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Aqueous enzymatic extraction of *Moringa oleifera* oil

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ABSTRACT

This paper reports on the extraction of *Moringa oleifera* (MO) oil by using aqueous enzymatic extraction (AEE) method. The effect of different process parameters on the oil recovery was discovered by using statistical optimization, besides the effect of selected parameters on the formation of its oil-in-water cream emulsions. Within the pre-determined ranges, the use of pH 4.5, moisture/kernel ratio of 8:1 (w/w), and 300 stroke/min shaking speed at 40 °C for 1 h incubation time resulted in highest oil recovery of approximately 70% (g oil/g solvent-extracted oil). These optimized parameters also result in a very thin emulsion layer, indicating minute amount of emulsion formed. Zero oil recovery with thick emulsion were observed when the used aqueous phase was re-utilized for another AEE process. The findings suggest that the critical selection of AEE parameters is key to high oil recovery with minimum emulsion formation thereby lowering the load on the de-emulsification step.

1. Introduction

Moringa oleifera (MO) seed oil, also known as Ben or Behen oil, is extracted from MO seed kernels which originated from India. The MO trees are also widely grown in Kenya, Pakistan, Bangladesh, Malaysia, Nigeria, and Philippines. The MO oil contains high amounts of tocopherols and oleic acid, which contribute to the oil's oxidative stability and lower the risk of developing coronary heart disease, respectively (Abdulkarim, Long, Lai, Muhammad, & Ghazali, 2007; Anwar, Hussain, Iqbal, & Bhanger, 2007; Lalas & Tsaknis, 2002; Rahman et al., 2009; Tsaknis & Lalas, 2002; Tsaknis, Lalas, Gergis, Dourtoglou, & Spiliotis, 1999).

A number of techniques are available for oil extraction from seed kernels of different origins, which include solvent extraction, cold-pressing, supercritical fluid extraction, and aqueous enzymatic extraction (AEE) methods. The advantages of AEE over solvent extraction include the use of water which is safer, benign, and economical. Moreover, the simultaneous extraction of phospholipids also reduces the refining steps (Latif, Anwar, Hussain, & Shahid, 2011; Rosenthal, Pyle, & Niranjan, 1996; Santos & Ferrari, 2005). Latif et al. (2011), Abdulkarim, Lai, Muhammad, Long, and Ghazali (2006) and Abdulkarim, Long, Lai, Muhammad, and Ghazali (2005) reported that AEE resulted in MO oil having lower free fatty acids and better oxidative characteristics, even though the oil yield – measured as the mass of oil extracted per unit mass of the seed taken – was lower than in the case of solvent extraction. Another disadvantage of AEE is that it results in the formation of oil-in-water cream emulsions which requires separation to recover oil (Chabrand, Kim, Zhang, Glatz, & Jung, 2008; Latif & Anwar, 2011; Long et al., 2011).

Mat Yusoff, Gordon, and Niranjan (2015) and Rosenthal et al. (1996) have reviewed AEE and explored the links between the microstructure of a seed or kernel and the choice of enzyme which can potentially be employed. The enzymes play an important role in rupturing the major components of the cell wall and facilitate oil release. Additionally, these papers summarized the factors affecting oil yield in AEE which include the particle size of the oil-bearing material, the amount of enzyme and water added, the pH of the mixture, and the incubation temperature, time, and shaking speed. These factors inevitably influence the nature and stability of the emulsions formed after extraction, and hence downstream processing. Statistical optimization of the AEE parameters resulting in the highest oil yield possible, was reported for pine kernels (Li, Jiang, Sui, & Wang, 2011), watermelon kernels (Sui, Jiang, Li, & Liu, 2011; Liu, Jiang, & Li, 2011), and bayberry kernels (Zhang et al., 2012). In the case of MO kernels, the effect of different AEE parameters on MO oil yield and recovery has been reported by Abdulkarim, Lai, et al. (2006) and Abdulkarim, Long, et al. (2005) and Latif et al. (2011). However, no systematic studies have been conducted so far on the microstructure of MO kernels

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and statistical optimization of its AEE parameters. According to Sineiro, Dominguez, Núnez, and Lema (1998), the main purpose in optimizing AEE parameters is to obtain the conditions that best incorporate with the enzymes added, besides leading to greater cell rupture. Although this statement is not incorrect, it is important to note that cell rupture does not only determine the amount of oil released from the kernel, but it also plays a critical role in the structure and stability of the emulsion formed. So far, no studies have been carried out on the formation of MO oil-in-water cream emulsion as affected by AEE parameters.

The main objectives of this study are to examine the microstructure of MO kernels and identify the type of enzymes which can be employed in AEE method. In addition, this work also reports on the effect of AEE parameter, identified above, on the yield and recovery of MO oil. This paper also discusses the effect of selected AEE parameters on the nature of MO oil-in-water cream emulsions. Finally, the resulted aqueous phase from the AEE conducted by using the optimized AEE parameters was re-used in another AEE process in order to evaluate the re-usability of the enzymes.

