
I	Animal-origin protein preparations	Pure whey protein unflavoured	Undenatured whey protein concentrate (milk, soy lecithin)
II		Egg white powder	Egg protein
III		Pure whey isolate tm 97 unflavoured	Undenatured 97% whey protein isolate (milk)
IV		Beef protein isolate 97 (hydrobeef) unflavoured	Hydrolyzed beef protein isolate
V	Plant-origin protein preparations	Organic pumpkin	100% pumpkin protein
VI		Soya protein isolate 90% unflavoured	100% soy protein isolate
VII		Brown rice protein 80% unflavoured	100% brown rice protein

Source: based on packaging information.

The research material consisted of seven selected protein Bulk Powders purchased in an organic food store in Gdynia (Table 1). The products were identified by codes from I to VII. Unflavoured products of animal and plant-origin, mostly consisting of one main ingredient, were selected for quality assessment. The nutritional value of the products declared by the producer on the unit packaging is presented in Table 2.

Table. 2. Nutritional value

Nutritional Value	**Product**						
	I	II	III	IV	V	VI	VII
Energy value (kcal/ kJ)	402/ 1708	375/ 1569	374/ 1564	392/ 1640	407/ 1699	378/ 1582	397/ 1683
Fat (g)	6.7	0.3	0.3	1.5	11	3.3	3.5
Saturated fat (g)	2.5	0	0.2	0.5	1.4	0.9	1,1
Total carbohydrates (g)	5	8.1	0	0	3	<1	3.8
	5	8,1	0	0	1.2	0	tq
Dietary fiber (g)	0	0	0	0	16	0	3.8
Protein (g)	80	85	97.6	97	58	91	80

Characterization of the physicochemical properties was carried out primarily by determination of bulk (untapped) and tapped density, which are the base values for determination of the Carr index and the Hausner ratio. According to the methodology of determining the coefficients, 100 g of the sample were weighed into a graduated cylinder of volume of 500 cm^3. After tapping 100 times, differences in the volume occupied by the powder were observed (Abdullah, 1999; Ruszkowska i Wiśniewska, 2017).

The bulk untapped and tapped density was calculated from the formula (1):

$$\rho = \frac{m}{v} \quad (1)$$

where:
m – product mass (kg),
v – product volume in the graduated cylinder (m^3).

The following formula (2) was used to determine the Hausner's ratio (Hr):

$$H_r = \frac{\rho_T}{\rho_L} \quad (2)$$

While Carr index (Ic) was calculated from the formula (3):

$$I_c = \frac{\rho_T - \rho_L}{\rho_T} \cdot 100 \quad (3)$$

where:
ρT – tapped density [kg/m^3],
ρL – untapped density [kg/m^3] [23].

Characterization of the physicochemical properties of the powders also included the determination of the static angle of repose (cone angle) (4) and the determination of the kinetic angle of repose (Leśmian-Kordas, 2001; Ruszkowska i Wiśniewska, 2017). The determination of the angle of repose was carried out by pouring freely the powder through a funnel with a diameter of 14.5 mm, from a constant height and determining the dimensions of the created cone. The equation was used to calculate the static angle of repose (4):

$$AOR = \arctan\left(\frac{2H}{d-a}\right) \quad (4)$$

where:
AOR – static angle of repose (o),
H – height of the cone formed by the product (mm),
d – cone base diameter (mm),

a – inner diameter of the funnel (mm) (Ruszkowska i Wiśniewska, 2017).

The determination of kinetic angle of repose – the angle at which the loose material begins to roll along the slope – was performed using the tilted surface method with a constant product volume and two types of surfaces: glass and metal (Beakawi et al., 2018, Cheng, 2018).

