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Microparticulation of Whey Protein Concentrates using the Hot Extrusion Process: The Influence of Protein Concentrations and Other Parameters

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Abstract

Microparticulation is influenced by many factors of the hot extrusion process, including process and system parameters as well as product properties. The key aim of this study was to investigate the influence of protein concentrations ($C_{protein}$) and process parameters on microparticulation of whey protein using the hot extrusion process. During the extrusion process, demineralized water was used to reach the desired $C_{protein}$ of 20, 25, 30 and 35% respectively. The experiments were carried out using a corotating twin-screw extruder (ZSE18HP) with an L/D ratio of 40:1 at 90°C as the maximum barrel temperature. The particle size of the microparticulate whey proteins (MWPs) was measured using laser diffractometry, and particle size distributions were calculated by Fraunhofer approximation. The colors of all extruded samples were determined using the CIE L*a*b* system. Additionally, the microscopic properties of particles were determined using a digital microscope with a high-magnification lens. The statistical analysis and data fitting were done using SPSS. Protein concentrations showed a significant ($p<0.001$) influence on the particle size distribution of microparticulated whey proteins. The particle sizes of MWPs declined by raising the $C_{protein}$ up by 30%. On the other hand, the increase of screw speeds caused the particle sizes to decrease. However, 35% of $C_{protein}$ and a screw speed of 200 rpm gave us the opposite results. The particles that were $d_{50} \leq 5$ µm and $d_{50} \leq 3$ µm in size were observed from the screw speed range of 400 rpm to 600 rpm and 800 to 1000 rpm respectively. In terms of particle size distributions, the screw speeds of the extruder were also statistically significant ($p<0.001$). In conclusion, the particle size distributions in the so-called microparticulation process can be controlled through the manipulation of the $C_{protein}$ as well as the screw speed of the extruder.

Keywords: Microparticulation; Protein concentrations; Hot-extrusion; Screw speed; Whey proteins

Introduction

Microparticulation of whey protein concentrates (WPCs) occurs because of protein denaturation under temperature and shear. Protein molecules are aggregated by irreversible protein denaturation. This process is controlled by several influencing factors, including product properties such as lactose, pH and calcium content, as well as protein concentrations [1-3]. Microparticulation is a controlled and thermally induced process in which shared forces are applied to limit the growth or to reduce aggregate sizes of microparticulated whey proteins [4]. Currently, this process is conducted industrially by a scraped surface heat exchanger [5] where two different units (e.g., a heat exchanger and homogenizer) are used separately. However, it is also possible to use the hot-extrusion process as an alternate sustainable technique for producing MWPs by considering different process parameters; for example temperature, screw speed and $C_{Protein}$ [4,6]. High moisture (about 40% of total dry mass) extrusion is also an appropriate technique that can optimize the MWP production process in the desired size range of 0.5 µm-10 µm at a near-neutral pH [7].

Many studies have investigated thermal denaturation as well as aggregation characteristics of whey $C_{protein}$. The thermal denaturation behavior of whey proteins has been intensively studied without shear stress up to 5% (w/v) $C_{protein}$ by Zuniga, Tolkach, Kulozik and Aguilera [8] and up to 10% (w/v) by Roefs and de Kruif [9]. Only a very small number of studies investigated a $C_{protein}$ of up to 20% (w/v) [10], and higher concentrations up to 40% (w/v) at maximum [11]. However, there are few studies that investigated the process and system parameters of the extrusion cooking at 30% of $C_{protein}$ with shear stress [4].

Microscopic observation of the particles describes the shapes of the particles using several parameters (e.g., elongation ratio, roundness, and sphericity). According to Houghton and Amidon [12], the values of elongation ratio and sphericity characterize the shapes of particles based on whether the particles resemble needles, rods, plates or cubes. The elongation ratio of ~1.2 and ~11 symbolize the cube- and rod-shaped particle respectively. However, the critical characteristics of MWPs and most of the protein-based fat replacers to create a creamy mouthfeel were reported to be uniform for spherical shape and size of 0.1-3.0 µm [13,14].

