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Water-binding capacity of protein-rich particles and their pellets

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ABSTRACT

The water-binding capacities (WBCs) of pea protein isolate, soy protein isolate, lupin protein concentrate and vital wheat gluten particles were investigated by hydrating them in excess water, centrifuging these dispersions, and calculating the WBCs from the weight of the pellets. It was found that, except for pea proteins, the pellet consisted of a notable amount of interstitial water. Furthermore, it seems that when particles were largely deformable a (semi-)continuous protein network was formed in which individual particles could not be distinguished anymore. Then, the WBC of the pellet did not represent the WBC of the original *particles* anymore. Consequently, it was concluded that the WBC of the pellet (WBC-P) differs from the WBC of the particles. Therefore, the characteristics of the particles and their pellets were further investigated with, among others, time domain nuclear magnetic resonance (TD NMR). TD NMR turned out to be a useful additional tool to do this, and has the potential to give an indication of the amount of water present in each water domain. From the information obtained about the characteristics of the particles and their pellets, it could be concluded that variations in the WBC-P were the result of differences in the deformability of the particles (i.e., their capability to swell and to withstand the centrifugal force), and their ability to bind water interstitially.

1. Introduction

An important property of proteins is their ability to interact with water. This affects various functional properties such as their ability to gel, to dissolve, to swell, and to act as stabilizer in emulsions (Chou & Morr, 1979; Zayas, 1997). A way to describe the interaction of proteins with water is by their water-binding capacity (WBC). Unfortunately, not only are several definitions utilized for this designation in literature, but other designations than "WBC" are used as well (Zayas, 1997). A further complication is that various methods are utilized to determine the WBC. A frequently employed technique to determine the WBC of proteinaceous particles is to make a dispersion of the particles in excess water and centrifuge it (Berghout, Boom, & van der Goot, 2014; Ige, Ogunsua, & Oke, 1984; Wang & Kinsella, 1976; Yu, Ahmedna, & Goktepe, 2007). The supernatant is then discarded and the WBC of the pellet (WBC-P) is determined via the weight of the pellet, with, for example, the following formula:

$$\mathrm{WBC_p\ (g\ water/g\ \ dry\ \ matter)} = \frac{\left(M_{\mathrm{p_{wet}}} - M_{\mathrm{p_{dry}}}\right)}{M_{\mathrm{p_{dry}}}} \quad (1)$$

in which $M_{\mathrm{p_{wet}}}$ is the weight of the pellet obtained after centrifugation, and $M_{\mathrm{p_{dry}}}$ the weight of the material after drying the pellet. In this way, the WBC is defined as *the ability of a protein sample to bind water when subjected to an external force*.

Previous research performed with whey protein microparticles showed that the weight of their pellets was a combination of water bound internally (within the particles) and interstitially (between the particles) (Peters et al., 2016, 2017). Actually, measurements performed with time domain nuclear magnetic resonance (TD NMR) revealed that interstitial water was an important contributor to the WBC-P. This means that the WBC-P was unequal to the WBC of the particles themselves.

These results may have consequences for interpreting the WBC of other proteinaceous powders as well. That is why the WBC-Ps of several protein-rich particles were investigated in this paper. These WBC-P values were compared to the values obtained via TD NMR data. In addition, swelling of the particles was studied and the morphology of the pellets examined with microscopy. These techniques allowed an estimation of the contribution of internal

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and interstitial water to the WBC-P. This information, together with the diffusivity of water in the pellet, was used to gain more insight into the microstructure–protein–water interactions in the pellets and to conclude whether water-binding by particles in pellets is governed by general principles.

2. Materials and methods

2.1. Materials

Pea protein isolate (PPI; Nutralys F85M, Roquette, Lestrem, France), soy protein isolate (SPI; Supro EX 37 IP, Solae, St. Louis, Missouri, USA), lupin protein concentrate (LPC; Fralu-Con, Barentz B.V., Hoofddorp, The Netherlands) and vital wheat gluten (VWG; Barentz B.V., Hoofddorp, The Netherlands) were used. These powders have a protein content of 72% (±2), 78% (±2), 41% (±1), and 70% (±1) respectively, according to Dumas analysis (N × 5.7). To visualize the proteins with a confocal laser scanning microscope (CLSM), Rhodamine B (Sigma–Aldrich, Germany) was used as a staining agent. Milli-Q water was used (resistivity of 18.2 MΩ cm at 25 °C, total oxidizable carbon <10 ppb, Merck Millipore, France) in all experiments.

