

Influence of the Combined Effect of Transesterification and Supercritical CO₂ Extraction for the Obtaining of Biodiesel and Pigments from Capsicum Annuum Oleoresins

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Abstract

According to the composition of Oleoresin, which is mainly based on lipid matter but incorporates a significant amount of bioactive pigments [1], the fractionation of oleoresin into biodiesel and pigments could be achieved by the combination of an oleoresin pre-treatment with a subsequent stage of supercritical CO₂ batch extraction.

In this work, a pre-treatment process consisting of transesterification using basic catalyst, was employed to turn the lipid matter of oleoresin into acylglycerols, which are compounds more easily extracted by supercritical CO₂ [2]. The transesterified oleoresin was then subjected to supercritical CO₂ batch extraction at 300 bar and 333 K, which were the most suitable operational conditions previously selected elsewhere [3]. A comparison of two different types of oleoresins, oleoresin paprika (OR_P) and oleoresin capsicum (OR_{Cap}), was carried out to determine the influence of the initial raw material on extraction yield and on the efficacy of fractionation of biodiesel and bioactive pigments. Moreover, in order to evaluate the benefits of the developed pre-treatment, the results obtained with the transesterified oleoresins were compared to conventional ones and solubilities were contrasted.

INTRODUCTION

The demand of bioactive compounds from natural sources has increased noticeably in the last decades. In fact, nowadays consumers demand compounds highly enriched in antioxidants, vitamins and any other component which can hold a potential benefit in human health. Within the wide range of compounds which own beneficial properties, carotenoids and capsaicinoids have been extensively studied [4-6].

The products derived from capsicum species, paprika powder and liquid oleoresin, are both enriched always in carotenoids. Besides, depending on the variety of pepper employed for their production, capsaicinoids pigments can also be found. The importance of carotenoids pigments is attributed to their provitamin A activity, but they also play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen [7] and inhibiting the development of certain types of cancers [8]. Furthermore, carotenoids are the responsible of colorant capacity of paprika powder and liquid oleoresin and can be employed as natural colorants.

According to the other main group of bioactive compounds of capsicum species, capsaicinoids, they belong to the family of alkaloids and can be found in pungent peppers (*C. frutescens*) with a wide range of pungency capacities. Capsaicin was described as a counter-irritant in lumbago, neuralgia, and rheumatic disorders but, recent interest has centred on the use of capsaicin as a topical analgesic for a variety of conditions characterized by pain not responsive to classical analgesics [9]. Attending to these properties, capsaicinoids present many applications in current pharmaceutical industry, besides provide the spicy taste to food.

Previous researches have demonstrated the feasibility of supercritical fluid extraction (SCFE) to achieve the fractionation of liquid oleoresin in two high added value products: an extract enriched in capsaicinoids and a raffinate enriched in carotenoids [3]. Nevertheless, due to the composition of liquid oleoresins, mainly composed by triglycerides, diglycerides and monoglycerides, in addition to the other minor compounds previously described (carotenoids and capsaicinoids), the enrichment of these minor constituents would be improved by removing acylglycerols from the mixture. Attending to the solubility of acylglycerols in comparison to that of fatty acid methyl esters, the latter one is magnitudes higher [2], thus the transesterification of acylglycerols into methyl ester enhances the extraction of these compounds and as a consequence the enrichment of carotenoids and capsaicinoids.

In this work, the transesterification of acylglycerols into methyl esters by means of conventional reaction using basic catalyst was studied as a previous pre-treatment of the SCFE of liquid oleoresin to get the enrichment of the bioactive compounds present in the raw material. Due to the higher solubility of fatty acid methyl esters (FAME's), an extract enriched in these compounds was obtained. Depending on the purity of this extract, it could be used as biodiesel. Moreover, two different types of liquid oleoresin, oleoresin paprika and oleoresin capsicum, were analysed to determine the influence of the raw material over the enrichment of bioactive compounds after a combined process of transesterification and SCFE.