The rheological behavior of WPCs varies at different pH levels with the same concentration of whey protein [7]. Thermal stability of protein denaturation differs according to the pH of WPCs. Lower pH values of WPCs indicate higher denaturation stability. The pH values 4 and 6 represent the viscosity of 911.3 and 53.3 mPa.s respectively at 25% of $C_{protein}$ [1]. However, at a temperature of 110°C and a pH value ≤ 5.5, it is possible to achieve an acceptable range of small particles [5]. A lower pH level also displays a better particle size distribution of WPCs using extrusion cooking [11,15,16]. The calcium content also plays a critical role in building protein particulates [5,17,18]. Lactose content also has a significant influence on the whey protein aggregation and particle size distributions [15,16]. At a low lactose content of 1.5%, the particle size of MWPs remains below 2 µm [19], which is characterized as a creamy and smooth mouthfeel. Aggregate diameters above 10 µm represent

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a slightly mealy mouthfeel, while an approximately 50 µm diameter represents a rough and sandy mouthfeel [3].

The descriptions in the paragraph above clarify that the quantity of raw materials and the properties also have an influence on the aggregation and microparticulation process. However, the influence of $C_{protein}$ on microparticulation under shear stress has not been clearly understood using the hot extrusion process so far. Hence, the key aim of this study was to investigate the influence of $C_{protein}$ on particle size distribution using the hot extrusion process. The impacts of product flow, screw speed, specific mechanical energy input, and barrel temperature were also considered.

Material and Methods

Materials

Whey protein concentrates (WPCs) supplied by Sachsenmilch Leppersdorf GmbH, Germany, with a $C_{protein}$ of 80% was used as the main raw materials in the experiments. They also contained 8.5% lactose, 6.5% fat and 4.0% ash, with a pH value of 6.9. Additionally, demineralized water was added during the extrusion process to reach the required $C_{protein}$ and was adjusted to 20, 25, 30 and 35% (w/w), respectively.

Extrusion process and parameters

Extrusion experiments were carried out using an intermeshing co-rotating twin-screw extruder (ZSE18HP, Leistritz Extrusionstechnik GmbH, Nürnberg, Germany) with an L/D ratio of 40:1. The barrel was composed of nine individual heating zones, with each zone (except for the first) having an independent heating and cooling control system. Water (at 27°C) was used to control the cooling system.

The screw profile was designed with the normal conveying elements and described in Figure 1. The screw speed of the extruder was maintained between 200 and 1000 rpm. The barrel temperature was set at 25°C, 30°C, 50°C, 90°C, 90°C, 90°C, 70°C, 55°C and 25°C ± 5°C of various zones 1 to 9 respectively. A volumetric screw feeder (Brabender Technology GmbH and KG, Duisburg, Germany) was used for feeding WPCs, and a single channel peristaltic dosing pump (Petro Gas Ausrüstungen Berlin GmbH, Berlin, Germany) was used for feeding water on a weight basis. The total feed mass flow rate was 3.6 kg/h.

In accordance with Onwulata, Mulvaney and Hsieh [20], the Specific Mechanical Energy (SME) was calculated using the equation (1):

$$SME = \frac{N}{N_0} \times \frac{Md[\%]}{100} \times \frac{P}{mf} \quad (1)$$

The calculation was used with the following figures: the value of maximum screw speed (N_0=1200 rpm), the actual screw speed N (rpm), Md to the torque linear load (%), the total mass flow rate (mf=3.6 kg/h) and the maximum power of the motor specific to the extruder (P=6.0 kW).

Sample collection

After establishing a stable condition of product temperature and motor torque, the samples were collected and cooled in normal atmospheric conditions. The product outlet temperature was measured using a thermocouple temperature sensor, and the motor torque of each sample was recorded during the extrusion process. All samples were stored at a temperature of 4°C until analytical analysis could be completed.

Particle size analysis

The determination of particle size distribution of the extruded samples was conducted using a Malvern Mastersizer with a Hydro 2000SM measurement unit (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom). The light sources used included a blue LED laser and a red He-Ne laser with wavelengths of 466 nm and 633 nm, respectively. Particle size was calculated using the Fraunhofer approximation [21]. The measurements of particle size were described on the volume basis as d_{10}, d_{50} and d_{90} respectively representing the diameters (µm) at 10%, 50% and 90% of cumulative volume [22].

