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- Singla, M., & Sit, N. (2021). Application of ultrasound in combination with other technologies in food processing: A review. *Ultrasonics Sonochemistry*, *73*, 105506.
- Singla, M., & Sit, N. (2022). Isolation of papain from ripe papaya peel using aqueous two-phase extraction. *Journal of Food Measurement and Characterization*, 1-8.
- Singla, M., Singh, A., & Sit, N. (2022). Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel. *Journal of Food Process Engineering*, e14119.
- Singla, M., & Sit, N. (2023). Theoretical Aspects and Applications of Aqueous Two-Phase Systems. *ChemBioEng Reviews*, 10(1), 65-80.
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- Oral presentation on "Partitioning of papain enzyme from papaya peel (*Carica papaya* L.) By aqueous two phase system" in the international conference organized by Department of Food Processing Technology, Ghani Khan Choudhury Institute of Engineering and Technology Narayanpur, Malda, 2022.
- Oral presentation on "Effects of microwave and enzymatic pretreatments on ultrasonic extraction of polyphenols from papaya peels" in international conference on "Sustainable Approach in Food Engineering and Technology (SAFETy)" organized by Tezpur University,Assam and University of Georgia, USA, 2021.

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Review

Application of ultrasound in combination with other technologies in food processing: A review

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ABSTRACT

The use of non-thermal processing technologies has been on the surge due to ever increasing demand for highest quality convenient foods containing the natural taste & flavor and being free of chemical additives and preservatives. Among the various non-thermal processing methods, ultrasound technology has proven to be very valuable. Ultrasound processing, being used alone or in combination with other processing methods, yields significant positive results on the quality of foods, thus has been considered efficacious. Food processes performed under the action of ultrasound are believed to be affected in part by cavitation phenomenon and mass transfer enhancement. It is considered to be an emerging and promising technology and has been applied efficiently in food processing industry for several processes such as freezing, filtration, drying, separation, emulsion, sterilization, and extraction. Various researches have opined that ultrasound leads to an increase in the performance of the process and improves the quality factors of the food. The present paper will discuss the mechanical, chemical and biochemical effects produced by the propagation of high intensity ultrasonic waves through the medium. This review outlines the current knowledge about application of ultrasound in food technology including processing, preservation and extraction. In addition, the several advantages of ultrasound processing, which when combined with other different technologies (such as microwave, supercritical CO_2, high pressure processing, enzymatic extraction, etc.) are being examined. These include an array of effects such as effective mixing, retention of food characteristics, faster energy and mass transfer, reduced thermal and concentration gradients, effective extraction, increased production, and efficient alternative to conventional techniques. Furthermore, the paper presents the necessary theoretical background and details of the technology, technique, and safety precautions about ultrasound.

1. Introduction

Food processing is the effective conversion of agricultural produce into consumer ready product with the objective of producing more appealing, shelf-stable, compact, and usable and value-added product [22], which can involve single or multiple operation. The raw food items can be processed using several conventional processes and preservation techniques namely drying, baking, boiling, salting, pickling, soaking, etc. which are still used commonly and efficiently. The most traditional food processing methods rely on the underlying theory of increasing heat in order to reduce microbial growth and retard growth of foodborne pathogens that make the food suitable for consumption [122]. Such thermal processing requires high energy, yield low productivity and involve time-consuming procedures that require high external heat input/ consumption [19]. There are numerous food items that are susceptible to viral or bacterial intoxication that are not suitable for heat processing. Thus, these heat-sensitive food items on subjecting to thermal treatment can undergo changes at physical, chemical and microbial level including taste, color and textural modifications. This has to lead to the demand for extensive research and development in optimizing the existing technologies and to develop creative and efficient alternative techniques [116]. Some of the novel and innovative methods used in food processing are supercritical fluid extraction, high-pressure processing, pulsed electric field, cold plasma, ultrasound, and ultraviolet irradiation.

The utilization of new "green and innovative" techniques typically involves less time, water and energy in processing, pasteurization and extraction. These techniques are ultrasound-assisted processing [23,171], microwave processing [145], extrusion [77], supercritical fluid extraction and processing [133,140], controlled pressure drop

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ORIGINAL ARTICLE

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Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel

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Abstract

The study was aimed to investigate the effect of pretreatment by microwave and enzyme and type of solvent on kinetics of ultrasound-assisted extraction (UAE) of bioactive compounds and antioxidant activity from papaya peels. The enzyme used was viscozyme and the solvents used were water, ethanol, and their combination (1:1). The effect of combination of pretreatments was also studied. The data from the experiments were fitted to second order kinetic model and Langmuir model. The second order rate constant (*k*) varied from 0.0005 to 0.0134 for total phenolic content (TPC), 0.0016–0.0080 for total flavonoid content, and 0.0004–0.0228 for antioxidant activity (2,2-diphenyl-1-picrylhydrazyl-radical scavenging activity). The comparative kinetic assessment illustrated that the progression of the extraction for TPC was higher in enzyme-water (E-W) treatment (98.91 mg Gallic acid equivalent (GAE)/100 g), followed by microwave-enzyme water (ME-W) treatment (93.98 mg GAE/100 g) while microwave-water:ethanol (M-WEt) treatment gave the lowest yield (42.56 mg GAE/100 g), respectively. The present study also investigated influences of ultrasonication on extraction kinetics and their yield. Ultrasound was clearly influencing the yield (over 72.95% increase in yield of polyphenols). Antioxidant activity of the extracts confirmed that the UAE extraction is suitable for preparing antioxidant-rich plant extracts. Ultrasound and pretreatment before extraction could provide many advantages like increased yield and quality, energy efficiency, faster extraction, and low solvent consumption.