MATERIALS AND METHODS

Chemicals

A commercial oleoresin Capsicum (OR_{Cap}) was provided by R. Sabater S.A (Murcia, Spain) with a pungency capacity of $\sim 1.0 \cdot 10^6$ Scoville Heat Units (SHU) and a colour capacity of 2500 Standard Colour Units (SCU). CO₂ with 99.8% of purity was supply by Carburos Metálicos (Madrid, Spain). All organic solvents grade HPLC were supplied by Sigma Aldrich, S.A. (Barcelona, Spain) and standards used for identification and quantification were also supplied by Sigma Aldrich, S.A.

Equipment and experimental procedure

a) Transesterification

Transesterification was carried out employing the procedure described by Ramos et al. [10]. The set-up employed for the reaction of acylglycerols (Figure 1) consists in a stirring hot plate equipped with a temperature controller and a cooler to avoid losses of methanol along the

reaction time. All the reagents (MeOH, oleoresin and catalyst) were introduced in a 250 mL vessel where the stirring was achieved magnetically. As catalyst, CH_3ONa in methanolic solution was employed. Once the mixture reached the suitable temperature (328 K), the reaction was left along 4 hours. After that, the purification of the resulted mixture had to be developed.

The purification step consisted in washing the reactive mixture with deionised water, separating both phases removing water, glycerine, methanol and catalyst and centrifuging the resultant product to discard any trace of unsuitable compounds. Finally, the evaporation of any trace of MeOH was achieved by rotary evaporation at 323 K along 30 minutes.

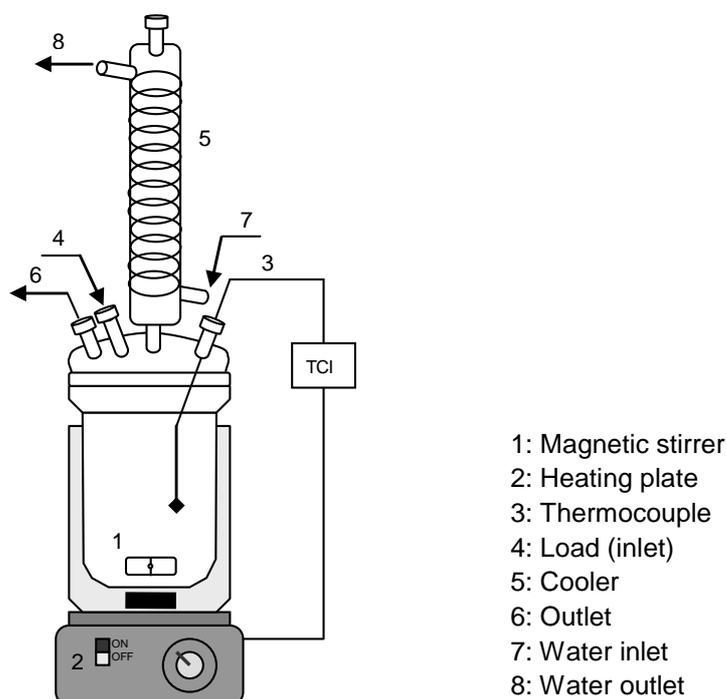


Figure 1. Schematic diagram of the set-up for transesterification reaction.

b) Supercritical fluid extraction (SCFE)

The experiments were carried out in a batch-type apparatus, as shows in Figure 2. Liquid CO_2 from a stainless-steel cylinder is filtered (F-1), cooled (E-1), and compressed by a positive-displacement pump (P-1). The pressure is regulated by a back-pressure regulator (BPR) and checked by a manometer (PI-1). The compressed fluid passes through a 100 mL stainless-steel cylinder (C-1) from the bottom to the top. At the top and at the bottom of the extractor a layer of glass wool is placed between two metallic meshes of 0.2 mm to prevent loss of particles. To keep the temperature of the extractor at the desired value, a digital controller (TIC) regulates the electric current through a resistance which is placed around the extractor cylinder. The oil-laden gas from the extractor passes through a heated metering valve (V-7) where the supercritical CO_2 is depressurized, and the extracted oil is collected in a cooled

container (T-1). The gas flow through the extractor was measured by a turbine flow meter (FI-1).

