Innovative Food Science and Emerging Technologies 39 (2017) 129–136

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

High pressure pre-treatment of *Moringa oleifera* seed kernels prior to aqueous enzymatic oil extraction

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ARTICLE INFO

Article history:
Received 16 July 2016
Received in revised form 19 October 2016
Accepted 22 November 2016
Available online 24 November 2016

Keywords:
Moringa oleifera
Seed kernels
Oil
Enzyme
High pressure processing
Emulsion

ABSTRACT

High pressure processing (HPP) was applied as a pre-treatment on *Moringa oleifera* (MO) kernels, for the first time, prior to aqueous enzymatic extraction (AEE) of the MO oil, and the effect of this pre-treatment is reported in terms of the free oil recovery and the nature of the cream emulsions formed. The HPP pre-treatments (50–250 MPa, 20–60 °C, 10–60 min) generally resulted in higher free oil recoveries and thinner emulsion layers from ground-sieved kernels than the whole kernels. Optimization of the HPP parameters indicated linear increment in free oil recovery with increase in temperature and time, but not the pressure level. Without the pre-boiling step in the AEE process, the use of HPP pre-treatment at 50 MPa and 60 °C for 35 min resulted in approximately 73% (w/w) free oil recovery with thinner emulsion layer than the use of AEE alone. These findings highlighted the ability of HPP in altering the MO protein structure into a form of less emulsifying functional properties, thus further de-emulsification method may not be necessary.

Industrial relevance: High pressure processing (HPP) applications are increasing in the food industry. High pressure application allows the use of relatively lower temperatures in processes, in order to achieve comparable outcomes of higher temperature processing. This study explores the application of HPP on *Moringa oleifera* (MO) kernels prior to aqueous enzymatic extraction (AEE) of oil. In general, the main disadvantage of AEE is its lower oil recovery in comparison with solvent extraction, which is attributed to the formation of a relatively stable cream emulsion after extraction. This study shows that the thickness of the creamy emulsion can be significantly reduced, and oil recoveries improved, by subjecting the kernels to HPP prior to extraction. The improvement in recoveries did not depend strongly on the level of high pressure applied, but downstream processes for free oil separation and recovery were considerably simplified.

1. Introduction

Aqueous enzymatic extraction (AEE) of oil from oil-bearing materials was reviewed by Mat Yusoff, Gordon, and Niranjan (2015) and Rosenthal, Pyle, and Niranjan (1996). This process involves addition of selected enzymes into a mixture of oleaginous material with pre-determined amount of water at a given pH value, followed by incubation of the mixture at a pre-set temperature, time, and shaking speed. The added enzymes function in hydrolyzing and breaking the cotyledon cell walls of the material, thus making the structure more permeable and further expose the oil component. The water-soluble components diffuse into the aqueous phase, while the released oil distributes itself between two phases: i) an oil-in-water cream emulsion in the aqueous phase and ii) a superficial oil layer on top of the emulsion (Rosenthal, Pyle, & Niranjan, 1998).

The advantages of AEE over solvent extraction were also pointed out by Rosenthal et al. (1998), which include lower environmental impact and lower process costs. In the case of *Moringa oleifera* (MO) kernels, the use of AEE was reported by Abdulkarim, Long, Lai, Muhammad, and Ghazali (2005), Abdulkarim, Lai, Muhammad, Long, and Ghazali (2006), Latif, Anwar, Hussain, and Shahid (2011), and Mat Yusoff, Gordon, and Niranjan (2016) which highlighted the importance of protein hydrolysis in the MO kernels for higher free oil recovery. Further, the kernels contain in excess of 35% protein content (w/w), and their microscopic structure indicates that the oil bodies in the cells are surrounded predominantly by protein (Mat Yusoff et al., 2016). Abdulkarim et al. (2006) also reported that the use of protease in combination with cellulase enzymes resulted in higher oil yield than the use of protease enzyme alone. Hence, the main enzyme used in earlier studies was mainly protease and cellulase. It is noteworthy that MO oil exhibits high oxidative stability due to the presence of high levels of tocopherols which also reduce the risk of developing coronary heart disease (Abdulkarim et al., 2006; Nguyen et al., 2011; Noakes, Nestel, & Clifton, 1996; Rahman et al., 2009; Zhao & Zhang, 2013).