2.2. Methods

2.2.1. Water-binding capacity of protein-rich particle pellets

The water-binding capacity of the pellets of protein-rich particles (WBC-P) was measured by first adding an excess amount of water to the particles, to obtain a 10% w/w dispersion of each of the various materials in 1.5 mL Eppendorf tubes. The dispersion was mixed with a vortex until the sample was thoroughly wetted. Subsequently, the dispersion was mixed with a rotator (Stuart SB3) at 16 rpm and 25 °C for 3 h (further referred to as hydrated for 3 h). Every 15 min, the dispersion was again mixed with the vortex. Next, the tube was centrifuged in an Eppendorf centrifuge at 3000 rpm (845*g*) for 20 min. The supernatant was discarded and the pellet weighed. Afterwards, the pellet was dried in an oven at 105 °C for 24 h and weighed again. The weight difference between the wet and dried pellet was divided over the weight of the dry pellet to calculate the WBC-P, according to Equation (1).

The 10% w/w dispersion was also centrifuged immediately after thoroughly wetting (further referred to as hydrated for 0 h) to investigate the effect of the hydration time on the WBC-P of the sample. After centrifugation, the sample was weighed and dried in an oven at 105 °C for 24 h. For both hydration times, the WBC-P was measured in quadruplicate.

2.2.2. Dissolution of protein-rich particles

The supernatants that were obtained after centrifuging the protein-rich particle dispersions after 0 h or 3 h of hydration (Section 2.2.1.) were dried at 105 °C for 24 h, to determine the amount of material that dissolved during the WBC-P experiments. This was performed four times for each hydration time per material. The dry matter concentration of the supernatants was calculated with the following equation:

$$C_{\mathrm{sup_{dm}}}(\%) = \frac{M_{\mathrm{sup_{dm}}}}{M_{\mathrm{sup}}}\,100 \tag{2}$$

in which $M_{\mathrm{sup_{dm}}}$ is the weight of the remaining dry matter after drying the supernatant, and M_{sup} the weight of the supernatant before drying. The solubility of the material was calculated using Equation (3):

$$\text{Solubility}\ (\%) = \frac{M_{\mathrm{sup_{dm}}}}{M_{\mathrm{dp}}}\,100 \tag{3}$$

in which M_{dp} is the weight of the material used to make a 10% w/w dispersion of the material (Section 2.2.1.). The dry matter content and solubility of SPI hydrated at 0 h was not given, because the supernatant was enveloped by the pellet.

2.2.3. Size and shape of dry protein-rich particles

The size and shape of the unhydrated protein-rich particles (further referred to as dry samples) were analyzed with a high-resolution field emission scanning electron microscope (SEM; Phenom G2 Pure, Phenom-World BV, Eindhoven, the Netherlands). Carbon tabs were used to fix the samples on aluminum pin mounts.

In addition, the size of the dry particles was measured with static light scattering (Mastersizer 2000, Malvern Instruments). This was done with a Scirocco 2000 dry dispersion unit (Malvern Instruments, Malvern, UK), connected to the Mastersizer. The scattering data were analyzed with the Mie-theory to obtain the size distribution and the average particle diameter $d_{4,3}$ (volume mean diameter). The size of the dry particles was measured at least in triplicate.

2.2.4. Size, shape and water-binding capacity of the protein-rich particles in their pellets

The size and shape of the protein-rich particles in their pellets were examined with CLSM, using pellets obtained after a hydration time of 3 h (Section 2.2.1.). In this case, however, 200 μL of Milli-Q water was replaced by 200 μL of a 2×10^{-3}% w/w Rhodamine B solution, and the samples were covered with aluminum foil during hydration. After centrifugation, the pellets were visualized with a LSM 510 microscope (Zeiss, Germany). Fluorescent emission of Rhodamine B was induced with a 543 nm laser for excitation and detected at 580 nm.

The area of water between the protein-rich particles in a pellet in the images was determined with Image-J (ImageJ 1.49°, National Institute of Health, USA), using 5 images per treatment. It is assumed that the area fraction obtained from an image is equal to the volume fraction (Russ, 2005). To roughly calculate the WBC of the particles themselves, the volume of the pellet was first estimated with Equation (S2) (Appendix). Next, this volume was multiplied by the area fraction of the hydrated protein particles, to obtain the total volume of all hydrated protein particles. Subsequently, the amount of dry matter between the particles, the WBC of the particles themselves, and the ratio of water *inside* and *between* the protein particles were approximated with Equations (S3), (S4), and (S5) (Appendix). Then, the WBC of the particles in the pellet was converted to the dry matter content of the hydrated particles. For these calculations, it was assumed that the density of proteins was 1330 kg m^{-3} and the density of water 1000 kg m^{-3}, and that the dry matter concentration within the supernatant was equal to the dry matter concentration between the particles.

2.2.5. Dynamic adsorption isotherms

Dynamic water vapor adsorption isotherms for all four kinds of particles were obtained by using an automatic multi-sample moisture sorption analyzer SPSx-11μ (Projekt Messtechnik, Ulm, Germany). The relative humidity (RH) within the climate chamber of the moisture sorption analyzer was controlled by mixing a dry nitrogen gas flow with a water-saturated gas flow. A dew point analyzer and a microbalance (WXS206SDU Mettler-Toledo, Greifensee, Switzerland) were present in the moisture sorption analyzer to monitor the RH and weight respectively. The variations

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