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COMPARATIVE CHARACTERIZATION OF SURFACE HYDROPHOBICITY AND OTHER PHYSICO-CHEMICAL PROPERTIES OF SELECTED PROTEIN PREPARTIONS

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ABSTRACT

Modeling physicochemical properties plays an important role in the function of animal and plant preparations which are widely used in the food processing. The study presents some functional characteristics of 21 randomly selected protein preparations subjected to the examination under the same analytical conditions. All examined preparations were highly variable in their composition, solubility, emulsifying and foaming properties. It has been confimed that the evaluation of protein preparation hydrophobicity is possible using different empirical or computational methods. The surface hydrophobicity value may be used to extend complex laboratory characterization of protein preparations.

Key words: protein preparations, functionality, surface hydrophobicity

INTRODUCTION

Protein preparations obtained commercially from raw materials of vegetable, animal or microbiological origin as a result of different technological processes create a group of functional additives with a long tradition in foodstuffs production. Because these preparations demonstrate different form, hydration degree, level and

quality of protein, it is obvious, that the qualitative effect of their applying is not equal. Technological usefulness of protein preparations is considerably determined by their physical and physico-chemical properties [5, 15].

Since protein preparations have to meet most critical functional features, which determine their ultimate value as ingredients in formulated food, extensive reviews are available on functional properties of such preparations, both in aqueous solutions and in model food products, as well [4, 7, 11]. All of these studies, however, are mostly difficult to compare due to differences in processing conditions and analytical methods employed.

Attempts to find one, most important factor responsible for protein functionality, highly correlated with parameters characterizing especially surface properties of protein dispersion systems and simultaneously giving a chance of predicting various functional properties of protein compositions, have been made by some authors [10,16,17]. It is generally accepted that so called "hydrophobicity", especially surface or effective hydrophobicity is so essential for understanding protein functionality [27].

However measurement of surface hydrophobicity is still quite controversial so that no standard method has ever been establish, valuable additional information is expected from comparing this parameter for various protein preparations [14].

The objective of this work was, therefore, to investigate and compare functionality of different protein preparations under the same analytical conditions and methods, including their surface hydrophobicity determined by selected methods.

MATERIALS AND METHODS

21 different protein preparations appropriate for use in food processing were obtained for this study. More detailed information concerning types of examined preparations and preparing method are given in Table 1.

Table 1. General characteristics of protein preparations

No	Code of preparation	General characteristic of preparation
	ALB	bovine blood albumin, fraction V
	OL	under lab conditions freeze dried blood plasma,
	OSR-POL	commercially made spray dried blood plasma of Polish production
	OSR- HOL	commercially made spray dried blood plasma of Dutch production
	OSR-LAB	under lab conditions spray dried blood plasma
	MBL	lean beef meat, freeze dried, after removal of fat by the use of petroleum ether
	MPC 1	spray dried all protein milk concentrate, ultrafiltrated, of Dutch production
	MPC 2	spray dried all protein milk concentrate, ultrafiltrated, spray dried, of Polish production
	ESP	whey protein concentrate, ultrafiltrated, spray dried, of Dutch production
	SERW	whey protein concentrate, ultrafiltrated, spray dried, of Polish production
	KS-POL	whey protein concentrate, ultrafiltrated, spray dried, of Polish production
	KS-HOL	commercially made spray dried sodium caseinate, of Dutch production
	KAZWA	commercially made spray dried calcium caseinate, of Dutch production
	KAZPO	commercially made potassium caseinate, drum dried, of Dutch production
	PPSZ	commercially made wheat protein concentrate, ultrafiltrated
	SOJA 1	soy protein concentrate, manufactured with extraction method, spray dried
	SOJA 2	commercially made soy protein concentrate of Dutch production
	SOJA 3	commercially made soy protein concentrate, manufactured with extraction method, spray dried
	SOJA 4	soy protein isolate commercially made with method of extraction and precipitation
	ŁUB	freeze dried lupine protein concentrate obtained from fat free flour
	BJAJ	commercially made spray dried egg white protein preparation, sugar free

The following physico-chemical features of selected protein preparations were determined in the first step of this study protein content (with Kjeldahl method), bulk density (with weighting method of known volume of sample) and pH value of 10% aqueous solution at 20°C (with pH-meter type N-517).