Microscopic observations

A digital microscope Keyence VHX 600 (Keyence Deutschland GmbH, Nei-Isenburg, Germany) and high-resolution lens with 1000X magnification were used for the microscopic observations of microparticulated whey proteins, and in particular, particle shape of the MWPs. Microparticulated whey proteins were diluted with demineralized water as 1% (w/w) solution of all $C_{protein}$ and screw speeds used in this experiment. In each case, a drop of the 20 µL sample was placed on microscope slides (76 mm × 26 mm) and concealed with cover glass (20 mm × 20 mm) (Carl Roth GmbH, Karlsruhe, Germany). According to Houghton and Amidon [12], the particle shapes were characterized using a connected digital display.

Color analysis

The colors of the extruded samples were determined using the

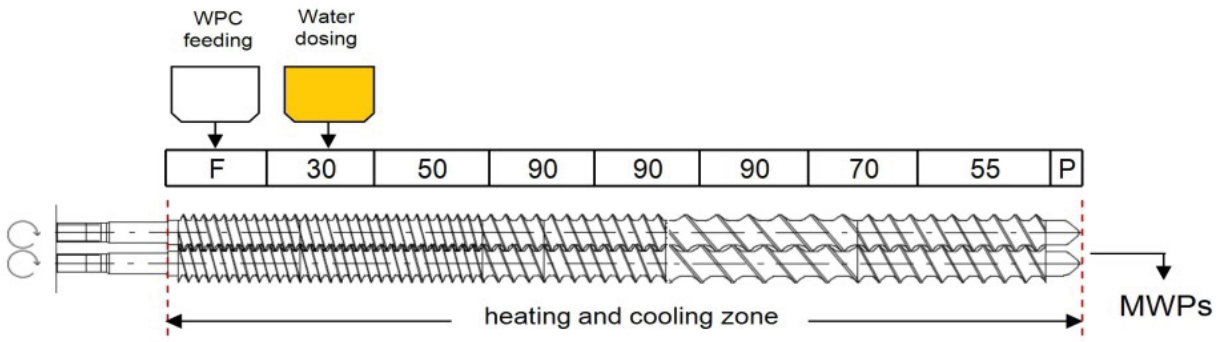

Figure 1: A co-rotating standard screw configuration used for microparticulation.

CIELAB (International Commission in Illumination L*a*b*) system. The CIELAB color system is an easy-to-use, reasonable and quantitative way to characterize color and better evaluate similarities and differences [8,23,24]. In the case of food products, it makes the achievement of improved accuracy regarding color parameters possible [25]. The L*a*b* values of each sample were measured using a portable spectral colorimeter (Hach Lange GmbH, Germany). The samples were placed in a petri dish up to a height of 10 mm. The colorimeter was pressed on top of the sample and the reading of L*a*b* values was recorded. After each measurement, the contact surface of the colorimeter was rinsed with tap water.

Statistical analysis

All analyses and experiments were carried out as triplicates. The statistical analysis and data fittings were conducted using Microsoft Excel, XLSTAT and SPSS Version 24.0 (IBM, New York, US). The significance ($p<0.05$) of all attributes was analyzed using an analysis of variance (ANOVA) by considering mean values of 95% confidence levels. Interaction and correlations among attributes were done by post-hoc analysis using the Least Significant Difference (LSD).

