Production of ethyl esters from radish seeds using a sequential process under pressurized fluid conditions

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1. Introduction

Radish (*Raphanus sativus* L.) is a plant of the Brassicaceae family, which seeds are intended for oil extraction with the aim to obtain biodiesel. The meal generated as a by-product is generally used as animal feed[1]. The sequential production of alkyl esters is considered an integrated process, in which the material from the oil extraction (oil + solvent) is used directly as a reaction mixture, eliminating the solvent removal and oil refining steps, as well as the addition of a new acyl acceptor reagent in the reaction[2]. The technology that uses fluids under pressurized conditions is an intensification process that promotes high solvating power to the fluids, since small changes in temperature and pressure cause changes in the physical properties of fluids such as density, viscosity, solubility and dielectric constant[3]. Due to this flexibility and ease of adjustment, the process under pressurized conditions can be applied both for oil extraction and production of biodiesel[4].

This work aims to investigate the sequential production of ethyl esters (EE) from radish seeds using pressurized processes as an integrated approach. Therefore, pressurized liquid extraction (PLE) and supercritical reaction were used for the oil extraction and EE production, respectively, using the material from the extraction directly in the reaction. Ethanol and ethyl acetate were used as extracting solvents and acyl sources and their effects were compared.

2. Materials and Methods

The extraction of radish seeds oil (RSO) was carried out in the extraction unit previously described[2], using ethanol and ethyl acetate as solvents at a flow rate of 3 mL min^{-1}, temperature of 135 °C, pressure of 50 bar, static time of 10 min and 50 min extraction time. For comparative purposes, the conventional extraction by the Soxhlet method was performed using *n*-hexane as a solvent[5]. The mass yield (Y_M) was calculated from the ratio between the mass of crude extract obtained and the mass of seeds used in each extraction. The non-lipid fraction of the material (w_{nl}) was determined according to the method reported by Rodriguez et al.[6] and the oil yield (Y_O) was determined considering the value obtained and Y_M. The extraction of defatted meal proteins was performed[7] and soluble protein content was determined by the method reported by Lowry et al.[8].

The material collected after the PLE was directly used as a reaction mixture and pumped to the experimental continuous reaction unit, reported by Postaue et al.[9]. The reactions were conducted to evaluate the effect of temperature (275 °C and 300 °C) at a residence time of 15 min and pressure of 20 MPa. To evaluate the effect of water in the conversion of the RSO into EE, experiments were carried out with the concentration of water adjusted to 10% and 20% in relation to the volume of ethyl acetate, at the same experimental processing conditions mentioned previously. The EE contents were analyzed by gas chromatograph (Shimadzu, GC-2010 Plus, Tokyo, Japan) using analysis conditions previously reported by Trentini et al.[10].

3. Results and discussion

From Table 1, it is observed that the extraction using pressurized ethanol resulted in higher Y_M, reaching a higher value than the Soxhlet extraction. However, the extracted material presented high w_{nl}, which shows that 37.18% of the Y_M is attributed to non-lipid compounds extracted during the process, resulting in a lower Y_O than the other extraction methods. This result is partially explained by the lower protein content retained in the defatted meal obtained after the PLE using ethanol, since it indicated that there was a concomitant extraction of oil and proteins in the extract phase. Studies showed that ethanol has the capacity to extract proteins[11]. The oil obtained by the PLE with ethyl acetate showed lower w_{nl} and higher Y_O compared to the same technique using ethanol as a solvent. This result showed the high selectivity of

pressurized ethyl acetate in obtaining RSO, which was able to reach 88.4% of the Y_O of the Soxhlet technique in just 50 min, on the other hand, the conventional Soxhlet technique required 480 min.

Table 1. Mass yield (Y_M), fraction of non-lipid material (w_{nl}), oil yield (Y_O) and soluble protein content (SPC) retained in the defatted meal from the oil extractions of radish seeds conducted with different pressurized solvents and by the Soxhlet method.

Technique	Solvent	$Y_M{}^1$ (wt%)	$w_{nl}{}^2$ (wt%)	$Y_O{}^3$ (wt%)	SPC (wt%)
PLE	Ethanol	33.09 ± 0.64^a	37.18 ± 0.66^a	20.85 ± 0.43^c	11.79 ± 0.56^c
PLE	Ethyl acetate	23.41 ± 0.43^c	4.28 ± 0.35^b	22.40 ± 0.33^b	22.81 ± 0.94^b
Soxhlet	*n*-Hexane	25.50 ± 0.59^b	0.65 ± 0.07^c	25.34 ± 0.59^a	31.09 ± 0.68^a

The sequential supercritical reaction conducted at 275 °C showed EE content of 10.05±0.63% and 12.76±0.95% when ethanol and ethyl acetate were used as acyl acceptors, respectively. With the increase of temperature to 300 °C, the EE content reached 20.76±0.20% (ethanol) and 38.67±0.46% (ethyl acetate). This behavior is related to the increase in the rate constant in the kinetics of the chemical reaction, since the increase in temperature favored the homogeneity of the reaction system, due to better miscibility of the reactants, as well as increasing the kinetic energy of the molecules[12]. In addition, as the temperature increased, the degree of hydrogen bonds in the alcohol decreased, resulting in a reduction in the dielectric constant and polarity of ethanol[13]. When comparing the contents obtained in the sequential reaction with ethanol and ethyl acetate, it was observed that at 275 °C, there was no difference between the results obtained ($p>0.05$). However, at 300 °C, the reaction with ethyl acetate resulted in a higher EE content ($p<0.05$). Increasing temperature, the solubility of the oil in ethyl acetate was higher than in ethanol, promoting more synthesis of EE. Ethyl acetate has a lower polarity than ethanol, so the oil is more soluble and mass transfer limitations are circumvented[14]. Furthermore, in PLE, ethyl acetate had better selectivity in the extraction of neutral lipids (Table 1), which represented the real raw material for the production of biodiesel, since this lipid fraction was capable of reacting with alcohol or ester to produce EE.

Since ethyl acetate showed better performance in the sequential EE production, it was selected to investigate the effect of water addition on the reaction. At 275 °C, the EE contents were 45.17±085% and 43.82±1.65% for reactions conducted with 10% and 20% of water, respectively. But at 300 °C, these contents increased to 45.92±2.11% (10% of water) and 53.03±1.25% (20% of water) probably due to the effect of the three reactions that occured simultaneously in the presence of water, interesterification, hydrolysis and esterification, contributing to the formation of EE[14].

4. Conclusions

The use of ethanol in PLE promoted the extraction of non-lipid compounds and resulted in lower Y_O. On the other hand, ethyl acetate showed excellent selectivity for the oil extraction under pressurized conditions, reaching 88.4% of the Y_O of the Soxhlet technique. Furthermore, in the sequential reaction, increasing the temperature from 275 °C to 300 °C promoted higher levels of EE. When ethyl acetate was used as acyl source, higher EE content was reached at 300 °C where the addition of water favored the formation of EE.

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