Potential application of pre-processed whey protein isolate (WPI) for high protein food
Nanik Purwantia, b, Anouk Moerkensb, Atze Jan van der Gootb, a, *, Remko Boomb

a Top Institute Food and Nutrition, Wageningen, Netherlands
b Food Process Engineering, Wageningen University, Wageningen, Netherlands (atzejan.vandergoot@wur.nl *)

ABSTRACT
Whey protein is a prospective ingredient for high protein diets. Unfortunately, the development of high protein products is hampered by the negative sensorial properties of such products. A product that contains a high concentration of protein is often too firm, has a rubbery mouth feel and hardens in time. A possible solution for using whey protein as an ingredient in high protein food is through “pre-processing”. WPI is pre-processed at around neutral condition into so called “aggregates” and “microparticles”, prior to further application in a product model system. The aim of both methods is to induce (almost) complete denaturation of WPI and formation of short-range interacted networks, thereby reducing number of long range interactions and the ability to form a strong gel network. WPI aggregates were produced by heating a diluted WPI solution, 3% and 9% w/w. WPI microparticles were produced by heating and mixing a 40% WPI dispersion. The properties of the concentrated protein gels using those pre-processed WPI were investigated using small deformation or large deformation tests. By applying pre-processed WPI, concentrated protein gels that were softer than those using native WPI were obtained.

Keywords: high protein food; whey protein isolate; pre-processing; aggregates; microparticles

INTRODUCTION
Products with increased protein content could provide health benefits, but often those products lack good textural properties. In addition, those products harden in time [1]. The lack of good textural properties are caused by protein interactions during storage [2]. Pre-processing of proteins prior to the application in high protein food could be a method to delay or even stop the undesirable changes [3]. The aim of pre-processing should be to limit or inhibit total long-range interactions and replace them by short-range interactions. As a result, the total networks might be broken down easier. This paper presents two methods of pre-processing whey protein and describes their effects on the application in the model food product.

MATERIALS & METHODS
Materials
Bipro® whey protein isolate (WPI) lot no. JE 034-7-440 was obtained from Davisco Food International Inc. (Le Sueur, MN); sodium hydroxide (NaOH), disodium hydrogen phosphate (Na2HPO4), sodium dihydrogen phosphate (NaH2PO4) and isopropanol Emplura® were received from Merck (Germany); 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB), acetonitrile, trifluoroacetic acid (TFA) and dithriothreitol (DTT) were obtained from Sigma (USA).

Methods
Pre-processed WPI into protein aggregates
WPI solutions with 3% and 9% w/w protein concentration were heated for 30 minutes after reaching 90 °C. The pH was fixed at 7 using 2 M NaOH. Then, the suspensions with aggregates were cooled to room temperature with ice water before further use.

Characterization of WPI aggregates
Relative amount of the aggregates to their native form was quantified using reversed-phase chromatography (RP-HPLC) (Turbochrom, Perkin-Elmer). The samples were dissolved in 2% acetonitrile and 0.1% TFA and the detection was done using UV-absorption at 220 nm. The peak area revealed by WPI aggregates was compared to that by native WPI to determine the percentage of native being converted into aggregates.
Viscosity of the aggregates were measured using Ubbelohde viscometers (Poulten Selfe and Lee Ltd., USA), tube 0B 30451 or 0B 39709 in a controlled water bath at 25 °C (TV 4000, Tamson). The aggregates were measured at concentrations 0.25, 0.50, 0.75, and 1.0% in 10 mM phosphate buffer pH 7.0. The measurements were done in triplicate and the specific viscosity was calculated using the following equation

\[
\eta_s = \frac{t - t_0}{t_0}
\]  

(1)

where \(t_0\) and \(t\) are the times needed to flow through the capillary for the solvent and the suspension, respectively. The intrinsic viscosity \([\eta] (\text{L/g})\) was determined by extrapolating the reduced viscosity, i.e. \(\eta_s/C\), to zero concentration [4].

The sizes of the aggregates were measured using dynamic light scattering (Zetasizer nano series, Malvern), operated using 173° backscattering light. The aggregates were diluted with 10 mM phosphate buffer pH 7.0 using concentrations of 0.1% or 0.25%. The sizes reported are the effective hydrodynamic diameter of the largest intensity peak in the distribution analysis.

The degree of reactivity of the aggregates was determined by measuring the number of accessible thiol groups using Ellman’s reaction [5]. The aggregates reacted with DTNB solution leading to a colour change of the solution. The absorbance was measured using spectrophotometer (Carry UV-VIS 4000) at 412 nm. A molar extinction coefficient of 13600 M\(^{-1}\) cm\(^{-1}\) was used to calculate the number of thiol groups [6].

Pre-processing WPI into microparticles

A WPI dispersion of 40% w/w at its original pH, ~6.8, was heated at 90 °C for 50 minutes while mixing in a 40-L Stephan cutter mixer (Germany). After that, the resulted broken gels were cooled down to 30 °C. The broken gels were then dried in the oven at 50 – 60 °C until the moisture content was below 10% w/w. Subsequently, the gels were ground using an ultracentrifugal mill operated at 15000 rpm, in which an 80 µm-sieve was attached to the rotor.