Solubility NSI index was determined and expressed as % according to the method recommended by AACC [1].

Emulsifying capacity (EC) was determined according to method of Swift [35] modified by Webb [39], while emulsion stability (ES) was determined according to the method of Porteous [32] with slight modification.

Modified method described by Lin et al. [20] was used to measure foam capacity (FC), foam stability (FS) (foam volume remaining after 30 min expressed as %) and foam density (FD).

Surface hydrophobicity of examined protein preparations was determined using two fluorescence probes (ANS and CPA) according to procedures described by Kato and Nakai [8] and with the method of Lieske and Konrad [18].

Protein solutions were diluted to concentrations between 0.001% and 0.020 % protein using 0.01 M phosphate buffer, pH 7.0, containing 0.4 M NaCl. 15 μl of ANS (1-anilino-8-naphtalenesulfonate magnesium salt from SIGMA USA) methanol solution was added to 3 ml of diluted protein. Fluorescence intensity was measured with an spectrofluorometer PERKIN ELMER LS 50 at excitation wavelength λ_{ex} = 390 nm and emission wavelength λ_{em} = 480 nm. Pure methanol and diluted ANS solution were used in calibration procedure. The initial slope of the fluorescence intensity versus protein concentration (%) plot was calculated by linear regression analysis and used as an index of the protein hydrophobicity.

When surface protein hydrophobicity was determined using CPA (*cis*-parinaric acid from Molecular Probes Inc., USA), 10 μ l of CPA solution (3.6 mM in absolute ethanol containing 10 μ g/ml BHA) was added to 2 ml of diluted protein. Fluorescence intensity was measured at excitation and emission wavelengths λ_{ex} = 325nm and λ_{em} = 420 nm, respectively, using the same apparatus. The CPA solution diluted with 0.01 M phosphate buffer, pH 7.0 was used in calibration procedure.

Surface hydrophobicity of examined protein preparations was also determined using polyoxyethylene sodium monooleate (Tween 80, SERVA, Germany) as a ligand.

To determine surface hydrophobicity, the interference of dye binding in a protein assay (BIO-RAD Dye Reagent No 50000006) due to covering hydrophobic sites of the protein with Tween 80 was measured [18].
Detailed information about amino acid composition of the examined protein preparations were used for calculating their relative protein hydrophobicity values with two various approaches.

According to the first method, the side chain hydrophobicity values of individual aminoacids Δ f given by Ney [28, 29] and Bigelow [3] were applied. The average protein hydrophobicity Q value was calculated using the following formula

$$Q = \frac{\sum \Delta f \, x \, \% A}{100}$$

where:

Δ f – side chain hydrophobicity of individual aminoacid,
%A – content of aminoacid (%) in examined protein preparation.

Finally, a procedure given by Mangino [22] to estimate the relative hydrophobicity of a protein from aminoacid data and expressed as the *Net Polarity Index* (NPI) was used in the presented study. It was obtained by dividing the content of tryptophan + isoleucine + leucine + tyrosine + valine (most hydrophobic amino acids) by the total aminoacid content.
The experimental results were subjected to a statistical assessment including variance analysis and/or regression analysis. Correlations at significance level below $\alpha = 0.05$ were recognized as statistically significant.

RESULTS AND DISCUSSION

Data presented in Table 2 shown, that in the present study 21 different preparations in respect of origin, form (isolates, flours, concentrates) and content of total protein (from 54.1 to 99.9 % d. m.) were subjected to an examination. The pH values of examined preparations (10% aqueous solutions) were determined both by the preparation type as well as receiving method. Differentiation of origin, chemical composition and structure of selected protein preparations was main criterion during selection of examined raw material.

According to the data of another experiments [12, 37], characteristic, high pH values (equal to 7.9 – 9.0) were found for all blood plasma preparations, while the pH values of solutions of remaining preparations were located close to the neutral range (between 5.90 and 7.20).

