Behavior, Functionality and Digestibility of a Protein Concentrate, and Integration of Oil Bodies into Skim Milk: A Dual Study of Products from Hempseed Water-Only Fractionation

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Hempseed (*Cannabis sativa* L.) is emerging as a sustainable source of high-quality proteins and polyunsaturated fatty acid–rich oils [1]. Mild processing techniques, such as water-only fractionation, offer an environmentally friendly alternative to conventional extraction methods, such as alkaline extraction of proteins or oil bodies. In this study, hempseeds were fractionated using a water-only process to obtain a hempseed protein concentrate (HPC) and an oil body–rich cream [2]. The properties and functionality of these fractions were subsequently investigated and compared with their alkaline-extracted counterparts.

The HPC, which contained 73.8% dry basis (d.b.) of proteins, was compared to a conventional hempseed protein isolate (HPI, 83.6% d.b. proteins), prepared through hexane defatting, alkaline extraction, and isoelectric precipitation. Physicochemical analyses, including particle size, ζ-potential, and thermal properties, revealed distinct differences between HPC and HPI, suggesting that the extraction method significantly affects protein behavior. This effect is likely influenced by the high phytate content retained in the water-only process, as phytate interacts with proteins and divalent cations to form insoluble ternary complexes. Functional properties of HPC were evaluated across different pH levels and heat treatments, demonstrating distinct solubility patterns and promising gelling capacity. In vitro protein digestibility assays (INFOGEST) revealed that all hempseed protein samples reached high digestibility levels at the end of the intestinal phase, comparable to reference animal-based proteins, such as whey protein isolate. Notably, despite its considerably higher phytate content, HPC demonstrated digestibility profiles similar to HPI, suggesting that phytate did not significantly impair overall protein breakdown under simulated gastrointestinal conditions.

Separately, the cream fraction (97.4% d.b. oil), composed mainly of oil bodies (OBs) and obtained through the same water-only fractionation, was compared to a conventional alkaline-extracted OBs fraction to assess compositional and colloidal differences. The results demonstrated that water-only fractionation effectively yields a highly purified OBs fraction with higher oil recovery (55.7% yield, compared to 45.7% for alkaline extracted OBs), offering a simpler and more sustainable alternative to alkaline extraction. Both OBs fractions were separately incorporated into skim milk systems to investigate their potential to replace cow milk fat globules. Their behavior and colloidal stability were assessed by particle size distribution, ζ-potential and creaming stability measurements. After high-pressure homogenization (50 MPa), oil droplets in the 0.01–1 μm range were formed (measured by static light scattering), exhibiting enhanced colloidal stability, likely supported by adsorption of milk proteins onto the droplet surface. Rheological analysis confirmed that both OBs fractions were able to form milk gels with similar mechanical properties to those of whole milk gels, highlighting their potential as functional fat replacers in dairy formulations.

In conclusion, this dual study demonstrates that water-only fractionation can produce both protein- and lipid-rich hempseed ingredients with distinct functional properties. HPC offers a minimally processed protein source with promising techno-functional and nutritional properties, while the OBs fraction show strong compatibility with dairy systems, supporting their use as clean-label fat replacers for hybrid products. These findings support the development of sustainable, low-impact processing strategies for plant-based food products.

Keywords:

Water-only fractionation, Mild processing, Hempseed, Oil bodies, phytate, Hempseed protein isolate

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