Another important physicochemical parameter that characterizes the reconstitution property of protein preparations is static wettability. It means measuring wetting time of the tested products until they penetrate into the continuous phase. Determination was based on adding a constant amount of the product into 100 ml of water by freely pouring the powder through a funnel with a diameter of 14.5 mm (Jedlińska et., 2012; Ruszkowska i Wiśniewska, 2017, Selomulya i Fang, 2013). Time in seconds was an indicating parameter. Measurements were carried out in five repetitions at temperatures of 20, 40 and 85°C.

The research methodology included also determination of water activity (AquaLab 4TE, AS4 version 2.14.0 2017 by Decagon Devices, Inc. with an accuracy of ± 0.0003, at a temperature of 293K ((20°C) ± 2.5K) and determination of water content using the drying oven method at 105°C under normal pressure (Krełowska-Kułas, 1993). Statistical calculations and graphical processing of the results were performed in PQStat 1.8.0.476, Microsoft Excel 2007 and Statistica 12 software. Anova and post–hoc tests were prepared with use of PQStat 1.8.0.476. To analyse statistically significant difference Tukey test and least significant difference test for parameters: water content, water activity, bulk density, Hausner ratio, Carr index, cone angle, dynamic angle of repose were performed.

Results and discussion

Based on the research it was found that product V (organic pumpkin protein) and product VI had the highest water content – 8.52 and 7.47 respectively (Fig. 1). The lowest value of this parameter (3.81) was observed for product VII (brown rice protein). Products I, II, III and IV had a water content in the range of 5.30–5.81g /100 g d.s. The evaluated parameter was characterized by small values of standard deviation (0.02–0.08%). Using LSD and Tuckey test it was found that statistically significant differences in the mean water content were not observed between product I and III.Similar relationships were observed for the second assessed parameter – water activity. Product V (0.4354) – (organic pumpkin protein) was characterized by the highest water activity (aw) (Fig. 2). The preparations coded by numbers I (whey protein) and IV (beef protein isolate) were characterized by the lowest level of the tested parameter: 0.1943 and 0.1992. Moreover statistically significant correlation (0.9063) between aw and water content was observed. LSD and Tuckey tests calculated for aw parameter confirmed statistically significant differences between all products, with the exception of products I –IV, I–VII and III–VII.

Table. 3. Water content and activity in tested preparations

Product	Water Content (g /100 g d.s.)	SD	Water Activity	SD
I	5.4442	0.0008	0.1943	0,007
II	5.8062	0.0003	0.2572	0,002
III	5.5681	0.0002	0.2095	0,004
IV	5.3000	0.0005	0.1922	0,006
V	8.5239	0.0002	0.4354	0,001
VI	7.4742	0.0007	0.3377	0,005
VII	3.8122	0.0004	0.2017	0,002

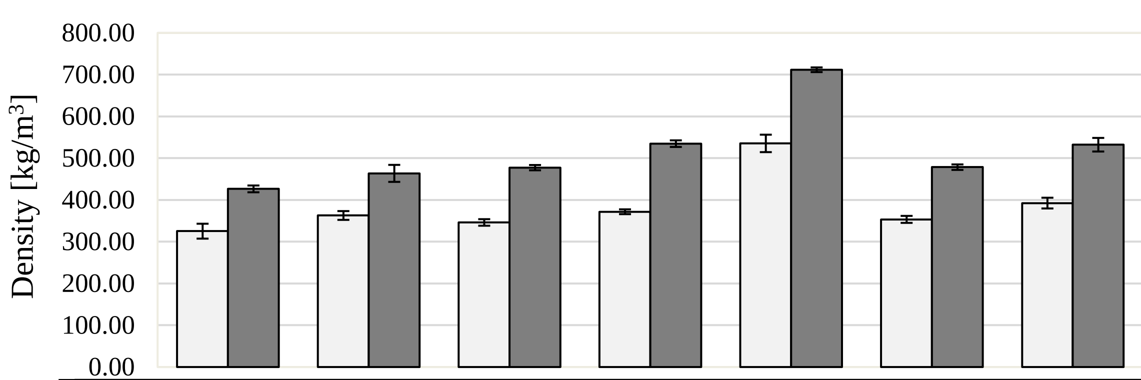
Source: Own study

Where: I – Pure whey protein, II – Egg white powder, III – Pure whey isolate, IV– Beef protein isolate, V – Organic pumpkin protein, VI - Soya protein isolate, VII– Brown rice protein.

