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*Abstract*

# Development of Aqueous Two-Phase Systems Based on Deep Eutectic Solvents for Continuous Protein Extraction in a Microextractor †

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**Abstract:** Currently, lipases are one of the most widely used enzymes, especially in catalysis, mostly due to their high activity in mild conditions and wide specificity. Therefore, obtaining the highest possible catalytic activity, which can be achieved through purification, is becoming more and more important. Since most of the purification techniques are time consuming, aqueous two-phase protein extraction is often investigated as a promising alternative. Additionally, this kind of extraction can be carried out in microextractors, which provides not only a continuous processing of raw materials, but also significantly higher efficiencies due to a high surface-to-volume ratio of microchannels. Extraction with deep eutectic solvents (DESs) fulfills all green chemistry principles, because DESs are biodegradable, non-toxic, and recyclable. In this research, the aqueous two-phase system based on natural DES for continuous protein extraction in a microextractor was investigated. The impact of salt concentration on extraction efficiency was investigated in batch experiments with six different previously characterized DESs. After determination of the optimal two-phase system features, the process was transferred to a microextractor. In addition, the selected DES was tested for recyclability while the developed extraction method was verified using raw lipase produced by *Thermomyces lanuginosus* solid-state cultivation on hull-less pumpkin oil pomace. The highest protein extraction efficiency achieved in a batch reactor was 94.70% for 30 min, while in a microextractor, the highest extraction efficiency obtained was 98.50% for 30 s. Obviously, the extraction process was significantly intensified by continuous microextraction. Additionally, the DES used in the microextraction experiments was efficiently reused in several extraction cycles.

**Keywords:** protein extraction; aqueous two-phase system; deep eutectic solvent; continuous microextraction

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