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 study were purchased from Sigma-Aldrich Company Ltd., Dorset, UK.

2.2. Methods

2.2.1. Preparation of Moringa oleifera kernels and determination of the kernels' protein content

Fig. 1 shows the processing steps involved in the preparation of MO kernels to be used in the oil extraction processes. The conditioned kernels (50 °C, 8 h) were kept in darkness at 4 °C until use (Zhao & Zhang, 2013). The protein content of the kernels was determined by Kjeldahl method – AOAC official method 955.04 (2000).

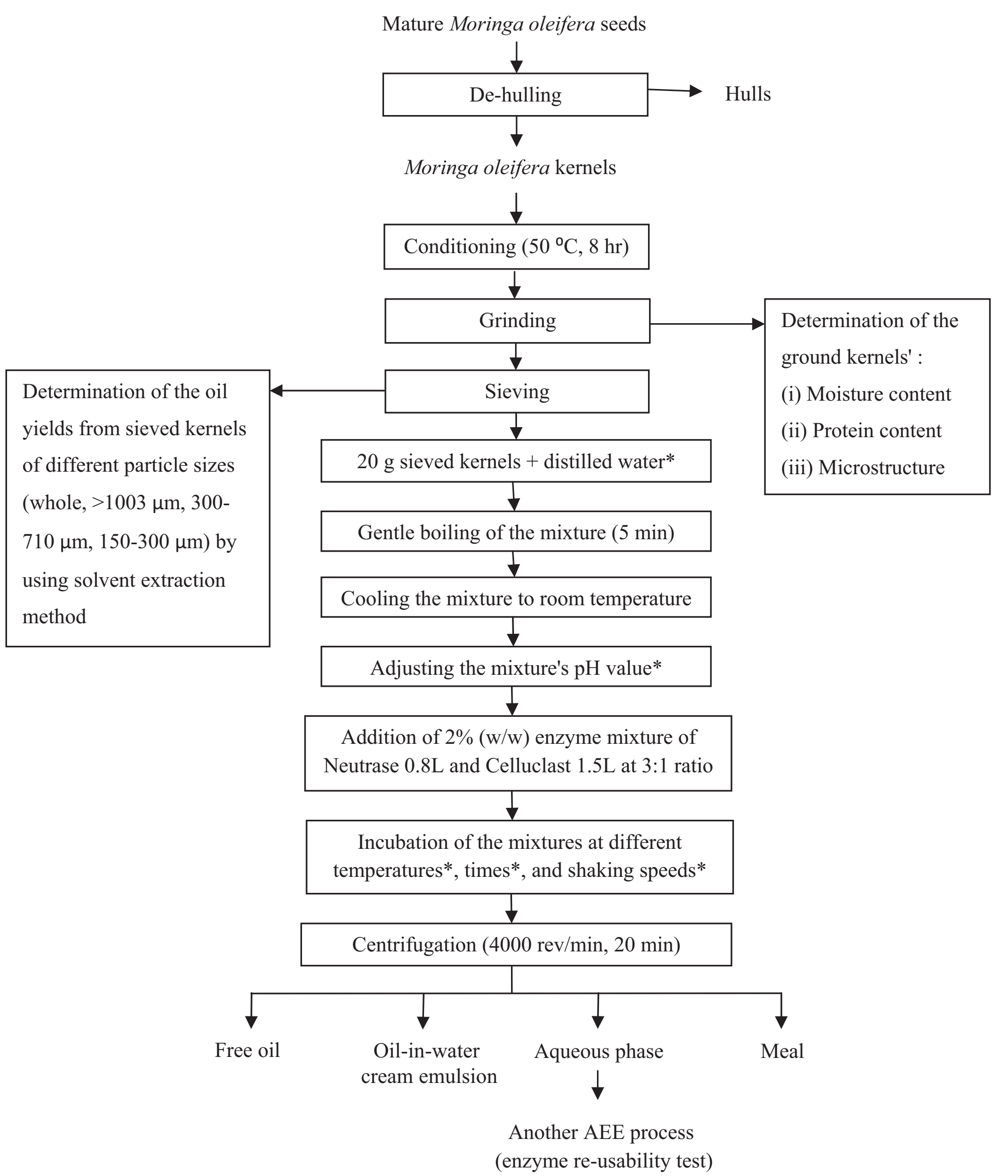

Fig. 1. Processes involved in the preparation of *Moringa oleifera* kernels, and extraction of *Moringa oleifera* oil via aqueous enzymatic (AEE) and solvent extraction methods. The (*) represents the AEE parameters tested.