Table 2. Selected properties of examined protein preparations

No	Code of preparation	Total protein [% d. m.]	pH value [10%, w/v, H_2O. 20° C]	Bulk density [kg/m^3]
	ALB	99.9	7.00	350
	OL	54.6	8.90	300
	OSR-POL	72.4	8.90	400
	OSR- HOL	70.0	9.00	400
	OSR-LAB	71.2	7.90	350
	MBL	85.4	7.20	300
	MPC 1	77.9	7.00	350
	MPC 2	67.0	6.50	360
	ESP	77.2	6.30	430
	SERW	54.1	6.20	350
	KS-POL	85.7	5.90	210
	KS-HOL	86.8	6.80	350
	KAZWA	90.5	6.70	475
	KAZPO	89.5	6.50	475
	PPSZ	71.2	6.50	580
	SOJA 1	71.8	7.00	380
	SOJA 2	71.0	6.90	380
	SOJA 3	67.0	6.70	400
	SOJA 4	89.5	6.80	350
	ŁUB	54.6	6.90	570
	BJAJ	81.5	6.60	350

***N x 6.25 (No.1-6. 15-21). N x 6.38 (No 7 – 14)**

Examined preparations demonstrated also different values of bulk density. The lowest bulk density (210 kg/m^3) was found for sodium caseinate of Polish production, and the highest value of this parameter was determined in case of wheat protein preparation (580 kg/m^3). The bulk density affected conditioned first of all by drying method of preparation, is an important feature of protein preparations used in food industry [13].

Differentiation of raw material used in this study was confirmed with results of determinations of their functional features (Table 3).

Table 3. Selected functional properties of examined protein preparations (0.01 M Na phosphate buffer pH = 7.0. 0.4 M NaCl) (mean values ± SD)

Code of preparation	Solubility [%]	Emulsifying properties*		Foaming properties*		
		EC [ml oil/ 100 mg of protein]	ES [% emulsion]	FD [kg/m^3]	FC [%]	FS [%]
ALB	99.9± 0.1	518.8±1.5	79.8±1.1	0.895±0.080	118.2±2.2	41.7±1.5
OL	91.9± 0.2	211.5±1.3	62.3±1.2	0.903±0.085	121.0±2.1	42.2±1.8
OSR-POL	81.9±0.2	139.3±1.9	64.8±1.3	0.893±0.090	124.2±2.0	40.3±2.0
OSR-HOL	87.6±0.4	245.0±1.8	60.3±0.8	0.880±0.085	120.4±2.1	45.0±1.1
OSR-LAB	89.5±0.2	157.5±1.2	57.5±1.2	0.830±0.090	122.0±2.5	41.5±1.2
MBL	55.2±0.3	131.3±1.3	46.0±1.3	0.850±0.085	124.8±1.6	16.8±1.1
MPC	52.0±0.4	158.5±1.3	55.7±1.1	0.896±0.090	118.4±1.8	14.7±1.2
MPC2	60.5±0.3	148.0±1.4	55.5±1.2	0.885±0.090	117.0±2.0	14.0±2.0
ESP	70.1±0.4	150.5±1.5	53.3±1.4	0.780±0.095	130.2±2.3	13.3±1.2
SERW	82.5±0.4	141.0±1.1	51.0±1.1	0.850±0.085	128.6±2.0	13.5±1.2
KS-POL	88.7±0.2	157.0±1.3	37.8±1.2	0.811±0.085	118.0±2.0	42.2±1.5
KS-HOL	91.0±0.2	171.8±1.3	43.3±0.9	0.820±0.090	129.0±1.8	44.0±1.5
KAZWA	74.4±0.3	197.5±1.2	50.3±0.9	0.893±0.090	118.6±1.7	4.4±0.6
KAZPO	78.1±0.3	211.3±1.3	48.7±1.1	0.868±0.090	122.0±1.8	5.5±0.9
PPSZ	54.4±0.4	155.8±1.6	37.2±1.5	0.850±0.085	76.0±2.0	10.8±1.0
SOJA1	42.2±0.2	188.3±1.5	38.0±1.0	0.950±0.080	116.0±2.2	16.1±1.2
SOJA2	36.5±0.2	192.5±1.5	43.2±0.9	0.910±0.095	84.0±2.0	27.1±1.6
SOJA3	45.0±0.3	165.0±1.4	42.5±0.9	0.875±0.089	95.2±1.8	18.5±1.8
SOJA4	75.6±0.2	180.5±1.3	47.0±1.2	0.855±0.090	110.4±1.8	25.0±1.0
ŁUB	42.6±0.4	127.5±1.6	32.0±1.5	0.832±0.090	80.4±1.1	12.4±1.1
BJAJ	91.0±0.2	241.3±1.2	51.7±0.9	0.846±0.085	125.6±1.1	47.1±1.5