Because of the dependence between water content and flow properties of hygroscopic powders, which is reduction of flowability with increasing moisture content because of stronger interparticle liquid bridges, worst flowability of product V and the best for product VII would be observed (Chinwan and Castell-Perez, 2019) (Table 3). Of particular importance is also water activity, for which in opinion of some researchers, flow factor can correlate better with water activity than with absolute moisture (Juarez-Enriquez et al., 2017). This finding was confirmed in this study by a statistically significant Pearson correlation between water activity and loose and tapped density (0.9280 and 0.8528 respectively). However, no significant correlations were observed between this parameter and the results of angle of repose, Carr's index and Hausner's ratio or between the water content parameter and all parameters determining the flow properties of preparates.

What is more, on the basis of the obtained values of water activity (aw), it can be assumed that there is a dependence between the protein content of the tested protein powder preparations and the value of the determined parameter (aw). Products with higher protein content, due to the proteins ability to bind water, were characterized by a lower water activity (Pearson correlation -0.8297) (Tables 2 and 3). Inverse relationship was observed between fiber content and water activity (Pearson correlation 0.9284).

Another assessed parameter related to the handling and transport of bulk products was the bulk untapped and tapped density (Figure 1). It was found that the product V – organic pumpkin protein was characterized by the highest bulk untapped and tapped density (Figure 1). What is more these values were observed despite the highest water content in product V, which may contribute to the particle agglomeration process and consequently reduce the value of the bulk density (Jedlińska et al., 2012). For the rest of the products bulk density was similar and ranged from 0.33–0.39 g / 100g d.s. (Figure 1).



	I		II		III		IV		V		VI		VII	
	Untap.	Tap.	Untap.	Tap.	Untap.	Tap.	Untap.	Tap.	Untap.	Tap.	Untap.	Tap.	Untap.	Tap.
I	-	-	ssd	ssd		ssd	ssd	ssd	ssd	ssd	ssd	ssd	ssd	ssd
II	ssd	ssd	-	-				ssd	ssd	ssd			ssd	ssd
III		ssd			-	-		ssd	ssd	ssd			ssd	ssd
IV	ssd	ssd		ssd		ssd	-	-	ssd	ssd		ssd		
V	ssd	ssd	ssd	ssd	ssd	ssd	ssd	ssd	-	-	ssd	ssd	ssd	ssd
VI	ssd	ssd						ssd	ssd	ssd	-	-	ssd	ssd
VII	ssd	ssd	ssd	ssd	ssd	ssd			ssd	ssd	ssd	ssd	-	-

Figure 1. Bulk untapped density

Source: own study

Where: ssd – statistical significant difference, untap. – untapped density, tap. – tapped density, I – Pure whey protein, II – Egg white powder, III – Pure whey isolate, IV – Beef protein isolate, V – Organic pumpkin protein, VI - Soya protein isolate, VII– Brown rice protein.

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The lowest tapped density was found for product I – pure, unflavoured whey protein. The highest percentage decrement in density as a result of mechanical operations (tapping) was found in product IV – hydrolyzed beef protein isolate. Besides relation between water activity and bulk density statistic calculations indicated almost full correlation (>0.9) between dietary fiber amount and bulk density. There were statistically significant differences between most of the samples (Figure 1.). Comparing the obtained values of loose and tapped density of the preparations with the results obtained by other authors, excluding product V, it was found that the parameter values were similar to those of powdered milk and whey (Ruszkowska and Palich, 2013). The bulk density of the tested preparations for most samples was higher than that of dried tomato pulp (Domian, 2005). The analyzed parameters for product V were similar to dried 40% glucose and fructose solutions with the addition of maltodextrin, dried chokeberry juice concentrate with maltodextrin, or dried sweet potato puree (Gawałek et al., 2017; Grabowski et al., 2006; Jedlińska, 2012).