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http://dx.doi.org/10.1016/j.ifset.2016.11.014
1466-8564/

Despite its obvious advantages, the MO free oil recoveries resulting from AEE are significantly lower than the recoveries observed by solvent extraction, ranging between 69 and 73% of solvent-extracted oil recovery (Mat Yusoff et al., 2016; Latif et al., 2011; Abdulkarim et al., 2006, 2005). Mat Yusoff et al. (2016) and Latif et al. (2011) found that the MO cream emulsion formed at the end of the AEE process required further de-emulsification in order to enhance free oil recovery, which was particularly difficult because of the added emulsion stability imparted by the proteins. Formation of creamy emulsion following an AEE process was also reported in the case of *Isatis indigotica* seeds (Gai et al., 2013), bayberry kernels (Zhang et al., 2012), and soybean seeds (Lamsal & Johnson, 2007); which in the latter case, extrusion was used as a pre-treatment on soybean seeds prior to AEE of soybean oil to prevent the formation of cream emulsion.

These findings therefore suggest that free oil recoveries can be enhanced by decreasing the emulsifying capacity of the proteins, possibly by altering their structures. The alteration of protein structure may be induced by both hydrolysis and denaturation, which can be promoted by subjecting the kernels to treatments such as high pressure processing (HPP). Upon denaturation, the peptide bonds of the protein become more available or more susceptible to hydrolysis by proteolytic enzymes, besides lowering the protein solubility (Anglemier & Montgomery, 1976). In addition, HPP also disrupts electrostatic and hydrophobic interactions in proteins (Messens, Van Camp, & Huyghebaert, 1997). HPP has been used in earlier studies to enhance oil yields. For instance, Jung and Mahfuz (2009) stated that the use of HPP at 200 MPa and 500 MPa, followed by AEE (protease enzyme), resulted in 3.20% and 1.30% (w/w) higher soybean oil yield, respectively, under otherwise identical conditions. In the case of tiger nuts on the other hand, Ezeh, Gordon, and Niranjan (2016) reported no significant difference in the oil yield from an AEE alone and from AEE with HPP pre-treatment at 50–700 MPa. It was reported that the parenchyma cells of the tubers exhibit cross linking of diferulic acid with arabinoxylans which contribute to its tough texture, even tougher than potatoes (Parker, Ng, Smith, & Waldron, 2000), thus the tuber's cells were not affected by the HPP pre-treatment. On the other hand, Omi, Kato, Ishida, Kato, and Matsuda (1996) reported that the solubility of soybeans' protein in aqueous phase increased with pressure from 100 MPa to 400 MPa, and decreased thereafter. Kato, Katayama, Matsubara, Omi, and Matsuda (2000) also reported considerable release of proteins from polished rice grains soaked in a mixture of enzyme and distilled water and treated with HPP at 100–400 MPa.

The role of protein as an emulsifier varies greatly as reported by Denda and Hayashi (1992), Kajiyama, Isobe, Uemura, and Noguchi (1995), Galazka, Dickinson, and Ledward (2000), Molina, Papadopoulou, and Ledward (2001), and Chapleau and de Lamballerie-Anton (2003a). Additionally, the factors affecting protein denaturation and hydrolysis may or may not affect the role of the protein as an emulsifier. Based on these theories and outcomes of earlier studies, it appears that a combination of protein denaturation caused by HPP, followed by protein hydrolysis employing AEE may significantly influence MO free oil recovery. This study aims to investigate this hypothesis as a novel application of HPP. To the best of our knowledge, there are, no studies reporting on the use of HPP pre-treatment to limit the stability of the cream emulsion formed after AEE. Therefore, the main objectives of this study are to explore the effect of different HPP parameters on the stability of MO cream emulsion formed after AEE and on the total recoveries of oil. Optimization of the HPP parameters has also been undertaken to determine the most suitable conditions which result in highest MO free oil recovery.