*** Determinations:**
S – solubility expressed as %
EC – emulsifying capacity expressed as ml of oil per 100 mg of protein
ES – emulsion stability expressed as percentage of emulsified phase
FD – foam density expressed as kg per m^3
FC - foam capacity as the percentage volume increase
FS – foam stability as the percentage of remained foam after 30 min
SD – standard deviation

Data given in Table 3 indicate the general possibility to receive protein preparations demonstrating specific, desirable functionality resulted from proper selection of raw materials and processing methods (f. e. drying technique). It was found, that under analytical conditions of the determination (phosphate buffer, pH =7.0, with addition of 0.4 M NaCl), the highest protein solubility was found for bovine blood albumin (99.9%), all examined blood plasma preparations (between 81.9 and 91.9%), sodium caseinates (88.7 i 91.0%) and egg white protein preparation (91.0%).

Considerably lower solubility value in comparison to remaining preparations were noticed for plant origin preparations, especially soy protein concentrate (SOJA 2) (36.5%) and lupine protein preparation (42.6%).

Among milk protein preparations the lowest solubility demonstrated all milk protein concentrate MPC (52.0%). The solubility results collected during this study correspond well with data of another reports focused on comparative evaluation of protein preparations used in food production [2, 15, 41].

Analysis of emulsifying properties of examined preparations indicated high differentiation of obtained results. All protein preparations demonstrated both ability to emulsify considerable amounts of oil (between 127.50 ml

and 241.25 ml per 100 mg of protein for lupine protein and egg white, respectively) as well as to create stable emulsions (between 32.0% for lupine protein and 64.8% for spray dried blood plasma of Polish production).

Under the same analytical conditions, pronounced highest emulsifying capacity (518.75 ml/100 mg of protein) and emulsion stability (79.8%) was observed in case of bovine blood albumin, fraction V (ALB).

All examined preparations were characterized with different foam ability and foam stability. It was found that both parameters, but also foam density, were conditioned by origin and receiving method of protein preparation. The highest amount of foam expressed in % was received for whey protein concentrate ESP (130.2%), while the lowest one – for wheat protein preparation (PPSZ) – 76.0%. Simultaneously, it was found that foam stability of examined preparations varied in wide range, however there was no statistically significant correlation between this parameter and either foam ability ($r = 0.298$, $\alpha > 0.05$) or foam density ($r = 0.076$, $\alpha > 0.05$).

It must be stressed, that foam properties of protein preparations are considered as a disadvantage in some cases f. e. during meat batter preparing, however the same properties increase technological usefulness of protein preparations in such products as creams or biscuits [40].

Literature review indicate, that on the area of protein functionality and its elucidation, new possibilities offers the determination of so called protein surface hydrophobicity [25, 26, 27]. In the presented study, this parameter was determined for all examined protein preparations using selected empirical or computational methods. Mean values of these determinations are presented in Table 4.

