Pressurized Gas eXpanded (PGX) drying of lysozyme and its composites

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Enzymes are natural catalysts capable of catalyzing specific reactions and versatile enough for widespread applications in the industry. To preserve the enzyme's activity, it is essential to isolate and dry them from aqueous solutions without degrading their structure or making their active sites inaccessible. The Pressurized Gas eXpanded (PGX) liquid technology is a novel process that was successfully used to dry, purify, and micronize various biopolymers from aqueous solution. In this process, a pressurized mixture of CO_2 and ethanol (the PGX liquid) at mild temperature is used as the drying medium generating open-porous structures and particles of low bulk density and large specific surface area (SSA). Since the PGX technology has not been previously used to process enzymes, this study was conducted to investigate if the PGX technology could be used for drying enzymes and if the PGX enzymes were still active once they were rehydrated.

Lysozyme was selected as the model enzyme tested in this study. Lysozyme is present in egg whites and has antimicrobial activity, making it suitable to be used in conjunction with other bioproducts to confer longer shelf life. Dried purified Lysozyme was purchased (Sigma-Aldrich), rehydrated in reverse osmosis water and then PGX dried at 10.0 MPa and 40 °C using a flow rate ratio of 10:30:10 g/min of aqueous solution:ethanol:CO_2. The resulting particles were evaluated for their bulk density, SSA (BET method), morphology (Helium Ion Microscopy, HiM), chemical modifications (FTIR), and enzymatic activity. After PGX drying, the bulk density of lysozyme was reduced from 3.5 (±0.19) × 10^{-1} g/mL to 8.4 (±0.8) ×10^{-3} g/mL (42-fold reduction) with SSA of 38 m^2/g. From the FTIR spectra, an intensification of the amide I and II bands (typical of proteins) was observed, which may indicate an exposure of the lysozyme surface after PGX processing. The HiM images of PGX processed lysozyme compared to lysozyme particles before processing demonstrated the profound effect of PGX processing on lysozyme microscopic morphology. While the purchased lysozyme particles were mostly spherical and in the order of tens of micrometers in diameter suggesting they were probably spray dried, the PGX lysozyme particles were reduced to less than 2 µm in size with open-porous structure and connected spherules in the order of tens of nanometers. These effects were also reflected on the enzyme activity. After PGX processing, an increase in activity was observed of about 4 times from ca. 8,000 Units/mg before PGX processing up to ca. 31,000 Units/mg for PGX lysozyme, which could most probably be attributed to the removal of non-enzymatic components with PGX, the increased SSA and the open-porous morphology, leading to exposure of active sites that were previously blocked inside the bulk of the original particles.

PGX processing was also applied to simultaneously dry aqueous mixtures (1:2 ratio) of lysozyme to polysaccharide (β-glucan or sodium alginate) to form composites. In both cases, the macroscopic structure of the composite was like that of the corresponding polysaccharide alone. In the case of β-glucan+lysozyme composite, a bulk density of 1.9 (±0.9)×10^{-2} g/mL and a surface area of 31.5 m^2/g were obtained. This was comparable to PGX processed β-glucan alone, with retention of the activity of lysozyme: 2,952 ± 200 Units/mg of composite. In the case of sodium alginate+lysozyme composite, bulk densities between 7.0 (±0.3)×10^{-3} g/mL and 1.0 (±0.15)×10^{-2} g/mL were obtained with a lysozyme activity of ca. 2,205 ± 226 Units/mg of composite. However, a reduction in the surface area was observed compared with PGX processed sodium alginate, which could be a result of the lysozyme partially covering the porous structure of sodium alginate. This study established that PGX is a preferred technology to dry enzymes, such as lysozyme, because it not only retains but also increases the enzymatic activity of the commercially available product by reducing the particle size and increasing surface area. Additionally, PGX can also be applied to composite preparation by simultaneously drying enzymes and polysaccharides, allowing the development of formulations and novel products consisting of a natural carrier with antimicrobial properties.