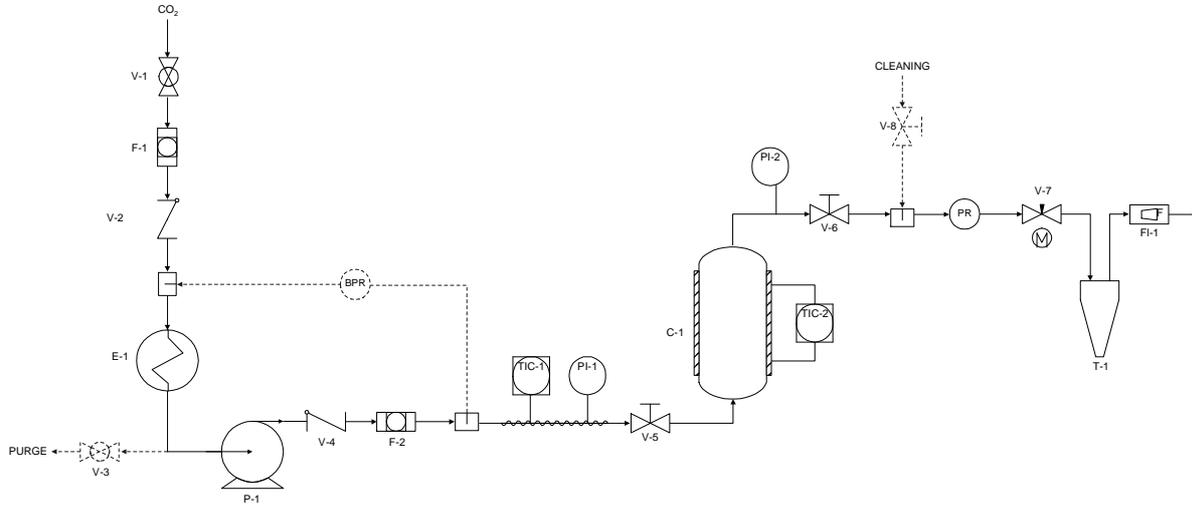


Figure 2. Flow diagram of the supercritical fluid extraction set-up.

Analytical characterization

a) Colouring capacity

The colour capacity was determined through the measurement of parameters ASTA and SCU in the extracts and raffinates. ASTA value of paprika oleoresin samples was evaluated according to the ASTA_{20.1} method [11] and using a Thermo Scientific spectrophotometer model Helios-Zeta UV-Vis (Madison, US). The ASTA colour value was then calculated as follows:

$$ASTA_{20.1} \text{ units} = \frac{A_{\lambda} \cdot 164 \cdot I_f}{m_s} \quad (2)$$

where A_{λ} is the absorbance of the sample at 460 nm, I_f is a correction factor of the spectrophotometer and m_s is the mass of raw material in grams.

Once ASTA units are obtained, Standard Colour Units (SCU) can be calculated from the relationship between both [12]:

$$SCU \text{ Units} = 40.24 \times ASTA_{20.1} \text{ Units} \quad (3)$$

b) Capsaicinoids concentration and pungency capacity

The concentration of capsaicinoids was determined by HPLC analyses. A known amount of sample was weighed and diluted with acetone. Prior to the injection into the HPLC column, the mixture was filtered through a 0.45 μm filter. The chromatographic analyses were carried

out using a Jasco Chromatograph (Essex, UK) equipped with a quaternary pump PU-1580, an autosampler AS-1550 and an in-line degasser DG-1580-53. For the determination of the capsaicinoids content in the samples the mobile phase used was water/acetonitrile (45:55, v/v) with a flow rate of 1 mL/min and a Discovery C18 column (15 cm × 4.6 mm, 5 μm) purchased from Analisis Vinicos (Tomelloso, Spain). A Diode Array MD-1510/1515 detector was used and the identification and quantification of peaks was made by comparison with standards.

Known the concentration of capsaicinoids, pungency capacity expresses as Scoville Heat Units can be calculated based on the following relationship [13]:

$$1 \text{ ppm of capsaicinoids} \sim 15 \text{ SHU} \quad (7)$$

c) Content of fatty acid methyl ester

The concentration of fatty acid methyl ester was determined according to the procedure described in the test method UNE-EN 14103 [14] by GC Chromatography employing a SupelcowaxTM 10 capillary column (Supelco, Madrid).