Table 4. Surface hydrophobicity of protein preparations determined by various methods (0.01 M Na phosphate buffer pH = 7.0. 0.4 M NaCl)[1]

Code of preparation	Computational methods		Empirical methods		
	Q	NPI	ANS	CPA	HP (%)
ALB	1333.31	0.2403	48250	24543	52.46
OL	1317.32	0.2245	45354	19200	60.10
OSR-POL	1325.76	0.2385	19624	9066	66.73
OSR-HOL	1423.60	0.2547	25430	19003	51.76
OSR-LAB	1329.62	0.2537	12460	15040	55.25
MBL	1272.55	0.2106	1726	1909	85.84
MPC	1438.55	0.2349	25961	10851	77.78
MPC2	1443.96	0.2653	10305	9959	70.45
ESP	1318.41	0.2311	9403	10276	82.04
SERW	1309.15	0.2580	9890	11250	81.02
KS-POL	1412.62	0.2329	12114	5643	56.40
KS-HOL	1418.27	0.2369	22082	7097	60.67
KAZWA	1416.32	0.2355	4705	5250	59.97
KAZPO	1344.80	0.2349	5200	5950	64.31
PPSZ	1378.01	0.1878	9753	5084	85.15
SOJA1	1268.01	0.2170	12350	1544	30.95
SOJA2	1253.18	0.2109	26764	2322	35.50
SOJA3	1271.14	0.2227	15600	2832	32.25
SOJA4	1254.11	0.2263	22630	3181	39.96
ŁUB	1152.77	0.1805	3918	925	72.74
BJAJ	1721.99	0.2544	10250	1167	63.10

[1] Determinations
CPA – fluormetrically determined using cis-parinaric acid [8]
ANS - fluormetrically determined using magnessium salt of 1-anilino-8-napthlenesulfonate and PERKIN ELMER LS 50 apparatus
HP – determined using detergent TWEEN-80 and test dye reagent of BIO-RAD company [18]
Q – total hydrophobicity calculated according to the method of [3] and [28, 29]
NPI – hydrophobicity calculated as index of hydrophobic aminoacid content [22]

The Q (average protein hydrophobicity) and NPI (Net Polarity Index*)* values presented in Table 4 were calculated basing on data of aminoacid composition earlier determined for all protein preparations examined in this study.

Ney [29] has found that proteins containing considerable amounts of strongly hydrophobic aminoacids like valine, leucine, isoleucine, proline, phenyloalanine, tryptophan and methionine are characterized by Q value > 1400 and there were casein (Q=1605), zein (Q=1480) and soy concentrate (Q=1540).

Q values obtained during this study are generally lower and f. e. for soy preparations (SOJA 1-4) they not exceeded 1280. The highest Q value was found for egg white protein preparation (BJAJ), while the lowest one value of this parameter demonstrated lupine protein preparation (ŁUB), it means Q = 1721.99 and Q = 1152.77, respectively. High conformability of obtained results were observed for own beef meat protein preparation (MBL) (Q = 1272.55) and beef meat preparation (Q=1300) reported by Ney [29].

The NPI values calculated for examined preparations according to the procedure proposed by Mangino [22] varied from 0.1805 to 0.2653, for lupine protein preparation (ŁUB) and all milk protein preparation (MPC2), respectively. Additionally, it was found, that for set of preparations examined in this study, correlation between Q and NPI values is statistically significant ($r = 0.566$, $\alpha < 0.05$).

Due to the opinion of Sikorski [33] and Nakai et al. [27] evaluation of average protein hydrophobicity basing on hydrophobicity of side chain of individual aminoacids does not allowed predication of conformation type of examined protein and their behavior in respect to solvent like water and other compounds of surround. Nakai et al. [27] indicate, that the protein molecule demonstrates three dimensional spherical molecule with different availability of hydrophobic or hydrophilic sites, and this fact is not taken into consideration by methods of hydrophobicity evaluation mentioned above.

Liwo et al. [21] indicate, that during examination of commercially made protein preparations representing blends of different proteins, hydrophobicity scales for proteins used in basic research of molecular biophysics are practically not useful. They required not only detailed data about protein composition but also about sequence and localization of individual aminoacid groups in protein macromolecules. For these reasons, authors mentioned above indicate superiority of empirical methods versus computational methods of hydrophobicity determination and, in particularly, of those which based on investigation of protein molecule surface subjected to influence of external or internal factors.

