Highly porous protein-based aerogels as carrier matrices for sensitive and sensory unpleasant substances in food products

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Background
The food industry shows a rising demand for transport or protection matrices for sensitive or sensory unpleasant substances. Micro encapsulation can protect the core material of extreme pH-values, oxidation, light and other detrimental effects or mask an unpleasant flavor. The properties of the matrix material must be a high biocompatibility, good biological digestibility and suitable for human consumption. This is given for hydrocolloids such as milk- and egg white proteins. Existing encapsulation technologies show a limited loading capacity due to a preloading of the core material. The gel formation may be hindered by the core material. In
contrast, aerogels allow a post loading step, where the gelation is independent of the concentration and sensitivity of the core material.

Preliminary experiments proved protein hydrogels as a suitable precursor for aerogels. Drying under ambient conditions or freeze drying will produce unavoidable tensions (shrinkage, breaking) within the matrix. Supercritical drying retains the protein network structure and the resulting aerogels have very large specific inner surface areas of up to 500 m$^2$ g$^{-1}$. High loading capacities can be expected when core material is loaded to this high inner surface area. Adsorptive loading was tested with ketoprofen and a loading ratio of 9.1% (w/w) was possible with aerogels, whereas only 0.4% (w/w) could be found on cryogels. The necessary solubility and stability in supercritical CO$_2$ for the impregnation process can also be attributed to fish oil, caffeine and enzymes, giving the technology a broad scope of application.

The aim of this research project was to develop a basic technology to produce biocompatible, food grade protein aerogels to encapsulate sensitive or sensory unpleasant substances. Exemplary core materials were fish oil (fat soluble, polyunsaturated fatty acids) and ascorbic acid (water soluble, Vitamin C). The work will contribute to a better understanding of interactions between process parameters and protein systems. Aerogels as encapsulation system should consequently be applicable for a number of core materials. In addition, the design of a coating process for the very light and small aerogels should enhance the protecting effect and controlled release properties.

**Results**

The structure of heat induced whey protein isolate (WPI) or egg white protein (EWP) hydrogels is strongly dependent on the pH during gel formation. Is the pH near the isoelectric point (IEP) when the gel is formed, hydrophobic interactions lead to random aggregation of the neutrally charged protein molecules. The gel is soft, shows low mechanical stability and high syneresis. The same effect is observed when salt is added to the protein solution. Ions shield the positive or negative charges of the protein molecules and reduce electrostatic repulsion. Random aggregates are the result. Acidic pH-values below the IEP induce a positive net charge of the proteins. Gel formation is less rapid but ordered, due to electrostatic repulsion. The gel appears firmer but still mechanically unstable. Negative net charges of the proteins are induced by alkaline pH-values. Gels with a stranded structure are formed. They are transparent and mechanically stable because of thiol-disulfide exchange reactions at alkaline pH-values. They withstand high degrees of deformation before the structure is irreversibly destroyed. Sodium caseinate solutions were enzymatically crosslinked to produce hydrogels as precursor to aerogels. Higher protein concentrations hereby raise the quiescent structure and gel hardness.

The emulsion method was used to form protein micro particles. A protein solution as hydrophilic component was emulsified in sunflower oil as hydrophobic component. The protein solution droplets were solidified by heat or enzymatic treatment. Process parameters were adjusted in a way that 60% of the droplets were in the desired size range of 50 to 100 µm. Centrifugation is used to separate the capsules from the oil phase. Repetitive centrifugation after adding an aqueous phase leaves the capsules as oil free sediment behind. Using NaCl- or
CaCl$_2$-solution as aqueous phase may reduce possible swelling of the capsules but lead to aggregation. In addition, pure water as aqueous phase did not show any negative effect on the specific inner surface and was, therefore, used as separation medium. A scale-up of the process was developed to produce larger amounts of aerogels.

To conduct supercritical drying a solvent exchange in the gel is necessary. The water in the hydrogels must be replaced by CO$_2$-soluble ethanol. Ethanol induces changes of the secondary structure in protein molecules. Measurements of the gel hardness as well as the breaking force of alcogels also revealed a change in structure of the heat denatured and enzymatically crosslinked gels. The gel structure became much firmer and shrinkage was observed, irrespective of type of protein and gel forming parameters. It is assumed that the gel structure in general is retained but compressed.