Results and Discussion

Influence of protein concentrations on the particle size distribution

A higher $C_{protein}$ has a strong impact on the aggregation rate under the thermal conditions and the rate of protein aggregation increases by the interaction of protein molecules [11]. The protein content of WPCs influences the particle size distributions of microparticulated whey proteins as shown in Table 1. The particle size 2.96 ± 0.08 µm, 2.71 ± 0.05 µm and 2.67 ± 0.05 µm at 50% of cumulative volume (d_{50}) of MWPs decreased at 800 rpm of screw speed following increases of $C_{protein}$ by 20%, 25%, and 30%, respectively. On the other hand, 35% of $C_{protein}$ showed the complete opposite at all screw speeds used in the experiment, which is more than the expected range of microparticles from 0.1 µm-10 µm [2,4]. Also, a similar outcome shows by the lower screw speed at 200 rpm. In that case, a higher number of protein molecules increased the probability of generating larger protein aggregates, whereas at 90% of cumulative volume (d_{90}) of MWPs particle size 8.20 ± 0.51 µm, 7.42 ± 0.80 µm, and 6.85 ± 0.25 µm reflected 800 rpm screw speed and 20%, 25% and 30% of $C_{protein}$ respectively. The screw speed range of 600-1000 rpm demonstrated the particle size distributions within the microparticulation range at d_{90} up to 30% of $C_{protein}$ as shown in Table 2. The significant influence ($p<0.001$) of $C_{protein}$ on microparticulation process and particle size distributions of MWPs is explained in Figure 2a.

Influence of screw speed on the particle size distribution

The screw speed of the extruder significantly influenced microparticulation [4,21] in the hot extrusion process. The sizes of the MWPs significantly decreased when the screw speed of the extruder was increased ($p<0.001$) for all $C_{proteins}$ used. In that case, at a screw speed of 200 rpm, the extruder had considerably less influence in order to produce the MWPs as shown in Figure 2b. Thus, a longer product residence time and less shear stress resulted in a higher degree of denaturation, which impacted the particle size distribution. On the other hand, a screw speed range of 400 rpm-1000 rpm had a very strong influence on the microparticulation process due to higher shear stress and less product residence time. However, based on particle size distributions of MWPs, the screw speed range of the extruder could be divided into two groups as shown in Table 1. There was a screw speed range of 400 rpm-600 rpm for d_{50} 3 ≤ 5 µm for the first group and a screw speed range of 800 rpm-1000 rpm for d_{50}<3 µm for the second group of MWPs within various $C_{proteins}$. According to Spiegel [3], the first group of particle sizes indicates a smooth texture for mouthfeel and the second group reveals a creamy and smooth texture for mouthfeel. Although the screw speed of 1000 rpm required more SME input than 800 rpm, both screw speeds were not significantly different. Thus, a screw speed of 800 rpm would be the optimized option used to achieve the targeted particle size of MWPs.

$C_{protein}$ (%)	Particle size distributions ± SD, d_{50} (µm)				
	screw speeds (rpm)				
	200	400	600	800	1000
20	20.02 ± 1.38	5.73 ± 0.14	3.95 ± 0.13	2.96 ± 0.08	2.57 ± 0.04
25	19.53 ± 1.87	4.75 ± 0.41	3.52 ± 0.06	2.71 ± 0.05	2.37 ± 0.03
30	12.33 ± 0.83	4.59 ± 0.24	3.41 ± 0.10	2.67 ± 0.05	2.21 ± 0.03
35	14.87 ± 7.24	13.56 ± 0.85	15.21 ± 2.31	11.66 ± 3.47	9.66 ± 1.53

Table 1: Influence of $C_{protein}$ and screw speed of the extruder on particle size distributions of MWPs at d_{50} volume (n=4).

$C_{protein}$ (%)	Particle size distributions ± SD, d_{90} (µm)				
	screw speeds (rpm)				
	200	400	600	800	1000
20	98.12 ± 3.64	17.31 ± 0.59	9.77 ± 0.47	8.20 ± 0.51	8.54 ± 1.53
25	105.59 ± 9.13	15.75 ± 0.95	9.46 ± 0.55	7.42 ± 0.80	6.84 ± 0.67
30	73.10 ± 8.12	13.59 ± 1.43	8.79 ± 0.23	6.85 ± 0.25	6.09 ± 0.18
35	50.59 ± 28.88	48.78 ± 8.99	58.12 ± 10.11	45.96 ± 13.49	35.80 ± 9.16

Table 2: Influence of $C_{protein}$ and screw speed of the extruder on particle size distributions of MWPs at d_{90} volume (n=4).