Practical Applications

Papaya peels are waste products generated during processing of papaya and are valuable sources of polyphenols and natural antioxidants such as caffeic acid, rutin, ferulic acid, p-coumaric acid, and many more bioactive compounds that have significant biological activity. These bioactive compounds of papaya peel can be extracted and utilized in food and pharmaceutical industry for development of functional food or nutraceuticals. Ultrasound extraction is regarded as a green technology as it is environment friendly and energy-efficient process. When ultrasound technology is combined with microwave or enzymatic treatment, the efficiency of such process may be further improved. The present study is therefore useful as it can provide a solution for utilization of papaya peel for extraction of valuable compounds using green technology.

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ORIGINAL PAPER

Isolation of papain from ripe papaya peel using aqueous two-phase extraction

Mohit Singla[1] · Nandan Sit[1]

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Abstract
Papaya peel is a good source of papain enzyme (cysteine protease) but currently unutilized for any commercial purpose and disposed of as waste, thereby becoming a source of pollution. Therefore, this study investigates the behavior of papain enzyme (cysteine protease) in an aqueous two-phase system (ATPS) comprising polymer/salt. Considering the excluded volume effect, polyethylene glycol (PEG) with a molecular weight of 6000 g/mol was used to form the ATPS. Sulphate salts (ammonium sulphate and sodium sulphate) were chosen to form phases owing to their ability to promote the hydrophobic difference between the phases. From the result, the best ATPS condition for the partitioning of cysteine protease was 10% (w/w) PEG 6000 + 18% (w/w) Na_2SO_4 at constant pH 9 and obtained the highest enzyme activity (K_e) 1.43 which increased the purity by 4.08-fold (PF) with the yield of 26.38%. PEG/salt ATPS has been shown to work well to purify partitioned cysteine protease, which can be scaled up and extracted with a high yield and purity factor. In addition, the paper studies the (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis) SDS-PAGE patterns of cysteine protease partitioned from papaya peels. The ATPS system is therefore effective in recovering and purifying papain enzymes from papaya peel.

Keywords Aqueous two-phase system · Cysteine protease · Papaya peel · Polymer · Salt

Introduction

Due to their ubiquitous nature, proteolytic enzymes (EC 3.4) are found in many organisms, including animals, plants and microorganisms [1]. In spite of the wide substrate specificity of enzymes from plants, they are also able to function in a wide range of temperatures and pH conditions as well as in the presence of additives and other organic compounds [2]. As a result, this enzyme is extensively used in various industrial processes, such as in food, detergents, waste, pharmaceuticals, and leather [3].

A tropical fruit of great economic importance, papaya (*Carica papaya L.*) is widely grown throughout the year and regarded as one of the most important commercial fruits among the Caricaceae family [4, 5]. With a total production volume of 15.36% of total tropical fruit production worldwide, the papaya is ranked third after mango and pineapple. Total production of papaya across the globe is 13,984,705 tonnes whereas in India, its total production is 6,011,000 tonnes [6]. Due to non-utilization of papaya peel for commercial purposes, it is disposed of as waste and contributes to environmental pollution across the globe [4]. Nevertheless, papaya peel has the potential to be used as it is a beneficial source for the production of natural enzymes such as papain (EC 3.4.22.2) [7].

The enzyme papain is a member of the cysteine protease family and generally appears as a minor component in papaya fruit latex [8]. The enzyme has been widely used in the food industries for meat tenderization and for various medicinal usages [7]. As a result, cysteine protease must be of high purity in order to meet the varied industrial and scientific requirements. In the traditional method, the purification process involves many steps, discontinuous operations, and a high labour intensity, all of which increases the cost and cause yield losses [9]. For the purification of protein products, industries are seeking fast and economical downstream processes, including those that can produce high yield and high purity [10]. The aqueous two-phase system

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ChemBioEng Reviews

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Theoretical Aspects and Applications of Aqueous Two-Phase Systems

Mohit Singla[1], Nandan Sit[1],*

Abstract

The aqueous two-phase system (ATPS) is commonly known as a technique that yields high-purity products in a single step. It is particularly advantageous for purifying biomolecules like proteins, nucleic acids, enzymes, viruses etc. Currently, aqueous two-phase extraction (ATPE), i.e., liquid-liquid extraction, involves the transfer of the solute from one aqueous phase to another. In ATPE, for recovery of biomolecules, polymer-polymer and polymer-salt type systems are used. The most recent developments with respect to recovery of biomolecules by ATPS are reviewed and discussed, covering the mechanism, which controls the phase formation, the conditions of solute partitioning in ATPS processes, and factors influencing the ATPS including concentration and molecular weight (MW) of polymers, types of salt, pH, and temperature. In addition, also the increasing applications of ATPS for the recovery of high-value bioproducts, the benefits of the ATPS recovery system, and the recent developments of alternative low-cost ATPS are highlighted.

