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Influence of Tribomechanical Micronisation on the Rheological Properties of Whey Proteins

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Summary

Investigations with powdered whey protein concentrates containing 60 % (WPC-60) or 80 % (WPC-80) of proteins were carried out. In this work proteins were treated using the laboratory equipment for tribomechanical micronisation with three different rotor speeds. Before and after the tribomechanical treatment, the analyses of the particle size and particle distribution as well as the specific area and scanning microscopy were carried out.

The influence of tribomechanical treatment as well as hydrocolloid addition on the rheological properties of model systems of whey protein concentrate (10 % of total solid) was studied. Rheological parameters, flow behavior index (n) and consistency coefficient (k) were determined by the power-law model.

The results obtained showed that the tribomechanical treatment involves a significant decrease in particle size, a change in particle distribution and an increase in specific area of powdered whey proteins.

Systems without hydrocolloid addition were Newtonian, but those with hydrocolloid addition exhibited pseudoplastic properties.

The viscosity of model systems containing tribomechanically treated whey protein concentrates (TWPC) was greater in comparison with the same systems prepared with untreated WPC. Systems with greater amount of proteins (WPC-80) had higher viscosity than the same systems containing lower amount of proteins. Hydrocolloid addition affected an increase of viscosity of model systems prepared with untreated WPC. The most significant increase of consistency coefficient (k) was observed in systems with carboxymethylcellulose (CMC) and addition of a special type of carboxymethylcellulose (CMC E466) known as DIKO, icecream binder 0911-E. In systems containing tribomechanically treated TWPC hydrocolloids had various effects. In systems with guar gum, carragenan, inulin, pectin or amid pectin, viscosity increased in comparison with those prepared with untreated WPC. CMC and DIKO affected a significant viscosity decrease of TWPC model systems, which is the consequence of the interaction between proteins and (CMC) hydrocolloids. The found effects were pronounced more strongly as the rotor speed of TMA equipment was greater.

Key words: tribomechanical micronisation, rheological properties, hydrocolloids, whey protein concentrate

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Introduction

The knowledge of rheological properties of foodstuffs and the influence of various ingredients and additives (hydrocolloids and emulsifiers) on those properties is important in defining the product and process quality control, predicting the product stability during storage as well as creating food texture (*1–5*).

Hydrocolloids are long-chain polymers, mainly polyanionic carbohydrates, which are used in small quantities (from 0.05 to 5 %) in food production in order to achieve appropriate rheological properties, prevent syneresis, increase the viscosity and stability of foodstuffs, inhibit crystal growth and to improve coating, film forming, whipping, *etc*. They do not have direct influence on taste or flavour of foodstuffs, but at the same time they have a significant effect on gel formation, water and aroma retention as well as emulsifying properties (*2,6,7*). Widely used hydrocolloids are starch, modified celluloses, alginate, carragheenan, various tree exudates (gums), pectin, agar. Their functions in dairy products are fast hydration that allows cold preparation and instant mixes of dairy creams, water retention, stabilizing whipped products, achieving pseudoplasticity, improving viscosity and texture (*8*). Because of various possible specific interactions among hydrocolloids, water, proteins, inorganic ions and other food components, the amount and type of hydrocolloid must be specified for each food product.

Whey proteins are an excellent source of all the essential amino acids and are easily digested. Typically, they are compact globular proteins with relatively comparable distribution of nonpolar, polar, charged and uncharged side chains of aminoacids. Particles of whey proteins produce three basic fractions: lactoglobulin, immunoglobulin and protease as well as peptone (*9–11*). They also contain relatively high fractions of branched chain amino acids – leucine, isoleucine and valine. Because of their nutritive value as well as due to their functional properties, whey proteins can be used as the main component in infant formulae, weight-gain and weight-reduction diet foods, protein-fortified fruit juices and other healthy foods and drinks.

Functional properties of whey proteins may be classified into three main groups: (a) hydration properties dependent on protein-water interactions that have an important bearing on wetability, swelling, adhesion, dispersibility, solubility, viscosity, water holding capacity; (b) interfacial properties including surface tension, emulsification and foaming characteristics; and (c) aggregation and gelation properties, which are related to protein-protein interactions (*12-15*). The functional role of proteins as food ingredients depends on complex interactions of many factors such as heating or cooling rate, protein concentration, pH, ionic strength as well as extent, and nature of various macromolecular interactions (*15-22*). Functional properties of proteins can sometimes be varied by mechanical means. Extensive whipping, ultraviolet light, high pressures and ultrasonic vibration are denaturation agents with a significant influence on the functional characteristics of proteins. Many studies have shown that high pressures (up to 800 MPa) can modify the protein structure and reactivity. β-lactoglobulin, the main whey protein component, seems to be more sensitive to high pressure treatment than other food proteins. High pressure changes and their conformational and aggregation properties cause a substantial loss of its emulsifying efficiency, improve its foaming ability and can be used for gel formation (*15,23,24*). Such gels have a sponge-like structure and are susceptible to exudation. Whey protein concentrate formed gels improve their structure with an increase in pressurisation temperature. The improvement is more noticeable at high protein concentrations (more than 10 %) (*25*). Because of these effects of high pressure treatments on the functional and structural properties of proteins, it is to be expected that the procedure of tribomechanical micronisation and activation can cause similar changes.

Therefore, the aim of this study was to determine the influence of tribomechanical micronisation and activation on the physical, structural and functional as well as rheological properties of whey protein concentrates and some whey protein model systems. The examinations of the influence of some hydrocolloids addition in whey protein model systems, with the aim to establish their interactions with proteins (treated and untreated) and their effects on the viscosity of the examined systems, were also performed.

Materials and Methods

In this investigation two samples (of different chemical composition) of powdered whey protein concentrate produced by »Meggle« GmbH, Wasserburg, Germany, were used. The samples were marked as WPC-60 and WPC-80. Their chemical composition was declared by the producer and is shown in Table 1.