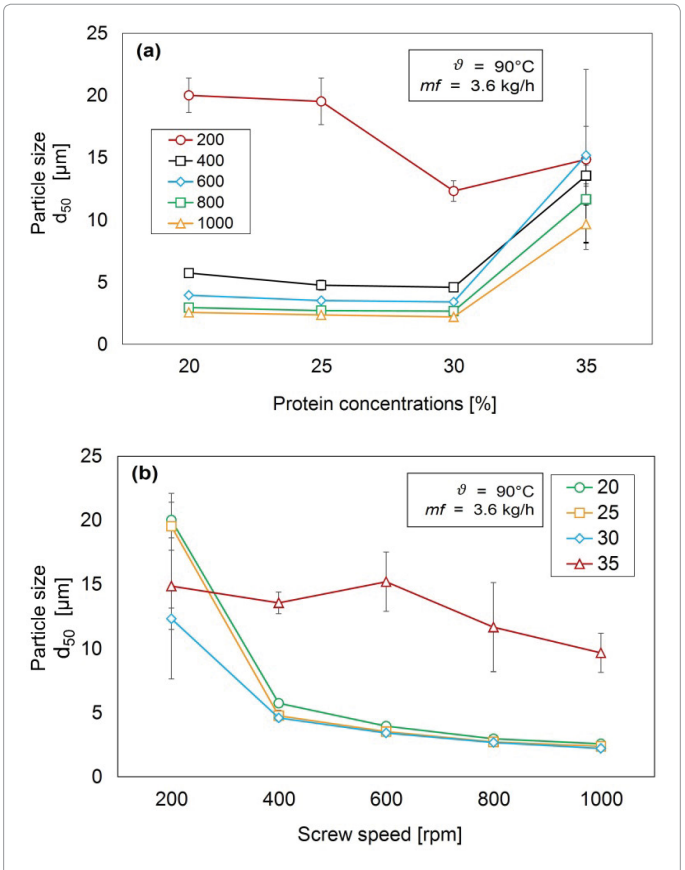

Figure 2: Influence of (a) protein concentrations and (b) screw speed on the particle size distributions at 90°C barrel temperature with a mass flow of 3.6 kg/h.

Influence of SME input on particle size distributions

Particle size distributions can easily be controlled by SME input in the extrusion process [21] which was not possible using SSHE in order to produce microparticles from whey proteins industrially. The screw speed of the extruder and SME input was strongly correlated (r^2=0.999), with 30% of $C_{protein}$ at a barrel temperature of 90°C, as shown in Figure 3a, whereas the particle size distributions of MWPs were strongly correlated with SME input as well as screw speed of the extruder. The significant influence ($p<0.001$) of SME input on particle size distributions is shown in Figure 3b. However, the size of microparticles 2.67 and 2.21 µm at two different point of SMEs 276 and 357 Wh/kg respectively were not significant ($p>0.05$); neither were the particle sizes of d_{50}<3 µm. Therefore, the screw speed of 800 rpm with 30% of $C_{protein}$ would be the better parameter to use to obtain an expected particle size of MWPs (rather than 1000 rpm).

Influence on the color of the extruded materials

According to the measurement of CIE L*a*b* system, there was no significant difference ($p>0.05$) of the L* value (brightness) between extruded samples in terms of different $C_{protein}$ and screw speeds, as shown in Figure 4. However, the a* (red) and b* (yellow) values were statistically significant ($p<0.001$). Nevertheless, the $C_{protein}$ and shear stress affected the color of extruded whey proteins [24]. The screw speed of the extruder had no influence on the color of extruded materials, and also there was no significance ($p>0.05$), which can be seen in Figure 4b. Furthermore, the a* value of extruded samples decreased in the direction of a less reddish color as screw speeds increased. On the other hand, by increasing $C_{protein}$, the a* value of extruded materials increased in the direction of a more reddish color. Generally, the temperature and high shear stress have an impact on the color of the extruded products, whereas the maximum barrel temperature was only 90°C in this study. This could be a reason behind the fact that there was no difference in the brightness of the extruded products.