Keywords: Aqueous two-phase extraction, Aqueous two-phase system, Biomolecules, Biomolecules purification, Polymers

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1 Introduction

1.1 General Remarks

Recent advancements in biotechnology have paved the way for the establishment of a wide range of biomolecules with applications in research, pharmaceuticals, clinical studies, and industry. Downstream processing forms an important aspect of any product development process, where the cost of the finished product is primarily determined by the costs incurred during the total recovery process. Conventional solid-liquid extraction is not effective for bioseparation since the size of microorganisms to be separated is small, especially when the cells are damaged and internal components are released, increasing the system's viscosity [1, 2].

Column chromatography and precipitation are not only expensive but also result in lower production. Centrifugation with other newer procedures, such as electrophoresis, possess significant scaling issues, rendering them uneconomical unless the commodity is of great importance. As a result, efficient, cost-effective, quick, easy, and environmentally acceptable downstream processing approaches for recovering biomolecules created by biotechnological means are required. Aqueous two-phase systems (ATPS) are one such approach for extraction. Although Albertsson invented this approach in the mid-1950s, its significance and uses were just recently understood.

In the chemical industry, liquid-liquid (L-L) extraction via organic-aqueous phase systems is widely employed. Despite its benefits, this approach has yet to obtain widespread industrial acceptance in the biotechnological field due to the lower solubility of proteins in organic solvents along with the tendency of organic solvents to denature them. Because it uses two aqueous phases, aqueous two-phase extraction (ATPE) has proved successful in overcoming the limitation of conventional organic-aqueous extraction to a great extent. For the extraction and purification of biomolecules, ATPE has been considered as a superior and adaptable approach [1, 3, 4]. Reverse micellar extractions [5], cloud point extractions [6], extractions using thermo-separating polymers [7], and micellar extractions are a few other L-L extraction methods used in the isolation and purification of various biomolecules [8].

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Chapter 21

Raising challenges of ultrasound-assisted processes and sonochemistry in industrial applications based on energy efficiency

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21.1 Introduction

Cavitation is the driving force behind sonochemistry and ultrasonic processing. It was originally recognized and classified in 1895 by Thornicroft, who discovered that it was the reason of inefficient propeller drive on the high-speed torpedo boat HMS Daring (Mason, 2012). The sort of cavitation created by propellers driving through water, that is, hydrodynamic cavitation, was the focus of this and subsequent early investigations.

Ultrasonics is the study of application of sound wave energy vibrating at frequencies greater than 20,000 Hz which is beyond the audible range for humans (Al-Juboori et al., 2015a, b). Sound is transmitted from one place to another by means of waves. The general method involved in the generation of ultrasonic waves is the rapid vibration of a dense material, which causes the surrounding air of the material to vibrate with the same frequency and spread out in the form of ultrasonic waves (Pokhrel et al., 2016).

In the industrial, medicinal, and other areas, ultrasonics has become a valuable instrument. It has widespread applications in materials science, industrial processes, oceanology, marine navigation, petroleum and mineral prospecting, medicine, dentistry, and noise cancellation (Al-Juboori et al., 2015a). The first widespread use was in underwater exploration for determining the depth of water. Ultrasonics has further industrial and diagnostic applications (Mason, 1992).

Since ultrasonic waves can vibrate the particles through which they pass, they are often used to shake, or even destroy, certain materials (Eskin and Tzanakis, 2018). An example of this procedure is ultrasonic emulsification. It can also be used for breaking up the kidney stones and gallstones, thus avoiding invasive surgery (Tripathi et al., 2019).

Ultrasonic energy has been used in industry since the middle of the 20th century, although only a few applications have been commercially commercialized thus far (Tripathi et al., 2019). However, in the last 10 or 15 years, there has been a resurgence of interest in ultrasonic processing, particularly in industries where ultrasonic energy may be used to enhance or create effects in a clean and efficient manner. Power ultrasound appears to be becoming an emerging technology for process development in key areas such as the food industry, environment, pharmaceuticals and chemicals manufacturing, equipment, and so on (Mason, 2012).

The design and development of efficient power ultrasonic systems (generators and reactors) capable of large-scale successful operation particularly suited to each particular process might be a key challenge in the use of high-intensity ultrasound to industrial processes.

This chapter examines some recent challenges in ultrasonic processing in order to demonstrate the current state and future promise of high-power ultrasonics as an innovative technology in a variety of industries.

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Title: Partitioning of papain enzyme from papaya peel (carica papaya l.) By aqueous two phase system

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Title : PARTITIONING OF CYSTEINE PROTEASE FROM PAPAYA PEEL (CARICA PAPAYA L.) USING AQUEOUS TWO PHASE SYSTEM

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Title: Effects of microwave and enzymatic pretreatments on ultrasonic extraction of polyphenols from papaya peels

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