Table 1. Chemical composition of whey protein concentrate (WPC)

Component	WPC-60	WPC-80
w (protein) / %	60.0	80.0
w (lactose) / %	25.0	5.0
w (fat) / %	6.0	6.0
w (water) / %	3.0	3.0
w (ash) / %	6.0	6.0

Investigations of rheological properties were carried out with eight model systems (Table 2 and 3), prepared by mixing powdered whey proteins (treated tribomechanically at 16 000; 20 000 and 22 000 rpm and untreated) with several hydrocolloids. Hydrocolloids used were:

- *Inulin*, Ljubljanska mljekara, Ljubljana – Inulin is a mixture of linear (2,1)-fructose-polymer with different chain-length and one glucose at each C2-end.
- *DIKO* – Special type of carboxymethylcellulose (CMC E466), Kristall Chemie, Wiener Neudorf
- *Guar gum*, Giullini Chemie, Ludwigshafen – Guar gum is a mixture of galactose and manose in proportion 1 : 2.

– *Carboxymethylcellulose* (CMC), Giullini Chemie, Ludwigshafen
– *Carragenan* (Aquagel GU 805), Werba Chemie, Handelsgellschaft m.b.H. Wien – high amount of κ-carragheenan.
– *Pectin classic* (AY 905), Werba Chemie, Handelsgellschaft m.b.H. Wien – low methoxyl Apple Pectin, standardized with dextrose, degree of esterification 35–42 %.
– *Amid pectin*, Werba Chemie, Handelsgellschaft m.b.H. Wien – low methoxyl, amidated Apple Pectin, standardized with phosphates and dextrose, degree of esterification 28–34 %, degree of amidation 16–22 %.

Hydrocolloids were purchased from several producers under their commercial names and with recommended advice for use.

These hydrocolloids, known as thickeners or gelling agents, were used with the aim of investigating their influence on the viscosity of protein dispersions containing untreated or tribomechanically treated whey proteins and their possible interactions with proteins.

Preparation of model systems

Model systems marked as A1-4 (WPC-60) or AA1-4 (WPC-80) were aqueous dispersions of powdered whey protein concentrate containing 10.0 % of dry matter. For this purpose 10.3 g of powdered WPC (97.3 % of solid matter) untreated and treated at 16 000, 20 000 and 22 000 rpm were dispersed in 89.7 g of distilled water by vigorous hand mixing at 20 °C.

Model systems marked as B1-4 to H1-4 or BB1-4 to HH1-4 were prepared as aqueous dispersions of a powdered whey protein concentrate and seven various hydrocolloids were added to the amount of 0.4 %. For this preparation, in 89.7 g of distilled water, 9.9 g of powdered WPC (97.3 % of solid matter) untreated and treated at 16 000, 20 000 or 22 000 rpm) were dispersed. Then 0.4 g of hydrocolloid were added with vigorous hand mixing at 20 °C. Dispersions containing 10 % of solid matter were chosen because of the possible changes of protein functional properties which are better observed in systems with higher protein concentration.

Tribomechanical micronization

Samples of powdered whey protein concentrate were tribomechanically treated with the laboratory equipment for tribomechanical micronisation and activation (TMA equipment) shown in Fig. 1. The equipment consists of housing that was cooled with cold water and two rotor disks placed one against the other. Each disk supplied with 3 to 7 concentric wreaths with especially constructed hard metal elements. Disks rotated in opposite directions at the same angular rate. The starting material entered the equipment through the central part of the rotor system by ventilating air streaming. Therefore, the particles were accelerated and, because of the repeated change of motion directions, they were in collision and friction at short time intervals (< 1 ms). The results of such treatment were fragmentation, increase of specific area and change of energetic potential (activity) of materials. This equipment, patented by Tihomir Lelas, was made by »Bauer Maschinenbau«, Hamburg, Germany and now it is situated in the Company »Geomin« Villach, Austria (*26*).

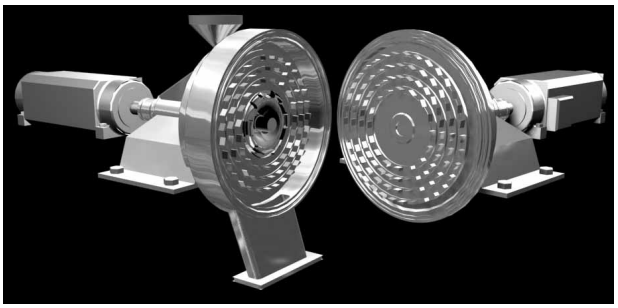

Fig. 1. Laboratory equipment for tribomechanical micronisation and activation (TMA equipment)

The treatment of WPC was carried out at three rotor speeds: 16 000, 20 000 and 22 000 rpm.

Input temperature of the material was 24 °C and outgoing temperature was 30.4 °C. This insignificant temperature difference achieved during micronisation was, in spite of high friction and collision that occurred among particles, the consequence of cooling of the housing of equipment and very short time required for passing through the equipment. For the micronisation of 1.0 kg of powdered material only 12 s were needed.

With such a low temperature increase we tried to eliminate its influence on the protein stability (denaturation) as well as on the viscosity of the examined protein dispersions.

Analytical methods

Before and after tribomechanical micronisation of the powdered whey protein concentrates, the following analyses were carried out: particle size and distribution, specific area and scanning microscopy.

Particle size determination

Particle size of the powdered whey protein concentrates before and after tribomechanical treatment was measured by Malvern Mastersizer X using a range lens of 100 mm. The angular dependence of the intensity of the laser light scattered from a dilute suspenion was measured and then the particle size distribution was indicated, which gave the closest fit between theoretical calculations (MIE theory) (*27*) and experimental measurements. This method was used also for a specific area determination.

Scanning microscopy

Scanning microsopy of powdered WPC before and after tribomechanical activation was made after sample preparation technique using Edwards S-150, sputter--coater unit. Electron micrographs were taken with microscope type JOEL-JSM-5800.