Characterization of WPI microparticles

Morphology of microparticles were observed using a light microscopy (Zeiss, Axiovert 200 MAT) at 200 x magnification. The size of microparticles was measured using static light scattering (Mastersizer 2000, Malvern Instruments). Isopropanol was used as the carrier fluid. Refractive indices of 1.545 and 1.39 were used for WPI particles [7] and isopropanol, respectively. The absorption of microparticles during measurement was set to 0.001. Density of WPI microparticles was measured using a manometric gas expansion pycnometer (Quanta Chrome, Ultrapycnometer 1000). The density of WPI powder was also measured and taken as a reference. Protein concentration of microparticles was measured using DUMAS method (Nitrogen analyzer, FlashEA 1112 series) using a conversion factor of 6.38.

Application of pre-processed WPI in the model of high protein food

The protein gels made from native WPI were used as control materials. Pre-processed WPI was used to make gels with similar protein content as those from native WPI. Suspensions of WPI aggregates originating from 3% and 9%w/w solutions were concentrated using rotor evaporator at 40 °C (Heidolph Laborora 4001) to obtain 15%w/w aggregates suspensions. After that, those concentrated suspensions were heated in a Couette geometry of the rheometer (CC-17, Anton Paar 301) to form heat-induced gels. The following heating profiles were used: heating up with 1 °C/min from 20 to 90 °C, holding at 90 °C for 30 minutes, followed by cooling to 20 °C with 1 °C/min, and holding at this temperature for 30 minutes. Subsequently, small deformation behaviour of the formed gels were characterized using strain sweep. Strain of 0.1 to 100% at a constant frequency sweep of 1 Hz was applied. A protein gel from 15%w/w native WPI was used as the control gel. The measurement was done in duplicate. A protein gel from 20%w/w native WPI powder was used as the control gel for the models with WPI microparticles. In the models, parts of native WPI powder were replaced by microparticles, keeping the total protein concentration at 20%w/w. The protein dispersions were heated at 90 °C for 30 minutes while stirring in glass containers. After heating, the gels were formed. Then, the gels were cooled in warm water for 5 minutes and subsequently in ice-water mixture for 20 minutes. The gels inside the glass containers were characterized under uni-axial compression tests (Instron 5564) using a constant speed 1 mm/s up to 15 mm depth. The gel stiffness was determined as the force increase needed for a compression strain from 0.3% up to 1%. This outcome of these measurements is similar to the Young Modulus.
RESULTS & DISCUSSION

Characteristics of WPI aggregates
WPI solutions that were heated using the conditions described above were (almost) completely converted into aggregates. The conversion was at least 95%. Properties of the aggregates are listed in Table 1. Aggregates that originated from the lower protein concentration (3% WPI) had smaller sizes and denser protein content. The later was shown by lower intrinsic viscosity per gram of protein aggregates. The number of thiol groups at this concentration, however, was similar to those from 9% WPI. This number indicates the potential of the thiol groups to form additional disulphide bonds.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter (nm)</th>
<th>$\eta$ (ml/g)</th>
<th>[SH] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%</td>
<td>49.30 (± 1.35)</td>
<td>8.4</td>
<td>0.49 (± 0.065)</td>
</tr>
<tr>
<td>9%</td>
<td>62.72 (± 0.91)</td>
<td>16.3</td>
<td>0.54 (± 0.011)</td>
</tr>
</tbody>
</table>

Characteristics of WPI microparticles
The WPI microparticles created had an irregular shape (Fig. 1 left) with an average size of ~ 70 µm. The particles density was 1.32 g/cm$^3$. This value was higher than that of native WPI powder, i.e. 1.13 g/cm$^3$. Protein concentration of the particles was around 93% which was relatively close to the protein concentration of native WPI.

Figure 1. Morphology of WPI microparticles (left) and the particle size distribution (right).

The application of WPI aggregates and microparticles in the model system
This section describes that pre-processing can be used to alter the properties of a concentrated WPI protein system. Fig. 2 shows the damping factor of protein gels made from native WPI, 3% aggregates, and 9% aggregates at around 15% w/w. The damping factor is the ratio between the viscous and elastic behaviour of viscoelastic material. A higher ratio indicates that the viscous property is dominant, thus, the material is softer or weaker than that with lower ratio. The protein gel from 9% WPIA is the weakest compared to the other two gels and the one from 3% WPIA is weaker than that from native.
The softer texture of high protein gels by applying WPI aggregates can be explained by considering the properties of the aggregates. The protein aggregates have a larger size than native protein and possess a lower ability to create additional disulphide bonds (i.e. pre-processing reduces the latent reactivity of the protein). As a result, the total long-range interactions in a gel obtained after reheating were reduced compared to gel formed from native WPI. This gave a gel with softer texture. This softening effect was increased when larger aggregates were used.

Figure 3 shows the changes in textures of the WPI gels with 20% w/w protein. It is shown that by replacing a certain percentage of native WPI by WPI microparticles, softer gel textures were obtained. This effect was caused by the fact that microparticles do not have any gelling ability anymore. When these particles were mixed with native WPI, gel formation would be dominated by native WPI that still had gelling ability. Thus, when more native WPI was replaced by the particles, the gel networks formed were weaker giving softer gel texture. The presence of WPI microparticles might have an additional effect as well being structural breakers in the protein matrix [3], giving the gel a more brittle character.
CONCLUSION
This work highlighted that improvement of the textural properties of high protein foods containing whey protein can be obtained by fine tuning the properties of the protein in the first place. This can be done by pre-processing of proteins in different ways, which basically aimed at reducing the number of long range interactions present in regular protein gels.

REFERENCES