Prior to the injection, the preparation of samples consists in weighing 0.250 g of sample and mixing it with 5 mL of the solution of the internal standard previously prepared attending to what is described in the test method.

RESULTS

Different experiments were carried out in order to determine the influence of the type of oleoresin and the influence of the pre-treatment on extraction yield, colouring capacity and pungency capacity of the extracts and raffinates collected along the process. Besides, the content of FAME's of the transesterified oleoresins and that of the extracts and raffinates collected after the SCFE process was also analysed.

Influence on extraction yield

Based on the results obtained in a previous research [3], which demonstrated the influence of the raw material on extraction yield, colouring capacity and pungency capacity of the products obtained after a single procedure of SCFE, in this work, the influence of the raw material on a combined process of transesterification, as pre-treatment, and SCFE, as second step, was analysed.

Transesterification reaction was carried out using the same catalyst and at the same conditions for both oleoresins, OR_P and OR_{Cap}. The obtained conversions after the process were 71.37 % wt. and 85.35 % wt., respectively. Besides, despite small differences between both profiles, distribution of fatty acid methyl ester was almost the same for both oleoresins.

Using the transesterified oleoresins as the raw material for SCFE at 300 bar and 333 K (these are the most suitable conditions for oleoresins without transesterification), the global

extraction yield increases as a consequence of the increase in solubility. Solubility of FAME's is higher in comparison to the solubility of acylglycerols, and hence a small amount of CO₂ is required to achieve equal extraction yields than when the pre-treatment was not carried out. Figure 3 illustrates this result for both types of oleoresin.

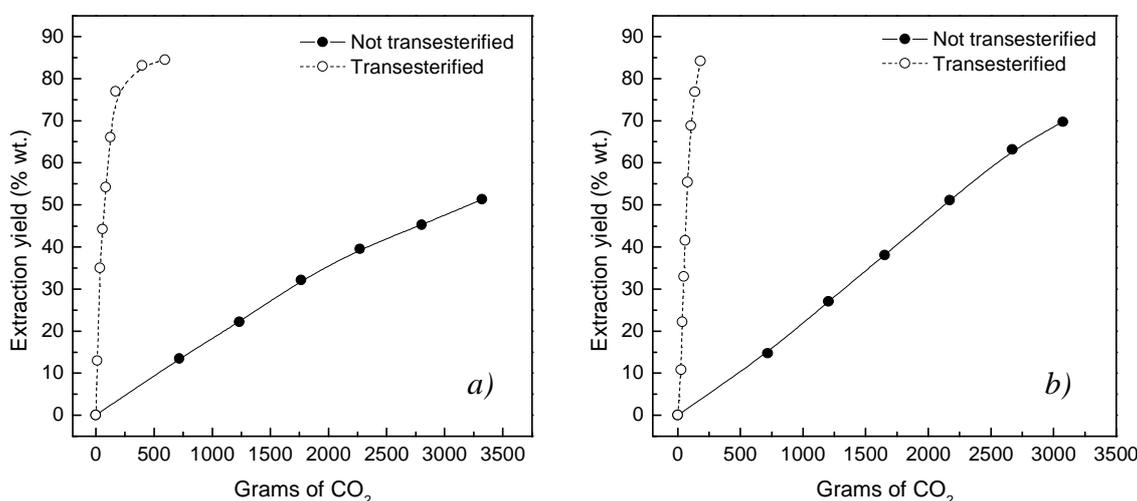


Figure 3. Evolution of the extraction yield for the SCFE process. P= 300 bar; T= 333 K. a) OR_P; b) OR_{Cap}.

Note that those experiments conducted without transesterification for different types of oleoresin (Figure 3 a) and b)) show different values of extraction yield for the same amount of CO₂ consumed (at 3000 g of CO₂, $Y_{ORP}= 47.6\%$ and $Y_{ORCap}= 68.6\%$). This can be explained because of the different composition and physical properties, especially viscosity, of the oleoresins employed as raw material. Nevertheless, when comparing the yields obtained after the pre-treatment of transesterification, results are quite similar for the same amount of CO₂ independently of the type of oleoresin. This result concludes that if a transesterification process is carried out prior to the SCFE, the type of oleoresin does not influence on the extraction yield, due to the fact that the pre-treatment converts acylglycerols into fatty acid methyl esters for both types of oleoresins and, as a consequence, the solubility behaviour of both types of transesterified oleoresins will be almost the same.