Rheology

Rheological properties of model systems were performed at 20 °C using a rotational rheometer, Brookfield

Table 2. Composition of model systems prepared with WPC-60 (samples A to H) and WPC-80 (samples AA to HH) and hydrocolloids

Sample	Type of hydrocolloid	*m* (WPC untreated)	*m* (TWPC treated at 16 000 rpm)	*m* (TWPC treated at 20 000 rpm)	*m* (TWPC treated at 22 000 rpm)	*m* (Water)
		g	g	g	g	g
A1, AA1	Without	10.3	–	–	–	89.7
A2, AA2	hidrocolloid	–	10.3	–	–	89.7
A3, AA3		–	–	10.3	–	89.7
A4,AA4		–	–	–	10.3	89.7
B1, BB1	0.4 g	9.9	–	–	–	89.7
B2,BB2	guar gum	–	9.9	–	–	89.7
B3,BB3		–	–	9.9	–	89.7
B4,BB4		–	–	–	9.9	89.7
C1,CC1	0.4 g	9.9	–	–	–	89.7
C2,CC2	CMC	–	9.9	–	–	89.7
C3,CC3		–	–	9.9	–	89.7
C4,CC4		–	–	–	9.9	89.7
D1,DD1	0.4 g	9.9	–	–	–	89.7
D2,DD2	carragenan	–	9.9	–	–	89.7
D3,DD3		–	–	9.9	–	89.7
D4,DD4		–	–	–	9.9	89.7
E1,EE1	0.4 g	9.9	–	–	–	89.7
E2,EE2	DIKO	–	9.9	–	–	89.7
E3,EE3		–	–	9.9	–	89.7
E4,EE4		–	–	–	9.9	89.7
F1,FF1	0.4 g	9.9	–	–	–	89.7
F2,FF2	inulin	–	9.9	–	–	89.7
F3,FF3		–	–	9.9	–	89.7
F4,FF4		–	–	–	9.9	89.7
G1,GG1	0.4 g	9.9	–	–	–	89.7
G2,GG2	pectin classic	–	9.9	–	–	89.7
G3,GG3		–	–	9.9	–	89.7
G4,GG4		–	–	–	9.9	89.7
H1,HH1	0.4 g	9.9	–	–	–	89.7
H2,HH2	amid pectin	–	9.9	–	–	89.7
H3,HH3		–	–	9.9	–	89.7
H4,HH4		–	–	–	9.9	89.7

DV-III with coaxial cylinders, type MV-I, where the radius of the outer cylinder was 21 mm and that of the inner cylinder was 20.04 mm. The height of the inner cylinder was 60 mm. Shear stress against the increasing shear rates from the lowest value 3.9 to 317 s^{-1} (rising measurements), as well as from 317 s^{-1} to the lowest shear rate value, were measured. At the highest shear rate, shear stress lasted two minutes and after that the rotational rate decreased successively to the initial value. All measurements were carried out three times and mean values of the obtained results were recalculated in the flow behaviour index (n) and consistency coefficient value (k). The calculations were performed using the Ostwald de Waele (power-law) model (using Brookfield´s computer program) as follows:

$$\tau = k \cdot \gamma^n \qquad /1/$$

where: τ = *shear stress (Pa)*, γ = shear rate (s^{-1}), k = consistency coefficient (Pa s^n), n = flow behaviour index.

This model is the most useful model applicable to a wide variety of fluid foods, especially time-independent non-Newtonian fluids, over a range of intermediate shear rates (*28*).

Suitability of the Ostwald de Waele model for data analysing was determined after the regression analysis according to the least square method. Correlation coefficients were calculated on 10 experimental data.

Apparent viscosity at shear rate of 40 s^{-1} was calculated using the Newtonian law.

Results and Discussion

In the equipment for tribomechanical micronisation, the intensive mechanical strains cause friction and collision among particles of hard mineral substances and the rupture of their crystal grates occurs. The consequence of this procedure is the decrease of the particle size, increase of a specific area and change of energetic potential (*26*).

We supposed that similar effects could be obtained using organic macromolecules such as proteins. Therefore, our investigations were carried out with whey protein. The main whey protein component is β-lactoglobulin, a compact globular protein with relatively comparable distribution of nonpolar, polar, charged and uncharged side chains of aminoacids. The formation of aggregates is most probably due to the generation of intermolecular disulfide bridges through SH/-S-S- interchange reactions (*29,30*). Cysteine side chains are mostly situated inside the proteins (hydrophobic part of proteins), while the outer side of the proteins is made of hydrophilic side chains of aminoacids (*31*).

Tribomechanical micronisation has manifested a significant influence on the structure of whey proteins

(Figs. 4–7), their particle size, its distribution and specific area.

The results of the particle size determination have shown that tribomechanical treatment of powdered whey proteins significantly decreased the particle size and changed their distribution. The intensity of these changes depends upon the kind of WPC and the rotor speed of TMA equipment. In general, the particle sizes were lower and the specific area was greater, as the rotor speed increased (Table 3, Figs. 2 and 3). Significantly greater effect of the rotor speed was observed using WPC-60. At 16 000 rpm 90 % of particles had a diameter lower than 68.68 μm, but at 22 000 rpm 90 % of particles were lower than 33.90 μm. Mean values of particle size distribution at the highest rotor speed showed that the greatest number of particles had diameters of 1.96 and 20.90 μm, respectively (Fig. 2). Tribomechanically treated WPC-80 had greater particles than the sample of WPC-60 treated in the same way. This different effect of tribomechanical micronisation on WPC-60 and WPC-80 could be explained by their chemical composition. Namely, WPC-80 contains 80 % of proteins, while WPC-60 contains only 60 % of proteins. Therefore, a stronger effect, at the same process conditions, was obtained in the sample with lower protein concentration.

Specific area of particles was greater in TWPC-60 than in TWPC-80 and at higher rotor speed of TMA equipment (Table 3).

In Figs. 5 and 7, the size and shape of tribomechanically treated whey protein globules obtained by scanning microscopy are shown. From these pictures the places where the rupturing of particles area has occurred can be seen as well as the presence of greater amounts of smaller fragments of globules. Such exchanged structure of proteins can cause changes of their physico-chemical and functional properties. It is known that high temperature and high pressure treatment can also change the protein structure. When most globular pro-

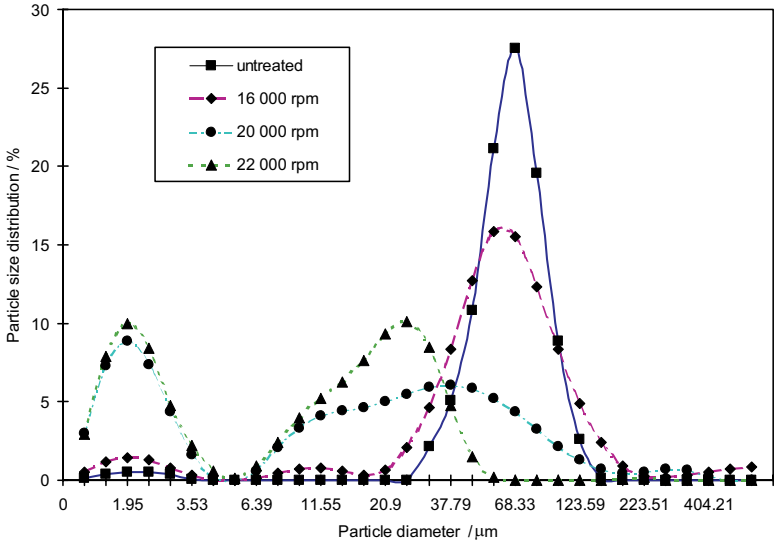

Fig. 2. Particle size distribution of powdered WPC-60 before and after tribomechanical treatment

