

THESES OF PhD DISSERTATION

**APPLICATION OF THREE-PHASE PARTITIONING
FOR INVESTIGATION OF FOOD PROTEINS**

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INTRODUCTION

Investigation of food protein composition becomes more and more significant in our days, that is motivated by the claim for verification of food origin and/or identification, growing cultivation of genetically modified plants, commercialization of functional food, stress on plants, etc.. Numerous methods are used for investigation of food proteins, however, the need for rapid data collection and for a better recognition requires a steady development and use of novel methods. In the dissertation the development of the simple protein fractionating step, three-phase partitioning, to a method suitable for investigation of plant and animal proteins is described.

Three-phase partitioning (TPP) is a salting-out type fractionation procedure, in which proteins dissolved are precipitated by addition of ammonium sulfate and tert-butanol to the solution. Since 1984, when the method was published (Odegaard et al.) it was applied for purification of various enzymes, nevertheless, the mechanism of TPP is not known wholly (Dennison, Lovrien, 1997).

Experimental work on TPP began in the CFRI in 1988, where a purification process of horseradish peroxidase based on TPP was elaborated (Szamos, Hoschke, 1992). Three-phase partitioning of neutral proteinase led to the recognition that the interfacial tension of the protein free, two-phase systems of identical composition correlates with the amount of protein partitioned in the third phase (Szamos, Kiss, 1995). Centrifugation of partitioned systems at different g-values offered a novel approach in characterization of drip samples (Szamos et al. 1998).

TPP coupled with electrophoresis of proteins partitioned into the third phase may render it a novel screening method.

The following aims were set:

- 1./ determination of parameters characteristic of TPP of ovalbumin, and β -lactoglobulin in systems of various composition (salt, alcohol).
- 2./ investigation of the effect of mechanical energy transmitted to TPP systems
- 3./ study of TPP of plant (corn) and animal (carp) proteins and separation of proteins of the third phase by nonequilibrium pH gradient electrophoresis (NEpHGE)

METHODS

Three phase partitioning of ovalbumin, and β -lactoglobulin were carried out in nine different systems, respectively, while the effect of transmitted mechanical energy was investigated in a partitioning system of definite composition.

For investigating of plant (corn) proteins and animal (carp) proteins partitioning systems of low salt and alcohol contents were used assuring an easy measurability of phase composition.

Compressibility of proteins partitioned into the third phase was determined by centrifugation.

Protein content of aqueous and organic phases was determined by the dye binding method of Bradford.

Proteins partitioned into the third phase were separated by nonequilibrium pH gradient electrophoresis of slab gel format.

For two dimensional separation NEpHGE-SDS was used.

Proteins were detected in gels with Coomassie G 250 staining.

A digital camera was used for documentation.

Quantity One software was used for densitometry.

NEW SCIENTIFIC RESULTS

- 1./ A new parameter, the interfacial tension of the protein free, two-phase system, correlating with the amount of protein (neutral proteinase) partitioned in the third phase, has been described.
- 2./ Compressibility of the third phase on centrifugation has been introduced, and a qualitative method of discrimination of beef and pork drip has been elaborated.
- 3./ It was established that there is a linear correlation between the thickness of centrifuged third phase (C) and the amount of protein precipitated (pork), further on between C and the pH of the aqueous phase in the pH range of 3,7-8,0 (carp).
- 4./ TPP proved to be a special enzyme immobilizing procedure which is suitable for preparation of bioactive layers.
- 5./ Application of TPP for concentration and purification of macromolecules was extended.
- 6/ It was established that the amount of partitioned protein, and the compressibility of the third phases, differ significantly during the partitioning of ovalbumin and β -lactoglobulin, respectively. The composition of the systems ranged from 30/17 (relative saturation of ammoniumsulfate / % (v/v) of tert-butanol) ($\gamma=0,86$ mN/m) to 70/50 ($\gamma=6,8$ mN/m), and the range of protein concentration was 2-10 mg/ml. Independent of the composition of the systems the whole amount of β -lactoglobulin partitioned into the third phase, that is, the TPP of this protein shows no correlation with the interfacial tension of the protein free, two phase reference systems.
- 7./ It was established that 65 % of ovalbumin was partitioned into the third phase in the system of $\gamma=0,86$ mN/m. In the range of 2,8-4,9 mN/m, the amount of partitioned ovalbumin shows a close dependence on the interfacial tensions of the protein free, two-phase reference systems ($y=23,14x-11,98$ ($r^2=0,94$))
- 8./ It was established that the amount of sarcoplasmic proteins partitioned into the third phase shows no dependence on transmitted mechanical energy (number of shaking) in case of silver carp, chicken beef and pork. The compressibility of the third phase shows different changes with increasing amount of energy: 1./ at first it

increases, then reaches a plateau 2./ it decreases. Because of this energy dependence, nearly the same amount of energy must be transmitted to compare compressibilities of third phases.

9./ It has been established that three-phase partitioning of carp sarcoplasmic proteins is pH-dependent.

10./ It has been established that three phase partitioning of corn proteins of varieties Borbála, Gazda and NK 643 is pH-independent.

11./ It has been established that structure of the third phase is influenced to a great extent by the composition of the partitioning system and the presence of foreign proteins (e.g. ovalbumin).

12./ A new sampling separator was constructed to facilitate isolation of sarcoplasmic proteins from muscle.

13. Two screening methods, one for corn meal and another one for fish muscle were elaborated.

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