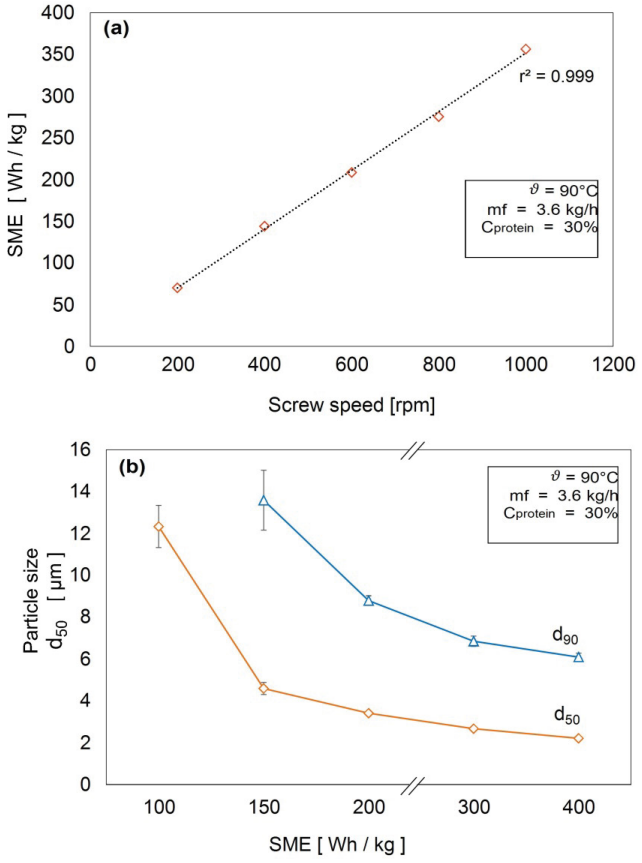

Figure 3: Influence of (a) SME input and (b) screw speed on the particle size distributions at 90°C barrel temperature and 30% of protein concentration with a mass flow of 3.6 kg/h.

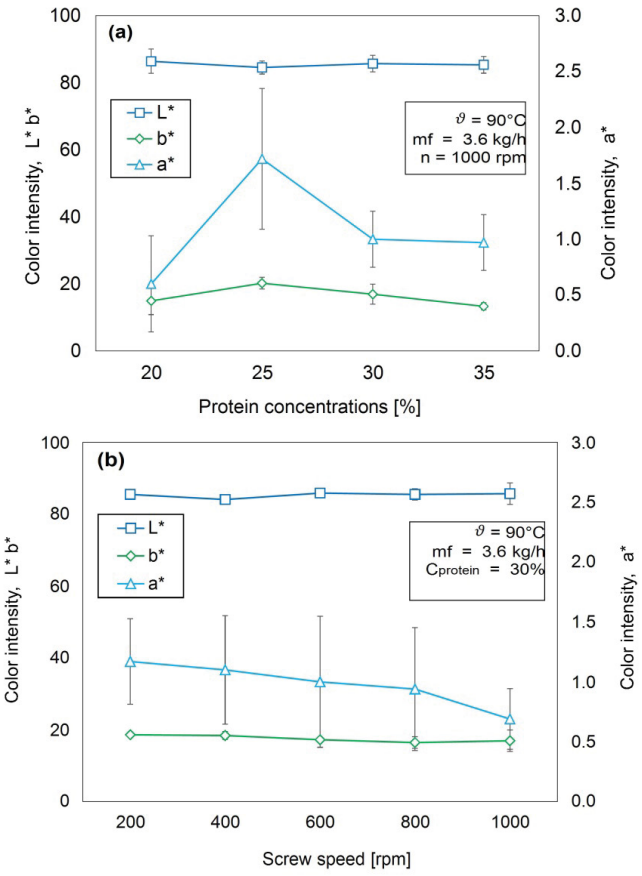

Figure 4: Influence of (a) protein concentrations and (b) screw speed on color of MWPs at 90°C barrel temperature with a mass flow of 3.6 kg/h.

