INTEGRATED BIOTECHNOLOGICAL APPROACHES FOR THE PURIFICATION AND CONCENTRATION OF LIQUID FOODS, PROTEINS AND FOOD COLORS

A thesis submitted to the

University of Mysore

For the award of the degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

by

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Dedicated to My Beloved Parents......

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DECLARATION

I hereby declare that the thesis entitled **"Integrated Biotechnological Approaches for the Purification and Concentration of Liquid Foods, Proteins and Food Colors"** submitted to the **University of Mysore** for the award of the degree of **Doctor of Philosophy in Biotechnology**, is the result of the research work carried out by me in the Department of Food Engineering, Central Food Technological Research Institute, Mysore, India under the guidance of Dr. **KSMS Raghavarao**, during the period 2000-2004.

I further declare that the results of this work have not been previously submitted for any other degree or fellowship.

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Certificate from Guide

I hereby certify that this thesis entitled **"Integrated Biotechnological Approaches for the Purification and Concentration of Liquid Foods, Proteins and Food Colors"** submitted by Mr. Naveen Nagaraj for the degree of **Doctor of Philosophy** in **Biotechnology**, University of Mysore, is the result of the research work carried out by him in the Department of Food Engineering, Central Food Technological Research Institute, Mysore, India, under my guidance and supervision during the period 2000-2004.

KSMS Raghavarao
(KSMS Raghavarao)
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(Naveen Nagaraj)

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Synopsis

SYNOPSIS

Recently, efforts are in progress by research and industrial community for the production of biological products through the application of biotechnology. However, the technology for downstream processing (DSP) of biomolecules from the broth has not kept pace with the advances in the upstream operations, despite the fact that in many cases DSP contributes major share (50-80%) of the total production cost. Existing DSP techniques such as chromatography, electrophoresis, precipitation etc., pose scale-up problems, and are prohibitively expensive on large-scale, unless the product is of high value. Therefore, current research in the area of DSP is directed towards the development of efficient and scaleable alternative bioseparation processes with flexibility for continuous operation.

Aqueous two-phase extraction (ATPE) has been recognized as superior and versatile technique for DSP of biomolecules. A wealth of information has been reported in the literature on various aspects of ATPE for the isolation and purification of proteins/enzymes and other biological materials. ATPE offers a better alternative to the existing methods of primary purification, providing low space-time yield, better enrichment of product, ease of scale-up and flexibility for continuous operation. This technique is effective also in removal of by-products such as other undesirable enzymes/proteins, unidentified polysaccharides and pigments. Furthermore, application of ATPE permits easy adoption of the equipment and the methods of conventional organic-aqueous phase extraction used in the chemical industry. ATPE is recognized as a primary purification step in the overall protein recovery train, since it is not selective enough to provide the desired purity of the enzyme/protein. Use of ATPE enables the desired product (enzyme/protein) to partition into one of the phases, thus reducing the volume of the process stream to be handled during the subsequent purification steps. Hence, final purification can be accomplished by highly selective techniques such as chromatography, electrophoresis.

After successfully partitioning the biomolecule to one of the phases in ATPE, it is desirable to separate the phase forming components and concentrate the solution containing the biomolecules. Membrane processes such as ultrafiltration (UF), dialysis can effectively do this job. The integration of ATPE with any one of the above membrane processes holds considerable promise to increase the productivity of the overall process.

The subject matter of this thesis is presented in five chapters.

Chapter 1- This chapter comprises of General Introduction and scope of the present investigation, literature review pertaining to fundamentals and application of ATPE for the purification of biomoelcules. Further, application of osmotic membrane distillation (OMD) for the concentration of biomolecules/liquid foods has been discussed followed by possible integration ATPE with OMD process.

Chapter 2 – It comprises of preamble for aqueous two phase systems (ATPSs), and the major hindrances for the industrial application of ATPE. Further, this chapter is divided into five sub-sections consisting of methods to enhance phase demixing rate by the application of external fields followed by field assisted extraction of natural food colorants and polymer recovery from spent phases.

Section 2A – Acoustic field assisted demixing has been employed to enhance the phase demixing rate in ATPSs. Application of acoustic field has increased the phase demixing rate up to 3.2 fold by varying the axial distance from the acoustic transducer in polyethylene glycol/potassium phosphate system. The enhancement of phase demixing rate with varying axial distance from the acoustic transducer has been explained based on acoustic field and material of the contactor.

Section 2B – In this section, it has been demonstrated for the first time that electric field can be applied to enhance phase demixing rates even in polymer/salt systems which otherwise was thought not possible due to high conductivity of the phases. The electrokinetic demixing of polymer/salt has resulted in significant enhancement in demixing rates up to 4 fold. The effect of electric field polarity, electric field strength, volume ratio, phase composition on phase demixing has been studied. Further, the influence of these parameters on phase demixing has been explained based on hydrodynamic flow electroosmotic flow (HEF) model.

Section 2C – In this section, microwave field has been explored for the first time to enhance the phase demixing rates (decrease the demixing times) in ATPSs. The microwave field assisted demixing process enhanced the phase demixing rates up to 4 fold in polyethylene glycol/potassium phosphate system and up to 6.5 fold in case of polyethylene glycol/maltodextrin system. The enhancement in demixing rate is explained based on dipole rotation, electrophoretic migration of free salts, multiple reflections at the interfaces, droplet-droplet collision and reduction in viscosity of the continuous phase that occur during the application of microwave field.

Section 2D – It was already shown that electric field can be successfully applied in enhancing the phase demixing of ATPSs. In the present study, an attempt has been made to apply electric field for the selective separation of betalaines (betaxanthin and betacyanin) derived from beet hairy roots.