Table 4 presents the values of Hausner's ratio and Carr's index for the tested preparations, compared with the classification proposed by Jinapong and others (tab. 5) (Jinapong et al., 2008; Ruszkowska and Palich, 2013).

Table 4. Values of Hausner’s ratio and Carr’s index for preparations

Product	**Hausner's Ratio (I_H)**	**SD**	**Carr’s Index (I_C)**	**SD**
I	1.31	0.05	23.78	3.03
II	1.28	0.04	21.69	2.29
III	1.38	0.04	27.47	2.22
IV	1.44	0.03	30.47	1.32
V	1.33	0.06	24.77	3.18
VI	1.35	0.05	26.10	2.51
VII	1.36	0.08	26.18	4.05

Source: own study

Where: I – Pure whey protein, II – Egg white powder, III – Pure whey isolate, IV – Beef protein isolate, V – Organic pumpkin protein, VI - Soya protein isolate, VII– Brown rice protein.

Product IV was characterized by the highest value of the Hausner's ratio (1.44). The lowest value of the parameter and at the same time the lowest cohesiveness were found in product II (1.28) – egg white powder (Table 4). The assessment showed the highest value of Carr's index for product IV (30.47) and the lowest for product II (21.69). Therefore based on the classification of Jinapong (Table 5) it was found that product II – (egg white) was characterized by the best flowability (Jinapong et al., 2008). Standard deviation values fluctuated within the range of 0.03–0.08 for the Hausner’s ratio and 1.32–4.05 for the Carr’s index. The values of the Hausner's ratio for most products and the Carr's index for products I and II were similar to some powdered dairy products (Ruszkowska and Palich, 2013). Furthermore the index values for brown rice protein were lower compared to brown rice powder in the studies of Qi and others (Qi et al., 2019). Calculation of correlation indicated relationship between Hr and IC and sugar content (-0.9090, -0.9195 respectively) and corelation between calculated indexes (0.9996). Significant statistical differences were observed between samples I–IV, II–III, II–IV, IV–V for Hausner ratio and Carr index parameters.

Table 5 shows the classification of the flowability and cohesiveness of powders based on the Carr index and the Hausner index.

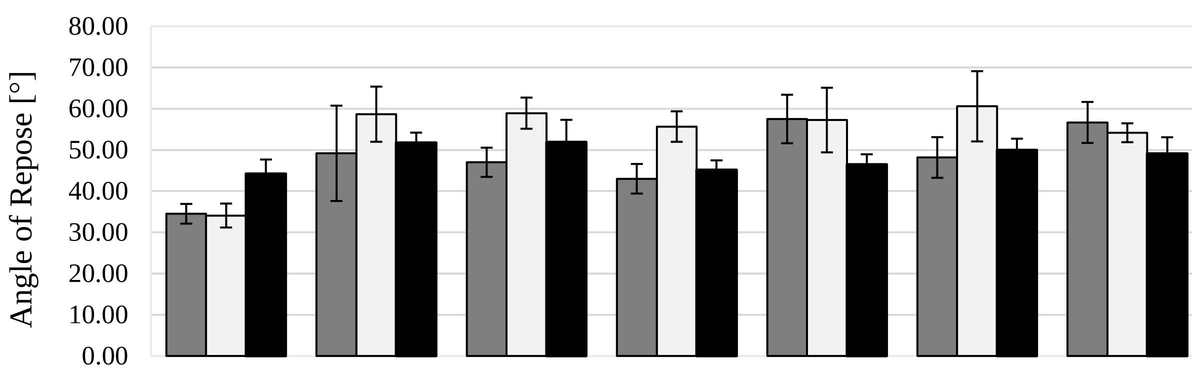
Table. 5. Cohesiveness and flowabillity based on HR and CI