Microstructural views of MWPs

The shape of the particles is shown in Figure 5 and indicates that they were primarily spherical and semi-spherical. On the one hand, it has been clearly observed from microscopic experiments that the size of microparticles decreased when the screw speed of the extruder was increased, as shown in Figure 5f. Although the shape of the particles was observed to be similar for all screw speeds, the volume of MWPs varied. As whey protein microparticulation was done through a thermomechanical and chemical process so-called hot extrusion [14] longer product residence time through the extruder barrel resulting a higher degree of protein denaturation and the probability of having bigger aggregates, as shown in Figure 5a. On the other hand, maximum screw speeds or higher shearing forces and short product residence times make the aggregates smaller, as shown in Figure 5b. Following this phenomenon, the volume of particles increased and the sizes of the particles decreased for the rest of the screw speeds used in the study.

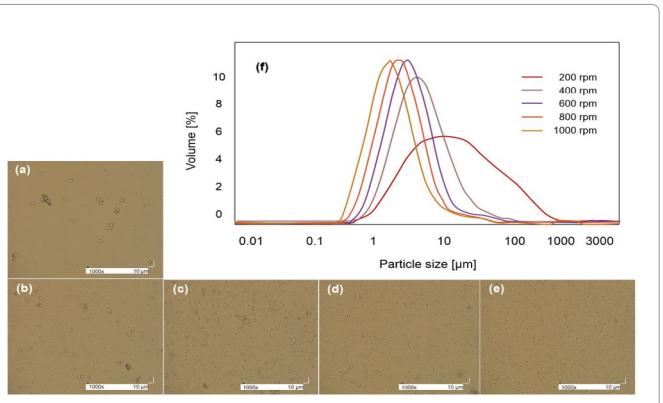

Figure 5: Microscopic observation with various screw speeds (a) 200 rpm, (b) 400 rpm, (c) 600 rpm, (d) 800 rpm, (e) 1000 rpm and (f) particle size distributions of MWPs at 30% of $C_{protein}$ and 90°C barrel temperature with a mass flow of 3.6 kg/h (n=5).

	$C_{protein}$	Particle size	Screw speed	SME
$C_{protein}$	1			
Particle size	0.307	1		
Screw speed	0.000	- 0.647*	1	
SME	0.000	- 0.646*	0.999**	1
*Strong correlation, **Significant correlation				

Table 3: Correlation among influencing factors in hot extrusion process.

Relationships among influencing factors

A significant correlation (r=0.9) between the screw speed of the extruder and specific mechanical energy (SME) input is shown in Table 3. Both parameters (screw speed and SME) were also strongly correlated (r>0.5) with particle size distributions, indicating that it influenced the microparticulation process of whey proteins. The SME input, in particular, directly impacted the final output of the process so-called MWPs [26]. However, there was no correlation (r=0.0) found among $C_{protein}$, screw speeds and SME input. There was a moderate (r>0.3) correlation observed between $C_{protein}$ and particle size distributions. Although the microparticulation was not influenced by raw material properties ($C_{proteins}$) in the hot extrusion process the relationships among relevant factors give us a clear explanation that the process parameter (i.e., screw speed) has a strong effect in the process. Finally, the evidence above indicated that the particle size distributions could be influenced by controlling for these factors in the extrusion process.

Conclusion

It has been clearly observed that there were significant influences of $C_{protein}$ and screw speed on particle size distributions of MWPs using the hot extrusion process. Thus, the particle size distributions of MWPs can be manipulated by varying the $C_{protein}$ as well as the other parameters discussed in this study. However, the screw speed of the extruder also had a very strong influence on microparticulation in order to obtain a targeted particle size range (0.1 µm-10 µm). The SME input during the extrusion process also had an influence on particle size distribution. Nevertheless, the higher screw speed (1000 rpm) obtained the smaller size of particles (d_{50}<3) at 30% of $C_{protein}$ only with transfer elements of the screw configuration, which delivers a smooth and creamy mouthfeel. Based on the findings of this study, it can be concluded that the raw material characteristics and process parameters strongly affected the microparticulation process. It also highlights the need for further investigation on other raw material properties, as well as process parameters, especially screw configurations, in order to understand and optimize particle size distributions. The findings of the current study leave us with an advanced understanding of the microparticulation process by hot extrusion technology compared to other existing techniques.

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