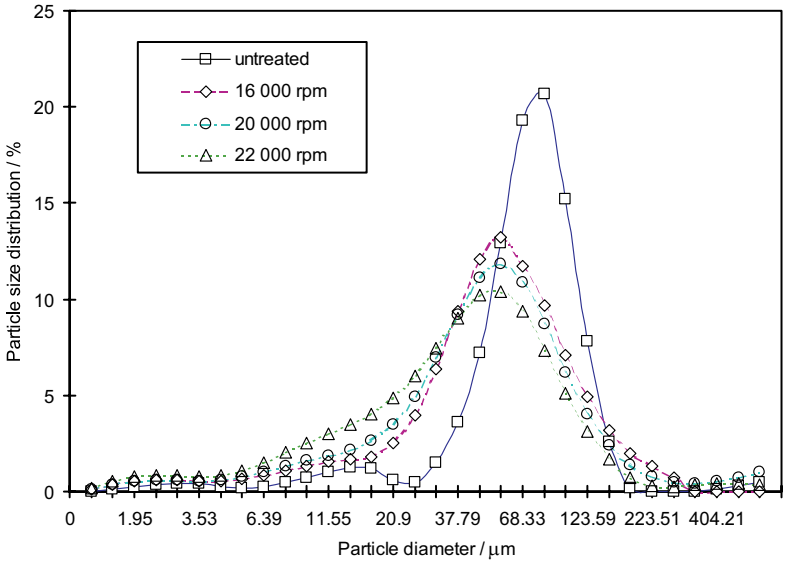

Fig. 3. Particle size distribution of powdered WPC-80 before and after tribomechanical treatment

Table 3. Particle size analysis and specific area of powdered whey protein concentrates (WPC) before and after tribomechanical micronisation

Sample	Treatment	Specific area	Particle size / μm		
		m^2/g	10 % under	50 % under	90 % under
WPC-60	untreated	0.1736	38.38	59.73	85.84
	16 000 rpm	1.2534	3.59	17.79	68.68
	20 000 rpm	1.3513	1.92	14.32	43.61
	22 000 rpm	1.3770	1.61	13.33	33.90
WPC-80	untreated	0.1643	32.29	66.69	104.25
	16 000 rpm	0.1917	13.55	46.59	109.90
	20 000 rpm	0.2436	10.94	45.78	101.33
	22 000 rpm	0.2786	9.92	45.07	97.41

Table 4. Rheological parameters of model systems prepared with WPC-60

Sample	Treatment	Apparent viscosity	Flow behaviour index	Consistency coefficient	Correlation coefficient
		mPa s*	n	k / mPa s^n	r^2
A1	Untreated	1.40	1.084	2.0	0.998
A2	16 000 rpm	1.50	1.083	2.0	0.998
A3	20 000 rpm	1.50	1.074	3.0	0.997
A4	22 000 rpm	1.60	1.068	3.0	0.996
B1	Untreated	53.00	0.723	166.0	0.994
B2	16 000 rpm	69.00	0.716	184.0	0.999
B3	20 000 rpm	73.70	0.714	197.0	0.999
B4	22 000 rpm	83.30	0.731	236.0	0.999
C1	Untreated	65.70	0.715	177.0	0.995
C2	16 000 rpm	40.70	0.731	109.0	0.997
C3	20 000 rpm	38.00	0.737	80.0	0.983
C4	22 000 rpm	23.40	0.817	47.0	0.999
D1	Untreated	2.20	1.097	14.0	0.998
D2	16 000 rpm	2.20	1.095	16.0	0.999
D3	20 000 rpm	2.60	1.103	17.0	0.999
D4	22 000 rpm	2.80	1.101	25.0	0.999
E1	Untreated	33.00	0.572	202.0	0.971
E2	16 000 rpm	28.70	0.642	131.0	0.976
E3	20 000 rpm	27.20	0.699	94.0	0.983
E4	22 000 rpm	25.00	0.753	66.0	0.998
F1	Untreated	3.90	0.886	4.3	0.996
F2	16 000 rpm	3.90	0.888	4.9	0.998
F3	20 000 rpm	3.89	0.871	5.9	0.999
F4	22 000 rpm	3.98	0.855	6.5	0.996
G1	Untreated	12.00	0.845	11.4	0.999
G2	16 000 rpm	14.70	0.668	27.5	0.997
G3	20 000 rpm	17.30	0.738	32.4	0.998
G4	22 000 rpm	29.50	0.558	53.9	0.977
H1	Untreated	3.25	0.943	3.8	0.999
H2	16 000 rpm	3.60	0.966	3.9	0.999
H3	20 000 rpm	3.90	0.923	4.3	0.993
H4	22 000 rpm	4.30	0.901	5.8	0.999

*Apparent viscosity at shear rate 40 s^{-1} was calculated using Newtonian law

teins are heated, their structure changes, most often irreversibly. β-lactoglobulin and α-lactoglobulin show well-defined thermal transitions in the range between 60 and 80 °C, depending on the concentration of the protein. Once the proteins become denatured by heat, they can probably react further, forming complexes with other proteins (*32*).