HR – Hausner's Ratio (I_H)	**Cohesiveness**
<1.2	Low
1.2–1.4	Intermediate
>1.4	High
CI – Carr's Index (%)	**Flowability**
<15	Very good
15–20	Good
20–35	Fair
35–45	Bad
>45	Very bad

Source: (Junapong et al., 2008; Ruszkowska and Palich, 2013)

Based on the classification proposed by Jinapong and others it was found that almost all tested protein preparations were characterized by intermediate cohesiveness and fair flowability (Jinapong et al., 2008; Ruszkowska and Palich, 2013).One exception was product IV, which cohesiveness was high. What is more such characteristics of powders may be associated with certain difficulties in their handling. High cohesiveness powders are more difficult to transport because of the occurrence of short-range molecular interactions – Van der Waals forces and electrostatic forces, obstructing the flow by causing adhesion and agglomeration phenomena (Bhandari, 2013). Similarly, lower flowability of powdered food products may be associated with the formation of flow obstructions and difficulties with filling packages and other handling operations (Schulze, 2008).

To confirm such characteristics of the powders further parameters: static (cone angle) and kinetic angle of repose were determined (Tab. 6). According to the literature data, the static and kinetic angle of repose are not physically comparable and are characterized by different load characteristics. Moreover, these angles are highly dependent on the water content in the product (Leśmian-Kordas, 2001).



	I			II			III			IV			V			VI			VII		
	D.M.	D.G.	St.	D.M.	D.G.	St.	D.M.	D.G.	St.	D.M.	D.G.	St.	D.M.	D.G.	St.	D.M.	D.G.	St.	D.M.	D.G.	St.
I	-	-	-	ssd	ssd	ssd	ssd	ssd	ssd	ssd	ssd		ssd	ssd		ssd	ssd	ssd	ssd	ssd	
II	ssd	ssd	ssd	-	-	-						ssd	ssd		ssd				ssd		
III	ssd	ssd	ssd				-	-	-			ssd	ssd		ssd				ssd		
IV	ssd	ssd				ssd			ssd	-	-	-	ssd						ssd		
V	ssd	ssd		ssd		ssd	ssd		ssd	ssd			-	-		ssd					
VI	ssd	ssd	ssd										ssd			-	-		ssd		
VII	ssd	ssd		ssd			ssd			ssd						ssd			-	-	

Figure 2. Values of repose angle of examined protein preparations

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Source: own study

Where: where: ssd - statistical significant difference, D.M. - dynamic angle of repose on metal surface, D.G. - dynamic angle of repose on glass surface, St. -static angle of repose, I - Pure whey protein, II - Egg white powder, III - Pure whey isolate, IV - Beef protein isolate, V - Organic pumpkin protein, VI - Brown rice protein.

The tested powders were characterized by the cone angle (static angle of repose) at the level of 44.20°–51.93° (Figure 2). The highest value of the assessed parameter was characteristic for product III (whey isolate) and product II (egg white powder), while the lowest value for product I (whey protein preparation). On the basis of the obtained values (>41°) and Mirhosseini and Amid classification it was found that all the powders were characterized by passable or poor flow character (tab. 7) (Mirhosseini and Amid, 2013). Additionally, taking into consideration Carr scale used in Abdullah and others article, they were mostly (preparations I–VII) highly cohesive products, reaching the static angle of repose in the range of 45–55° (Abdullah et al., 2010) Tukey's and LSD test indicated that most of the samples were not statistically significantly different in terms of the cone angle parameter (Fig. 2).