All mentioned hydrogels were successfully dried with supercritical extraction within 2-24 h with respect to size. Macroporous structures with very low inner surface areas are found with EWP gels formed near the IEP. Mesoporous structures with surface areas up to 380 m$^2$ g$^{-1}$ are the result of low and high pH-values. The addition of NaCl reduces the surface area. WPI aerogels also show mesoporous structures with specific inner surface areas of up to 460 m$^2$ g$^{-1}$. The mechanical stability increases for EWP and WPI aerogels with higher pH-values. NaCas-aerogels have the smallest surface area with 150 m$^2$ g$^{-1}$. An increasing protein concentration of 12.5% instead of 10% did hereby not alter the surface area in a mentionable way. The emulsion method was suitable to produce aerogel micro particles with similar characteristics to the monoliths. To analyze the drying kinetics, the ethanol detection during the drying process was improved substantially. Drying kinetics were identified for EWP-, WPI- and NaCas-gel particles. Within 30 min 80% of ethanol was extracted for all types of protein alike. The extraction process for the remaining 20% is diffusion limited and, therefore, slower. Drying times for particles in a packed bed were extended to a maximum of 7 h. It was observed that supercritical drying for 2 h causes the desired aerogel properties and a final, more cost-effective drying is possible during storage at low relative humidity.

Freeze drying experiments were conducted to compare cryogels and aerogels. The developed drying process showed reproducible results for native and acidic pH-values. Collapsed gel structures were observed for gels formed at alkaline pH-values, proposing a pH-dependency of the process. All dried gel variants were fragile and brittle with an specific inner surface area of under 10 m$^2$ g$^{-1}$. Cryogels and Aerogels are, therefore, not comparable in their techno-functional properties.

Supercritical impregnation with fish oil is dependent on the specific inner surface area of the aerogels. For EWP- and WPI-aerogels a loading ratio of up to 0.74 g fish oil/g aerogel is possible. NaCas-aerogels only adsorb 0.17 g fish oil/g aerogel. Much less effective is the adsorption of ascorbic acid where a maximum of 5 mg ascorbic acid/g aerogel was achieved. This may be ascribed to the lower solubility of ascorbic acid compared to fish oil in supercritical CO$_2$. A raise in pressure along with the resulting higher solubility of fish oil and ascorbic acid in supercritical CO$_2$ induces higher loading ratios on the aerogels. Following the impregnation the aerogels remain as free flowing particles.
Storage of particles without impregnation and impregnated with fish oil during 12 weeks at relative humidities of 0.11 and 0.33 did not result in negative product characteristics. The free flowing properties were retained. The fish oil remained within the particles, analyzing only very little changes in odor. The particles shranked. The water uptake was 1% at the maximum.

Process parameters were determined for injection of a 15% (w/w) shellac-ethanol solution in the spouted bed apparatus. Key factors were a low injection rate and the use of a syringe pump. To reduce formation of aggregates, drying intervals were integrated. Following the coating process, the particles remained as single particles and kept their free-flowing properties. Scanning electron microscope pictures confirmed the efficiency of the process as a shellac layer was visible on the particles. The shellac layer was around 1 µm thick.

To investigate the gas and particle dynamics during spouting numerical simulations of the used spouted bed apparatus were carried out by means of the coupled CFD-DEM (Computational Fluid Dynamics and Discrete Element Method) approach. To reduce the computational time a scaling approach was applied. The simulations were used to predict the elutriation point of particles from spouted bed apparatus.

In-vitro digestion experiments were conducted for aerogel particles loaded with fish oil. Uncoated particles showed a release rate of 10% at the maximum during the oral and gastric digestion phase. The subsequent intestinal phase induced for heat denatured WPI- and EWP-aerogels a release rate of 56% and 78% respectively. NaCas-aerogels were almost entirely digested and all encapsulated fish oil was released. Fractions of ω-3-unsaturated fatty acids in the released fish oil were comparable to the initial product. Shellac coated particles showed similar behavior. The coating may, therefore, enhance the protective effect of the capsules but still allows the release of the core material in the intestine.

**Economic importance**

The mesoporous aerogel structures may primarily function as transportation matrix for sensitive and sensory unpleasant substances (fish oil (polyunsaturated fatty acids), caffeine, vitamins, anthocyanins, xanthohumol). Aerogel particles are suitable as carrier system for any number of low molecular or bioactive components. The results provide relevant information particularly to producers of dietary supplements and special functional food.

Aerogel microcapsules are applicable in a variety of food products, as for example yoghurt, buttermilk/whey drinks, instant powder for drinks (sports nutrition) or backing products and cereals. The technology may just as well generate new market areas for suppliers in the food industry. The results are also relevant for mechanical engineers and plant construction companies as they may use the knowledge to develop and improve CO₂-high pressure plants and spouted bed coaters. The usage of highly porous, water insoluble protein aerogels generates new application areas and improved properties in comparison to existing encapsulation technologies in protein hydrogels. This broadens the field of application for microencapsulation technologies with regard to the core material as well as food products enriched with microencapsulated valuable components.
Publications


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