Many authors have reviewed the effects of high pressure on proteins. Pressure-induced denaturation of proteins is a complex phenomenon that depends on the protein structure, pressure, temperature, pH, ionic strength and solvent composition (*33*). High pressure applied to the globular proteins, like β-lactoglobulin, can lead to aggregation and ultimately, under appropriate conditions (pH, temperature, pressure, hydrocolloid addition) and high enough concentrations, to gelation and precipitation. Disulfide bonds play the main role in this process, because high pressure increases the reactivity of

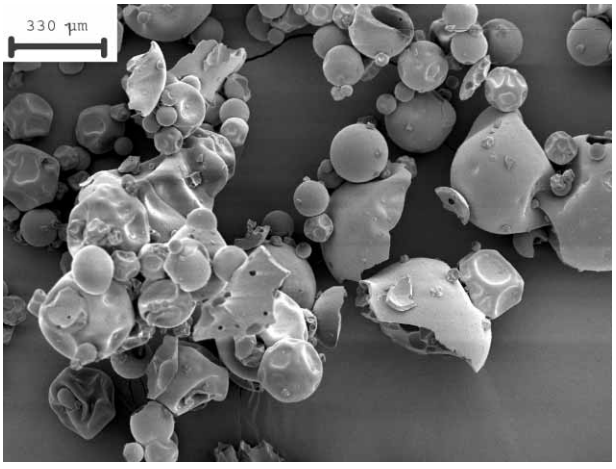

Fig. 4. Size and shape of untreated protein globules of powdered WPC-60

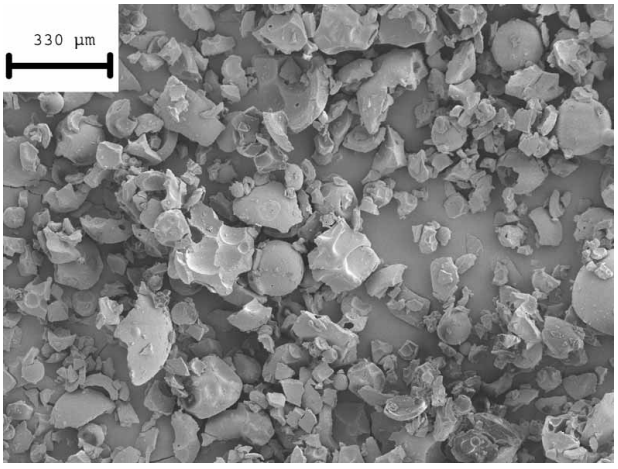

Fig 5. Size and shape of tribomechanicaly treated (22 000 rpm) protein globules of powdered WPC-60

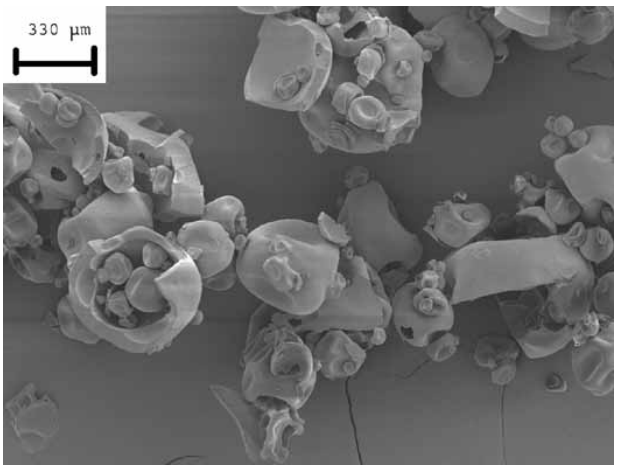

Fig. 6. Size and shape of untreated protein globules of powdered WPC-80

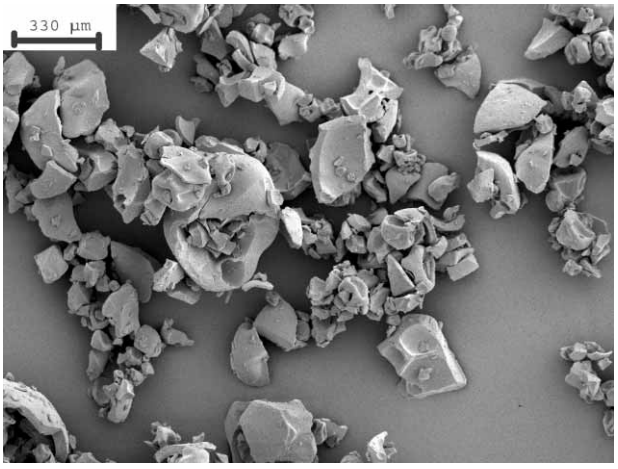

Fig 7. Size and shape of tribomechanicaly treated (22 000 rpm) protein globules of powdered WPC-80

-SH groups. Some investigations have shown that the combined use of elevated temperature and pressure also give variable results, which is highly dependent on the kind of protein (*15*).

Based on this knowledge, the investigation of the influence of the process of tribomecahnical micronisation on the structure and functional properties of whey proteins and, consequently, on the rheology of protein dispersions was performed. The advantages of this process, when compared with high pressure or heating processes, are: short time of treatment (several seconds), low temperature (< 31 °C), simple procedure and low cost.

The investigations of rheological properties have been carried out on various model systems prepared with tribomechanically treated WPC and some hydrocolloids. With the purpose of eliminating the influence of solid matter content, all model systems were prepared according to the recipe that enables reaching the same fraction of solid matter in all systems (10 %).

Rheological properties of the examined model systems are adequately described according to the Ostwald de Waele power-law model (correlation coefficient was between 0.887 and 0.999) and expressed as the consistency coefficient (k) and flow behavior index (n). The apparent viscosity at shear rate 40 s^{-1} has been determined against the Newtonian law.

Model systems containing only WPC-60 or WPC-80 were Newtonian ($n \approx 1$). The hydrocolloids addition changed this Newtonian character and almost all systems exhibited pseudoplastic properties ($n<1$). This is in agreement with the fact that pseudoplasic fluids are mostly dispersions containing asymmetrical particles or high molecular weight polymers. At low shear rates all systems are pseudoplastic and no zero shear viscosity has occurred. On the contrary, at higher shear rates systems have exhibited a Newtonianlike constant viscosity, the so-called infinite shear rate viscosity (η_∞). This Newtonianlike plateau for all investigated systems began at shear rate 39.6 s^{-1}, except for the systems with guar gum addition where it has begun at shear rate 52.8 s^{-1}. Therefore, the apparent viscosity was determined at shear rate 40.0 s^{-1}.