Section 2E – Apart from slow rate of demixing, another major hindrance for the adaptation of ATPE on industrial scale is the high cost of phase forming polymers such as polyethylene glycol (PEG) and also, the environmental problems arising due to the disposal of PEG rich phase after the extraction of biomolecules. In order to overcome the above drawbacks, there is a need to recover and recycle PEG from spent phases. In the present study an attempt has been made to separate and recover PEG from spent phases by the application of microwave field. The exposure of PEG rich phase to microwave field has resulted in phase separation of water (liquid phase) and PEG (solid phase). The separation of water from PEG rich phase is explained based on the decrease in PEG solubility at higher temperature and subsequent increase in PEG hydrophobicity. The separated PEG was dried to obtain it in the powder form. Studies were carried out to examine the physical and also chemical characteristics of PEG after recovery in order to ensure its suitability for reuse.

Chapter 3 – In recent years membrane based processes are gaining importance for the processing of biomolecules/liquid foods, in order to achieve value addition to the produce without product damage, to decrease the wastage and to facilitate preservation/transportation. Membrane processes like microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) are advantageous as they operate under relatively lower temperatures, thus minimizing product damage unlike in the thermal evaporation. Also, the water is separated without phase change thereby conserving energy. However, the existing membrane processes suffer from the drawbacks of concentration polarization, membrane fouling and maximum achievable concentration (only up to ~ 25°B). Even, newer membrane process like membrane distillation (MD) suffers from the drawbacks of low flux and temperature polarization. Hence, there is a need to develop an alternate/complementary process for the concentration of the solutions of proteins/natural food colors and other thermolabile biomolecules.

Osmotic membrane distillation (OMD) is a novel athermal membrane process that facilitates the concentration of solutions/liquids to the maximum achievable extent at mild operating conditions. In the present study, the effect of various process parameters such as type, concentration and flow rate of the osmotic agent, type and pore size of the membrane, temperature with respect to transmembrane flux was studied. Experiments were performed with real system (pineapple juice) in a flat membrane module. Osmotic agents (OA's) namely Sodium chloride and Calcium chloride dehydrate at varying concentrations are employed in the study. Higher transmembrane flux was observed at maximum osmotic agent concentration in case of both OA's. In comparison with sodium chloride, higher transmembrane flux was observed in case of calcium chloride. Experiments were carried out to study the effect of osmotic agent flow rate (25-100 ml/min) on transmembrane flux during concentration of pineapple juice by maintaining maximum osmotic agent concentration. Transmembrane flux increased with an increase in flow rate. A mass transfer-in-series resistance model has been developed considering the resistance offered by the membrane as well the boundary layers (feed and brine sides) in case of real system for the first time. The model could predict the **transmembrane flux** and also the effect of different parameters studied on flux.

Like any other membrane process, OMD also suffers from relatively low flux. Studies have been undertaken to apply acoustic field for the enhancement of transmembrane flux. Acoustic field from an acoustic transducer having a frequency of 1.2 MHz was applied perpendicularly to the membrane. Experiments were carried out for 5M Sodium chloride/pure water, 5M Calcium chloride dihydrate/pure water, Sodium chloride/sugarcane juice and Calcium chloride dihydrate/sugarcane juice systems both in the presence and absence of acoustic field in lab-scale flat membrane test cell. An enhancement of 22 - 205% in transmembrane flux by the application of acoustic field was observed.

Chapter 4 – This chapter has been categorized into three sub-sections with Section 4A dealing with purification and concentration of C-phycocyanin, Section 4B on Concentration of pineapple juice by OMD and Section 4C on large-scale studies for the concentration of pineapple juice employing hybrid process.

Section 4A – C-phycocyanin is a natural blue colorant derived from blue-green algae which finds application in food coloring, cosmetics and therapeutic uses. C-phycocyanin extract when derived from its source has high load of impurities and is in dilute form. Hence, C-phycocyanin needs to be purified and concentrated, so as to obtain C-phycocyanin suitable for food/pharmaceutical applications. Conventional methods employed for the purification of C-phycocyanin are inefficient, expensive and cumbersome due to involvement of more number of unit operations. Further, C-phycocyanin which is also protein needs to be concentrated under mild operating conditions since it is sensitive to heat/shear. Hence, studies have been undertaken to purify C-phycocyanin with lesser number of unit operations. Further, concentration of C-phycocyanin to higher levels has been undertaken by employing OMD process under mild operating conditions.

Section 4B – Pineapple is a popular non-citrus tropical and seasonal fruit. Pineapple has refreshing sugar-acid balance, attractive flavor and aroma. The fruit needs to be preserved suitably preferably in the form of fruit juice so as to cater to the consumer demand throughout the year all over the globe. Conventional thermal concentration process employed for concentrating pineapple juice leads to loss of color, flavor and aroma resulting in low quality end product. Hence, concentrated pineapple juice having all the organoleptic properties of the original fruit can find applications in the production of juice blends, carbonated soft drinks etc. Hence, alternate/complementary membrane process like OMD has such potential since it facilitates the concentration of pineapple juice to higher levels with minimal product damage. Studies have been undertaken to concentrate pineapple juice (>60°B) employing OMD process in a flat membrane module. The concentrated pineapple juice obtained from OMD process was analyzed for its sensory qualities by Kramer's rank sum method which confirmed that there was no difference in the quality of juice when compared to control sample.

Section 4C – OMD has low transmembrane flux like any other membrane process. Hence, it becomes inherently uneconomical to operate OMD process as a single step unless the product is of high value. In view of the above there is a need to enhance the overall productivity during large-scale processing of biomolecules/liquid foods. Attempts have been undertaken to concentrate pineapple juice on large-scale by employing hybrid membrane process involving UF, RO followed by OMD.

Chapter 5 – It comprises of other applications/constraints, suggestions for future work, followed by References.