Influence on colouring capacity: enrichment of carotenoids

Among the compounds present in liquid oleoresins, carotenoids are the less soluble in CO₂ [1], thus remaining in the raffinate after the extraction. In this sense, the higher the extraction yield is, the higher the enrichment of these pigments in the raffinate is, due to the decrease in the dilution effect which other non extracted compounds could produce in the concentration of carotenoids.

Attending to this fact, and taking into account the results obtained for extraction yields when a pre-treatment of transesterification is carried out, it is predictable that enrichment of carotenoids in transesterified oleoresins will be higher. Nevertheless, transesterification produces a decrease in the colouring capacity of both types of oleoresins, more noticeable in

the highest coloured oleoresin, OR_P , caused by the loss of some carotenoids during the washing step of purification.

Despite this initial loss of carotenoids, the colouring capacity in the raffinate for OR_P considerably increases respect to its extraction without transesterification (482,709.8 SCU and 296,613.3 SCU, respectively). Furthermore, the remaining raffinate obtained after the SCFE appears as small particles of solid, which characterization was developed by optical microscopy. Figure 4 illustrates the size and morphology of particles.

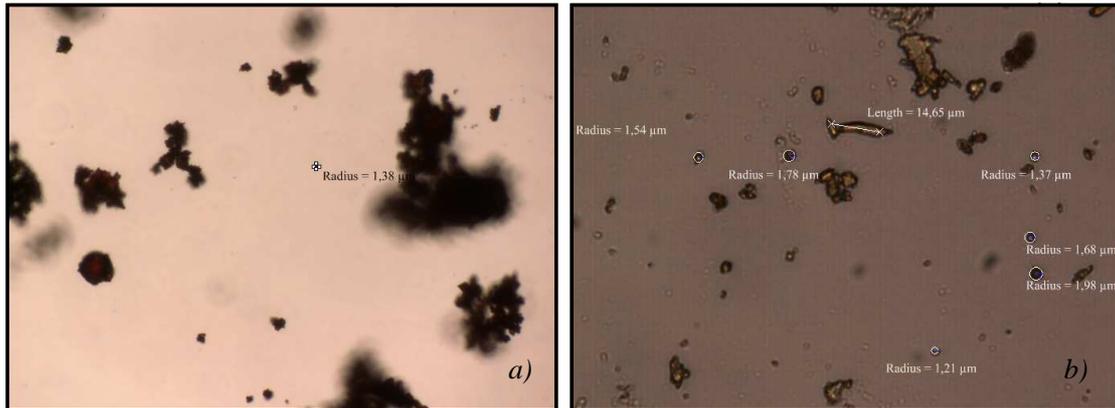


Figure 4. Solid particles obtained as raffinate after the SCFE of transesterified oleoresin paprika. a) Aggregates; b) Single particles.

It can be observed that many of the particles form aggregates of higher size, but observing a single particle, the average size is around 1.5-2 μm .

Influence on pungency capacity: enrichment of capsaicinoids

Attending to the pungency capacity of oleoresin capsicum, the transesterification process produces a loss of capsaicinoids pigments during the washing step of purification. This reduction implies to lose the half of the initial content of pungent principles of the oleoresin.

Despite this fact, the evolution in the pungency capacity of the extracts recovered along the SCFE at 300 bar and 333 K (see Figure 5a) follows the same trend as that obtained when the oleoresin was not previously transesterified: they keep almost constant along the extraction procedure. Nonetheless, whereas for non transesterified OR_{Cap} the average SHU of the extracts collected is higher than its initial value, for transesterified OR_{Cap} the average SHU is slightly lower than initially. This can be explained attending to the different solubility between acylglycerols and FAME's. As the first one are less soluble in CO_2 than FAME's, when OR_{Cap} is not transesterified the most soluble compounds in CO_2 are fatty acids and capsaicinoids and hence, their extraction is easier respect to the rest of compounds (although greater amounts of CO_2 are required). Nevertheless, when OR_{Cap} is transesterified, the extraction of FAME's respect to the rest of compounds is favoured and capsaicinoids which are extracted with them are diluted respect to the non transesterified oleoresin.