From the results shown in Tables 4 and 5, it can be seen that all model systems prepared with WPC-80 (with or without hydrocolloid addition) have had a significantly higher viscosity (higher consistency coefficient

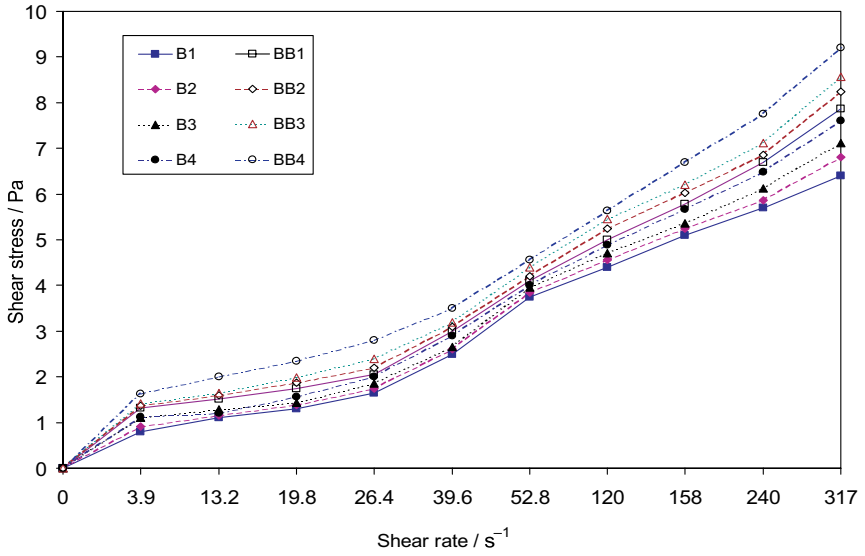

Fig. 8. Shear rate and shear stress relationship of model systems with addition of guar gum

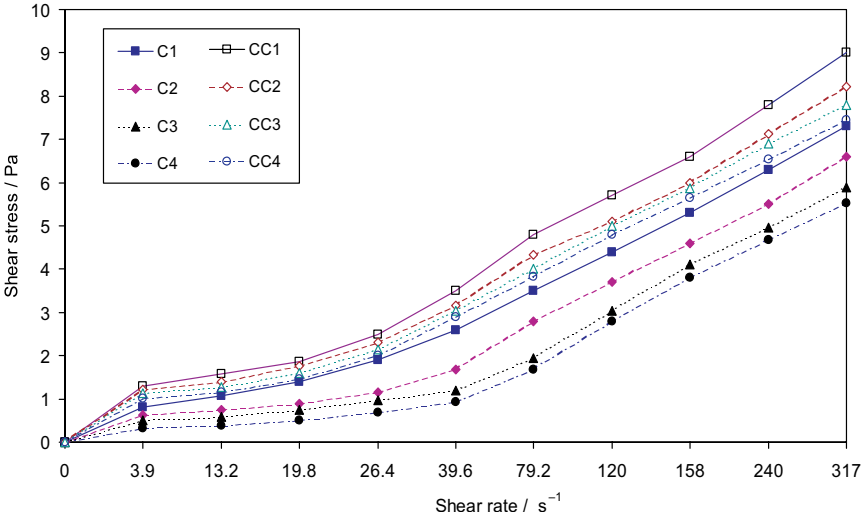

Fig. 9. Shear rate and shear stress relationship of model systems with addition of CMC

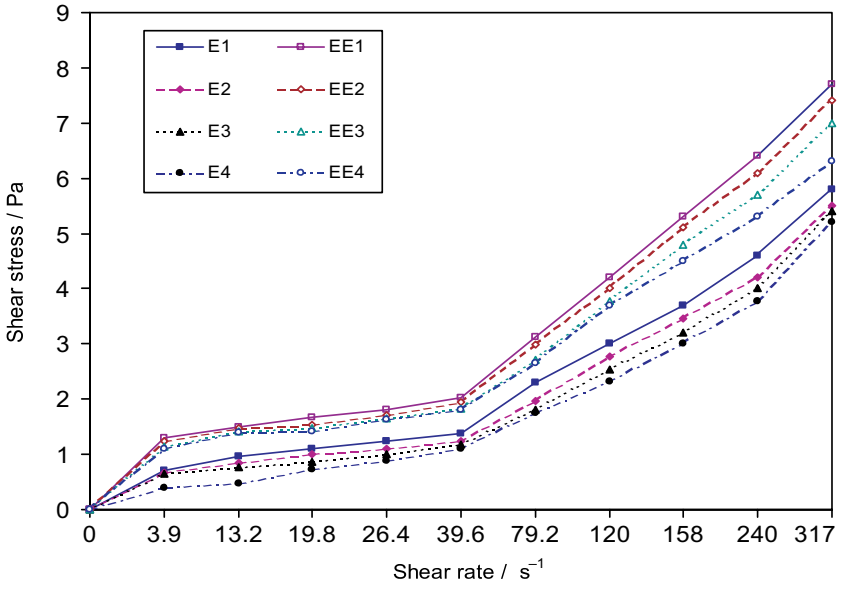

Fig. 10. Shear rate and shear stress relationship of model systems with addition of DIKO

Table 5. Rheological parameters of model systems prepared with WPC-80

Sample	Treatment	Apparent viscosity / mPa s*	Flow behaviour index / *n*	Consistency coefficient / *k* / mPa s^n	Correlation coefficient / r^2
AA1	Untreated	4.80	1.048	4.0	0.996
AA2	16 000 rpm	5.20	1.025	5.0	0.999
AA3	20 000 rpm	6.80	0.986	7.0	0.999
AA4	22 000 rpm	6.90	0.898	13.0	0.998
BB1	Untreated	92.60	0.723	223.0	0.987
BB2	16 000 rpm	95.10	0.716	242.0	0.997
BB3	20 000 rpm	98.60	0.764	252.0	0.998
BB4	22 000 rpm	99.70	0.550	610.0	0.999
CC1	Untreated	100.00	0.495	488.0	0.949
CC2	16 000 rpm	82.00	0.628	281.0	0.984
CC3	20 000 rpm	80.00	0.730	252.0	0.971
CC4	22 000 rpm	78.00	0.742	207.0	0.887
DD1	Untreated	10.30	0.897	15.3	0.999
DD2	16 000 rpm	10.90	0.865	18.6	0.999
DD3	20 000 rpm	11.30	0.870	24.4	0.998
DD4	22 000 rpm	15.40	0.805	32.8	0.999
EE1	Untreated	51.20	0.405	517.0	0.973
EE2	16 000 rpm	50.00	0.399	503.0	0.970
EE3	20 000 rpm	48.00	0.365	498.0	0.971
EE4	22 000 rpm	44.00	0.366	488.0	0.964
FF1	Untreated	10.20	0.801	21.4	0.999
FF2	16 000 rpm	11.90	0.804	23.0	0.999
FF3	20 000 rpm	11.80	0.780	26.6	0.999
FF4	22 000 rpm	14.40	0.776	33.0	0.999
GG1	Untreated	10.80	0.886	17.1	0.999
GG2	16 000 rpm	12.20	0.829	24.7	0.999
GG3	20 000 rpm	14.00	0.788	36.2	0.999
GG4	22 000 rpm	18.60	0.712	69.8	0.999
HH1	Untreated	23.60	0.585	75.4	0.999
HH2	16 000 rpm	27.40	0.636	111.3	0.999
HH3	20 000 rpm	28.20	0.662	127.2	0.999
HH4	22 000 rpm	46.60	0.685	138.8	0.989