2. Materials and methods

2.1. Materials

Mature MO seeds (PKM1 hybrid) were purchased from Genius Nature Herbs Pvt. ltd., Coimbatore, India. All solvents and enzymes used in this project were obtained from Sigma-Aldrich Company Ltd., Dorset, UK.

2.2. Preparation of Moringa oleifera kernels for oil extraction methods

The process flow for MO sample preparation, solvent extraction, HPP, and the AEE methods are illustrated in Fig. 1. Mat Yusoff et al. (2016) revealed that ground MO kernels of <710 μm particle size resulted in highest hexane-extracted oil yield of 41.03% (g oil/g kernel), thus samples having this particle size range were used as the starting material in this study. The kernels of this particle size was prepared by grinding in a coffee grinder (De'Longhi KG49 Electric Coffee Grinder, Hampshire, UK) and sieved using a vibratory sieve shaker (Fritsch, Analysette 3E) of 710 μm sieve. The solvent extraction method used is explained in Section 2.5. In the HPP pre-treatment prior to the AEE, for comparison purpose, the HPP was also conducted on the MO kernels which were not ground, termed as whole kernels.

In this study, the term “oil yield” refers to the percentage of oil in the MO kernels as determined by using hexane as the extracting solvent. On the other hand, in all AEE-related processes, the term “oil released” refers to the percentage of oil extracted which includes free emulsified oil. The “free oil recovery” refers to the percentage of oil in the top layer above the cream emulsion layer that is formed after centrifuging, the percentage being expressed relative to amount of solvent-extracted oil.

2.3. Statistical analysis

Minitab® 14.12.0 Statistical Software: MINITAB Release 14.12.0, New York, USA was used for all statistical analyses and determination of significant differences between the data obtained in this study. A 1-Sample *t*-test was applied when one datum is compared with a sample with replicates data. When two replicates were compared, a 2-Sample *t*-test was used, while analysis of variance (ANOVA) with Tukey's multiple comparison test was applied when more than two samples (each sample with replicates data) were involved. The confidence level used in this study was 95.0.

2.4. High pressure processing

The HPP parameters involved in this study were the pressure (50–450 MPa), temperature (20–60 °C), and processing time (10–60 min). The pressure range was determined based on earlier studies, where the amount of protein released from soybean seeds (Omi et al., 1996) and rice grains (Kato et al., 2000) increased up to 400 MPa only, besides decrease in the amount of soybean oil extracted at 500 MPa pre-treatment prior to an AEE method (Jung & Mahfuz, 2009). A preliminary study on the effect of HPP pre-treatment (pressure, 200 MPa, 400 MPa, 500 MPa, 600 MPa; 25 °C; 15 min) was also conducted prior to solvent extraction of MO oil using hexane (Section 2.5). It was found that the MO oil yield was highest at 400 MPa, and slightly decreased afterwards at higher pressures (Section 3.1). This finding further justified the use of lower pressure range (50–450 MPa) for the optimization purpose. In terms of the temperature range, most types of protein are fully denatured at 60 °C (Scopes, 1994) and above if held for sufficient time. In the case of the processing time, due to adiabatic temperature increase during the pressure treatment, the temperature was unstable in the first 2–3 min of pressurization period based on a preliminary study conducted. Therefore, a minimum of 10 min was used in order to allow enough time for the temperature to stabilize.

The statistical software (Section 2.3) was used to generate the Box Behnken experimental design used in this study, where the pre-determined HPP ranges resulted in 15 design points (i.e. 15 run orders) with 3 centre points (Table 1). A mixture of MO sample and distilled water (1:1 w/w ratio) was transferred into a polyester bag and vacuum-sealed in order to minimize the headspace. The solid-to-liquid