According to the enrichment in pungency capacity of the raffinate in comparison to the initial SHU value of loaded oleoresins (Figure 5b), transesterified OR_{Cap} present a higher value of SHU in the raffinate than non transesterified OR_{Cap} . This is as a consequence of the combined effect of the increase in extraction yield and of the extraction of more soluble compounds (FAME's), which produces the enrichment of capsaicinoids in the raffinate.

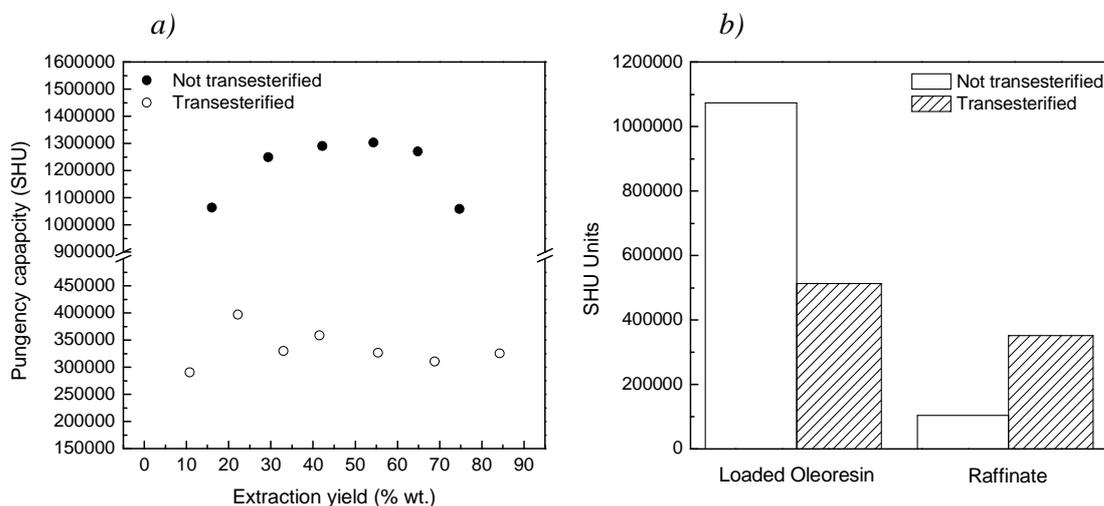


Figure 5. Comparison of pungency capacity of oleoresin capsicum. *a)* Evolution of SHU units for extracts recovered at different extraction yields; *b)* Comparison of SHU units between loaded and raffinate material.

CONCLUSION

The combined effect of transesterification pre-treatment and SCFE at 300 bar and 333 K produces better results of extraction yields, colouring capacities of OR_P 's raffinates and pungency capacities of OR_{Cap} 's raffinates than when using a single step of supercritical fluid extraction.

Despite using different types of oleoresins to develop the transesterification process, the solubility of both of them after the pre-treatment is quite similar, owing to the conversion of acylglycerols into fatty acid methyl esters, which makes that the obtained extraction yields would be almost the same. Nevertheless, when the SCFE is carried out without a pre-treatment step, the influence of the raw material on the extraction yield is significant. This can be explained attending to the different solubility of OR_P and OR_{Cap} as a consequence of their different properties (OR_P owns higher viscosity than OR_{Cap}).

Moreover, the type of oleoresin affects to the colouring and pungency capacity of the extracts and raffinates collected after the combined process. Although the pre-treatment of transesterification produces the loss of some carotenoids and the half of capsaicinoids present in OR_P and OR_{Cap} , respectively, the enhancement in solubility produces the extraction of FAME's and the enrichment of carotenoids and capsaicinoids in the raffinate.

Actually, when oleoresin paprika is used as raw material, the enrichment of carotenoids in the raffinate can be described by the enhancement of its colouring capacity, which increases 5 times respect to its initial value. Besides, the raffinate appears as solid particles of 1.5-2 μm mean average diameter. Attending to the recent interest in the production of solid particles enriched in bioactive compounds, the result obtained in this work provide a new way to produce a raffinate composed of solid particles highly enriched in carotenoids.

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