Apparent viscosity at shear rate 40 s^{-1} was calculated using Newtonian law

values) than model systems prepared with WPC-60 (with or without hydrocolloid addition), which can be explained by a dramatically higher protein fraction in WPC-80. The greatest differences in consistency coefficient values, as well as apparent viscosity values have been observed in samples that contained amid pectin (H), CMC (C), DIKO (E) and inulin (F).

In our preliminary investigations it was found that as the result of whey protein and carboxymethylcellulose interaction, the viscosity of model systems, prepared with WPC (10 % solid matter), was significantly lower than aqueous hydrocolloid solutions (0.4 % solid matter) (*7*,*20*,*34*,*35*).

However, it has been observed that the viscosity change depends on the kind of hydrocolloids used, a possible interaction between whey proteins and hydrocolloids as well as on the rotor speed of TMA equipment. The greatest influence on the rheological properties of model systems prepared with untreated WPC was exhibited with guar gum, CMC and DIKO, while the influence of other hydrocolloids (carragenan, pectin classic, amid pectin) was siginificantly lower.

All model systems prepared with tribomechanically treated whey proteins have greater viscosity when compared to those prepared with untreated WPC (22 % model systems prepared with carragenan, 25 % model systems prepared with inulin and others), except model systems prepared with CMC or DIKO addition (Figs. 8–10).

Systems prepared with CMC or DIKO addition have had lower viscosity than the same systems prepared with untreated WPC (Figs. 9 and 10). This phenomenon can be explained by the interactions between the positive active groups of proteins and the negative groups of hydrocolloids. It is also important to take into consideration the presence of a significant amount of mineral components in WPC as well as the ability of polyanion polysaccharides (CMC and DIKO) for cation binding. Such interactions can obviously improve the homogeneity of systems (*36*). But in this case the contact of ion pairs (CMC – whey proteins) had a negative effect on the rheological properties of the model system (*7*,*20*,*34*,*35*). This can be explained by the decrease of the number of active groups of hydrocolloids that bind water molecules.

Namely, the procedure of tribomechanical treatment of whey proteins affects the appearance of a greater amount of protein particles and, consequently, causes an increase of the number of protein active groups available for interactions with polyanion polysaccharides (CMC and DIKO). The result is lower water binding capacity of hydrocolloids and decreasing viscosity of the model systems. The above observations confirm the fact that all model systems prepared with tribomechanically treated WPC and carragenan, guar gum, amid pectin, pectin classic or inulin addition have a distinctly higher viscosity than the same systems prepared with untreated WPC. This may be explained by non-existence of interactions between proteins and these hydrocolloids as well as by the specific molecular structure of whey proteins. After tribomechanical treatment, as it has been stated above, a greater number of protein fragments and peptides occurred. The outer side of protein fragments (particles), consists mostly of hydrophobic side chains of aminoacides making them more efficient in binding with water molecules and resulting in a significant increase of model systems viscosity.

Conclusion

The process of tribomechanical micronisation causes the change of granulometric composition of powdered whey protein concentrates. Their particle size decreases dramatically and specific area increases significantly. These changes are greater as the rotor speed increases.

Rheological properties of the examined model systems are adequately described according to the Ostwald de Waele (power-law) model. The systems without hydrocolloid addition were Newtonian, while those with hydrocolloids addition exhibited mostly pseudoplastic properties (non-Newtonian).

All model systems prepared with WPC-80 showed a significantly higher viscosity than model systems prepared with WPC-60. Model systems prepared with tribomechanically treated whey proteins had greater viscosity except for the model systems prepared with CMC as well as hydrocolloid DIKO addition, as the result of the interaction between whey proteins and carboxymethylcellulose. This behavior is more expressive when the rotor speed of TMA equipment is higher.

From the results obtained it follows that the procedure of tribomechanical micronisation could be used to improve some of the whey protein functional properties such as viscosity of protein dispersions, better interactions between proteins and hydrocolloids (except hydrocolloids based on cellulose), better gelling and foaming ability. This can lead to the production of various whey protein based food products (infant foods, athletes foods, dairy desserts, ice cream, etc.) of better organoleptic and nutritional quality as well as structural stability.