Surface hydrophobicity values measured using ANS fluorescence probe (1-anilino-8-naphtalenesulfonate magnesium salt) (Table 4) illustrate differentiation of examined protein preparations with respect to so called "aromatic hydrophobicity". It reflects localization of non-polar, hydrophobic groups of aromatic aminoacids (phenylolanine, tyrosine, tryptophan), reacting with ANS [27].

The highest value of aromatic hydrophobicity was found for bovine blood albumin, fraction V (ALB) (ANS = 48250) and spray blood dried plasma (OL) (ANS=45354), while the lowest value of this parameter was observed for beef meat protein preparation MBL (ANS= 1726) and lupine protein preparation (ŁUB) (ANS= 3918).

Protein surface hydrophobicity measured using cis-parinaric acid (CPA) represents so called "aliphatic hydrophobicity" reflecting availability of aliphatic chains of hydrophobic aminoacids on surface of examined protein molecules [27]. Due to Sklar et al. [34] and Kato and Nakai [8] the CPA using during determination of protein surface hydrophobicity may be superior to another methods because of its natural origin and similarity with native fatty acids taking part in natural interactions with proteins. Under conditions of this study hydrophobicity values obtained with this method varied in the wide range from 924.6 to 24542.5 for lupine protein preparation (PŁUB) and bovine blood albumin(ALB), respectively.

Because of different analytical conditions in respect to ionic strength of buffer applied, pH value, temperature, origin and purity of examined protein preparations both ANS and CPA hydrophobicity values collected in this study differ in comparison with data reported by Voutusinas et al.[38], Nakai et al.[27] and Haskard and Li-Chan [6].

The polyoxyethylene sodium monooleate (Tween 80) as a ligand was used for determination of protein hydrophobicity according to method of Lieske and Konrad [18, 19] and expressed as HP (%) values (Table 4). Alkanes [24], sodium dodecyl sulfate (SDS) [9, 30, 31] and triglicerydes [36] were used for determination of protein hydrophobicity with another binding methods.

HP values collected in this study confirm different character of examined protein preparations indeed, but they do not correspond with results of protein hydrophobicity measured fluorymetrically, with ANS or CPA probes. Table 5 shows statistically significant correlation of relationships between calculated hydrophobicity values Q and NPI (r = 0.566, α <0.05) and between the CPA and NPI values only (r = 0.494, α <0.05).

Table 5. Linear correlation coefficients r for hydrophobicities of protein preparations determined (CPA, ANS, HP) or computational (Q, NPI) by the use of various methods

	CPA	ANS	HP	Q	NPI
CPA	X	0.691*	0.075	0.069	0.475*
ANS	0.691*	X	0.349	0.027	0.137
HP	0.075	0.349	X	0.168	0.038
Q	0.069	0.027	0.168	X	0.566*
NPI	0.475*	0.137	0.038	0.566*	X

*** Statistically significant correlations for α < 0.05**

Results obtained in this part of study suggest that more complex evaluation of protein hydrophobicity with one uniform method is not possible. Aromatic hydrophobicity, aliphatic hydrophobicity, effective or total average hydrophobicity based on the various approaches to this characteristic and it should be taken into consideration during the research.

In case of comparative studies it is necessary, like in protein functionality studying, to standardize analytical conditions of surface protein hydrophobicity determination, especially in respect to pH value, ionic strength and temperature. Consumption of time and work and availability of required reagents and instruments are the next important criteria for selection of proper analytical method of the protein hydrophobicity.

As it is mentioned above, in spite of methodological difficulties, documented also with results of this study, using protein surface hydrophobicity for elucidation and predication of protein functionality is suggested. This problem will be also discussed in our next publication.

CONCLUSIONS

1. Randomly selected group of protein preparations of animal and plant origin obtained under commercial or laboratory conditions were characterized with high differentiation of physico-chemical properties and surface hydrophobicity determined using empirical or computational methods.
2. It is generally accepted, that determined selected properties of examined protein preparations reflect a complex effect of influences resulted from protein nature and processing parameters applied during receiving individual preparations f.e. extraction conditions, temperature and time of drying and their post production storage.
3. Determination of hydrophobicity of protein preparations could be done with different methods and use as a complementary characteristic in a complex laboratory characterization of protein preparations.

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