Tabela. 6. Dependence on angle of repose and flow character

Angle of Repose (°)	Flow Character
25–30	Excellent
31–35	Good
36–40	Fair
41–45	Passable
46–55	Poor
56–65	Very poor
>66	Very, very poor

Source: (Mirhosseini and Amid, 2013)

Moreover, higher values of kinetic angle of repose were observed on the glass surface as compared to the metal surface. The observed differences may result from the higher friction coefficient between the sample and the glass surface in comparison to the metal surface, which may affect the values of the angle of repose (Santos et al., 2014). The lowest kinetic angle of repose was characteristic for product I, while the highest values for this parameter were characteristic for product V (organic pumpkin protein) – on the metal surface and product VI (soy protein isolate) – on the glass surface. Significant differences were especially observed between product I (whey protein preparaton) and other products (Fig. 2).

The last physicochemical parameter connected with reconstitution properties of the tested powdered protein preparations was the wettability parameter. Determination was performed at various temperature ranges shown in table 8.

Tabela. 7. Wettability of products

Product	Wettability Time in Temperature of 20°C (sec.)	Wettability Time in Temperature of 40°C (sec.)	Wettability Time in Temperature of 85°C (sec.)
I	>300	>300	>300
II	>300	>300	>300
III	>300	>300	>300
IV	>300	>300	>300
V	20.20±2.39	16.86±3.10	5.96±2.07
VI	>300	>300	>300
VII	194.48±26.09	65.26±12.89	23.84±4.89

Source: own study
Notes: where: I – Pure whey protein, II – Egg white powder, III – Pure whey isolate, IV – Beef protein isolate, V – Organic pumpkin protein, VI - Soya protein isolate, VII– Brown rice protein.

Determination was performed at various temperature ranges shown in table 8. Based on the wettability assessment, it was found that the powdered protein preparations were characterized by low wettability. Most of the tested preparations (products numbered I–IV and VI) did not become completely wetted within 5 minutes (Table 8). It can be assumed that the products were denatured because of the high temperature, which made the wetting process difficult. Product V – organic pumpkin protein was characterized by the lowest wetting time among the tested (20.20 s) at the temperature of 20°C. The rate of wetting of the preparation particles increased with increasing water temperature. The wettability of products V and VII, which are proteins of plant-origin, was higher than in the case of powdered vegetable beverages tested by an-other team, however it was lower than the results achieved for dried tomato pulp (Goula, 2005, Ruszkowska and Wiśniewska, 2017). The high values of standard deviation were observed for some samples. The wetting process was accelerated by the use of mechanical operations – mixing, which is consistent with the manufacturer's declaration on the packaging.

Conclusions

Protein preparations belong to the group of powdered food supplements. For this reason, it is necessary to evaluate their physicochemical properties related to the dispersion of the product in the liquid, as well as the possibilities of handling and reloading operations. Ensuring their quality is key to reducing the negative impact on the environment and allows for the implementation of sustainable development postulates. Therefore products V and VI, which were proteins of plant-origin, had the highest water content and activity, which affected bulk density of the product. Product V was characterized by the highest untapped and tapped density. Other products in terms of this parameter fluctuated at a similar level. Based on the flowability and cohesiveness classification, resulting from the Carr's and the Hausner's indexes, it was found that almost all protein preparations were characterized by intermediate cohesiveness and fair flowability. An exception was product IV, for which high cohesiveness was observed. The possibilities of blockages on the processing line were emphasized in particular by the values of the angle of repose. Based on this parameter, it was found that most of the products were characterized by poor flowability and high cohesiveness. The lowest kinetic angle of repose was characteristic for product I, while the highest was for product V. Differences in the value of that parameter were observed depending on the surface on which the determination was made. The higher friction coefficient was observed on glass surface. Reconstitution properties of the powders were debatable. In the absence of mechanical mixing, the powders were characterized by a long wetting time. Only the products of plant-origin (pumpkin and rice protein preparations) dissolved in water. It should be considered if introducing improvements in production processes may result in reducing the internal and external failure cost occurring because of flow obstructions and poor solubility leading to consumer dissatisfaction.

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