References

1. V. Hegedušić, M. Carić, Z. Herceg, D. Rade, *Mljekarstvo, 45* (1995) 191–203.
2. V. Hegedušić, S. Rimac, Z. Herceg, M. Škreblin, *Mljekarstvo, 48* (1998) 87–96.
3. Z. Herceg, V. Hegedušić, S. Rimac, *Mljekarstvo, 49* (1999) 15–26.
4. K. Demetriades, J. N. Coupland, D. J. McClements, *J. Food Sci. 62* (1997) 462–467.
5. V. J. Davidson, H. D. Goff, A. Flores, *J. Food Sci. 61* (1996) 573–576.
6. Z. Herceg, V. Hegedušić, S. Rimac, *Mljekarstvo, 49* (1999) 83–94.
7. Z. Herceg, V. Hegedušić, S. Rimac, *Acta Aliment. 29* (2000) 89–103.
8. G. O. Phillips, P. A. Williams: Interaction of Hydrocolloids in Food Systems. In: *Ingredient Interactions*, A. G. Gaonkar (Ed.), Marcel Dekker, New York (1995) pp. 131–169.
9. P. Havea, H. Singh, L. K. Creamer, O. H. Campanella, *J. Dairy Res. 68* (1998) 79–91.
10. T. Y. Hung D. M. Smith, *J. Food Sci. 58* (1993) 1047–1058.
11. B. H. Webb, A. H. Johnson, J. A. Alford: *Fundamental of Dairy Chemistry*, AVI Publishing Company, Westport (1974).
12. L. M. Huffman, *Food Technol. 50* (1996) 49–52.
13. L. King, *Food Tech. Eur. 3* (1996) 88–89.
14. C. Corradini, *Scienzia e Technca Lattiero Casearia, 49* (1998) 204–213.
15. V. B. Galazka, E. Dickinson, D. A. Ledward, *Curr. Opin. Colloid Interface Sci. 5* (2000) 182–187.
16. D. M. Smith: Protein Interaction in Gels: Protein – Protein Interactions. In*: Thermal Analysis of Foods*, O. R. Fabbena, M. Karel, W. Sanderson, S. R. Tannanbaum, P. Walstra, J. R. Whitaker (Eds.), Elsevier Appl. Sci., New York (1994) pp. 209–224.
17. M. R. Alizadehfard, D. E. Wiley, *Iran J. Polym. Sci. Technol. 4* (1995) 126–133.
18. A. Bhargava, P. Jelen, *Int. Dairy J. 5* (1995) 533–541.
19. J. I. Boye, I. Alli, H. Ramaswamy, V. G. S. Raghavan, *J. Food Sci. 62* (1997) 57–65.
20. V. Hegedušić, Z. Herceg, S. Rimac, *Food Technol. Biotechnol. 38* (2000) 19–26.
21. Y. C. Ker, R. T. Toledo, *J. Food Sci. 57* (1992) 82.
22. Z. Herceg, V. Hegedušić, S. Rimac, B. Šimundić, *Mljekarstvo, 49* (1999) 139-152.
23. V. B. Galazka, E. Dickinson, D. A. Ledward, *Food Hydrocolloids, 10* (1996) 213–219.
24. E. M. Dumay, M. T. Kalichevsky, J. C.Cheftel, *Lebensm. Wiss. Technol. 31* (1998) 10–19.
25. J. Van Camp, G. Feys, A. Huyghebaert, *Lebensm. Wiss. Technol. 29* (1996) 49–57.
26. T. Lelas, Patent: PCT/1B 99/00757, Vorrichtung zum Mikronisieren von Materialien und neuartige Verwendungsmoglichkeiten derartig mikronisierter Materialien, Geneve (1998).
27. C. F. Bohren, D. R. Huffman: Absorption and Scatering of Light by Small Particles, Wiley, New York (1983).
28. M. A. Tung, A. T. Paulson, Rheological concepts for probing ingredient interactions in food systems. In: *Ingredient Interactions*, A. G. Gaonkar (Ed.), Marcel Dekker, New York (1995) pp. 45–83.
29. V. B. Galazka, I. G. Sumner, D. A. Ledward, *Food Chem. 57* (1996) 393–398.
30. V. Y. Grinberg, T. Haertle, *J. Biotechnol. 79* (2000) 205–209.
31. Lj. Tratnik: *Milk-Technology, Biochemistry and Microbiology*, Croatian Milk Society, Zagreb (1998) pp. 345–380.
32. D. D. Dalgleish, J. A. Hunt: Protein-Protein Interactions in Food Materials. In: *Ingredient Interactions*, A. G. Gaonkar (Ed.), Marcel Dekker, New York (1995) pp. 199–233.
33. P. Masson: Pressure denaturation of proteins. In: *High Pressure and Biotechnology, Colloque, INSERM*, Montrouge: John Libbey Eurotex Ltd. *224* (1992) pp. 89–99.

34. Z. Herceg, V. Lelas, S. Rimac, *Mljekarstvo, 51* (2001) 105–118.
35. Z. Herceg, V. Hegedušić, S. Rimac, *Proceedings of the 2nd International Symposium on Food Rheology and Structure*, Zurich, Switzerland (2000) pp. 378–379.
36. G. O. Phillips, P. A. Williams: *Interaction of Hydrocolloids in Food Systems*, Marcel Decker, New York (1995).

Utjecaj tribomehaničke mikronizacije na reološka svojstva proteina sirutke

Sažetak

U ovom su radu istraživanja provedena s praškastim koncentratima proteina sirutke koji su sadržavali 60 % (WPC-60) ili 80 % (WPC-80) proteina. Uzorci su obrađeni u laboratorijskom uređaju za tribomehaničku mikronizaciju i aktivaciju pri trima različitim brzinama okretaja rotora. Prije i nakon tribomehaničke obradbe određena im je veličina i razdioba veličine čestica, te specifična površina, a provedena je i pretražna mikroskopija.

Utjecaj tribomehaničke obradbe te dodatka hidrokoloida na reološka svojstva koncentrata proteina sirutke ispitivan je na modelnim sustavima koji su sadržavali 10 % suhe tvari. Reološki parametri, indeks tečenja (n) i koeficijent konzistencije (k), određeni su pomoću modela potencijalne funkcije.

Dobiveni su rezultati pokazali da se tribomehaničkom obradbom bitno smanjuje veličina čestica, mijenja njihova razdioba, te povećava slobodna površina praškastih koncentrata proteina sirutke.

Sustavi bez dodatka hidrokoloida imali su newtonski karakter, a oni s dodanim hidrokoloidima uglavnom pseudoplastična svojstva.

Utvrđeno je da je viskoznost modelnih sustava s dodatkom tribomehanički obrađenih WPC (TWPC) veća nego istih sustava s dodatkom neobrađenih WPC. Sustavi istog udjela suhe tvari, ali s većim udjelom proteina (WPC-80), imali su veću viskoznost od istih sustava s manjim udjelom proteina (WPC-60). Dodatak hidrokoloida utjecao je na povećanje viskoznosti modelnih sustava pripremljenih s neobrađenim WPC, pri čemu je najznačajnije povećanje viskoznosti izraženo koeficijentom konzistencije (k) utvrđeno u sustavu s dodatkom karboksimetilceluloze (CMC) i dodatka specijalnog tipa karboksimetilceluloze (CMC E466, poznate kao DIKO, vezivo za sladoled). U sustavu s tribomehanički obrađenim proteinima sirutke (TWPC) dodatak je hidrokoloida različito utjecao. U onima s guar gumom, karagenom, inulinom, pektinom i amidiranim pektinom viskoznost se povećala u usporedbi sa sustavima s neobrađenim WPC, a u onima s CMC i DIKO viskoznost se smanjila, što je posljedica međudjelovanja između proteina i hidrokoloida. Utvrđeni utjecaji bili su to jače izraženi što je bila veća brzina okretaja rotora pri obradbi WPC-a.