Analytical Methods

Extraction of β-carotenes from palm oil mesocarp using sub-critical R134a


ABSTRACT

Sub-critical extraction of palm oil from palm mesocarp using R134a solvent was conducted via the dynamic mode to investigate the ability of R134a to extract β-carotene. The yield of palm oil and the solubility of β-carotene were investigated at 40, 60 and 80 °C and pressure range from 45–100 bar. The extracted oil was analysed for β-carotene content using UV–Vis spectrophotometry. The results showed that palm oil yield increased with pressure and temperature. The maximum solubility of β-carotene was obtained at 100 bar and 60 °C while the lowest solubility occurred at 80 bar and 40 °C. The higher concentration of extracted β-carotene ranging from 330–780 ppm as compared to that achieved through conventional palm oil processing indicates that extraction of β-carotene using R134a is viable.

1. Introduction

Palm oil is well-known to contain a high amount of carotenoids. Typical crude palm oil contains 500–700 ppm carotenes, which contribute to palm oil stability and nutritional value. Conventional processing of palm oil leaves about 3–7% residual oil in the fruit fibre (França & Meireless, 2000; Lau, Choo, Ma, & Chuah, 2008; Nik Norulaini et al., 2008) and a high content of carotenoids in the pressed palm fibres. Residual fibres from palm oil production contain between 4000 and 6000 ppm of carotenoids, about six times higher than that found in crushed palm oil (Franca & Meireless, 1997, 2000). Until now, palm oil fibre residues that are rich in valuable carotenoids are treated as a waste product, which is destined to be burnt together with empty fruit bunches (Birtigh, Johannes, Brunner, & Nair, 1995) or transported to the plantation for field mulching (Lau et al., 2008). Effective recovery of the carotenoids would therefore give a significant advantage to the palm oil industry.

Supercritical fluid extraction (SFE) technology has become an increasingly popular method for the recovery of food ingredients and products over the last 20 years, due to its unique advantages, including low temperature use, selective extraction, simpler and cleaner (solvent-free) product recovery. SFE is also an environmentally benign technology since the process typically generates no waste. Supercritical fluid exhibits high density like liquids, which contributes to greater potential for solubilisation of materials, and low viscosity similar to gases, which enables its penetration into the solid.

Extraction of palm oil from its mesocarp using supercritical fluid technology has been considered a promising way to recover carotenones and prevent their high losses during palm oil milling. Application of SFE technology for crude palm oil extraction, and for the recovery of carotenones from palm mesocarp is expected to have a great future outlook (Bharath, 2003) was and is seen to have great potential for replacing the conventional screw-press extraction, clarification and vacuum drying processes (Lau, Choo, Ma, & Chuah, 2006). Therefore, the extraction of palm oil using supercritical extraction technology is set to become more popular in the next 10 years.

Various studies aimed to investigate the potential of some marker compounds to be extracted using supercritical solvents, such as carbon dioxide (Cygarnowicz, Maxwell, & Seider, 1990; Franca & Meireless, 1997; Lau et al., 2008; Markom, Singh, & Hasan, 2001; Nik Norulaini et al., 2008; Puah, Choo, Ma, & Chuah, 2005; Saldaña, Temelli, Guigard, Tomberli, & Gray, 2010; Sovová, Stateva, & Galushko, 2001; Škerget, Knez, & Habulin, 1995), ethane (Mendes, Nobre, Coelho, & Palavra, 1999) and nitrous oxide (Sakaki, 1992; Subra, Castellani, Ksibi, & Garrabos, 1997). Hansen, Harvey, Coelho, Palavra, and Bruno (2001) reported the solubility of carotene in halocarbon (R134a).

Carbon dioxide (CO$_2$) has been the most popular supercritical solvent, due to its non-toxic, non-hazardous and non-flammable properties, but typically requires pressure of up to 500 bar for satisfactory extraction of food products. In view of the economic and environmental needs, it is desirable to explore alternative SFE...
solvents that enable operation at less intense conditions (Catchpole & Proells, 2001; Wood & Cooper, 2003), thereby allowing designers to exploit the typical SFE benefits at more reasonable costs (Perrut, 2000).

R134a (1,1,1,2-tetrafluoroethane) is non-toxic, non-reactive, non-flammable, and non-ozone depleting. It also has a high volatility and a boiling point at atmospheric pressure of –26.2 °C, which means that it leaves negligible solvent residues in the products. The use of R134a as the extraction solvent at sub-critical conditions can address the shortcomings of supercritical CO2 processes (Corr, 2002). Sub-critical fluid, which is also known as a high-pressure liquid, exhibits similar behaviour to, and can be exploited in the same manner as, supercritical fluids (Brunner, 1994; Brunner, 2005).

To-date, only Mustapa, Manan, Mohd Azizi, Nik Norulaini, and Mohd Omar (2009) have studied the use of sub-critical R134a as an alternative to CO2 solvent for the recovery of palm oil. They performed the extraction of palm oil from palm mesocarp, using R134a at sub-critical conditions. The extraction of palm oil was found to increase with temperature and peaked at a maximum of 100 bar and 80 °C. Mustapa et al. (2009) also reported that the extraction of palm oil using R134a has great potential for application in the palm oil industry. The objective of this study is to investigate the potential of using R134a to recover valuable minor components by determining the yield of palm oil extracted from palm mesocarp and the solubility trend of β-carotene in R134a over the range of temperatures and pressures studied.

2. Methodology

2.1. Materials and sample preparation

Fresh palm fruits were provided by MALPOM Industries Sdn. Bhd palm oil mill in Nibong Tebal, Penang, Malaysia. The fresh fruits were put in vacuum plastic bags and stored at –20 °C. Prior to extraction, a bag of the sample was randomly selected and was left at room temperature for about 30 min. Commercial liquefied R134a gas (purity 99.7%) was supplied in a gas cylinder by Malaysia Oxygen (MOX), Penang. The mesocarp of fresh palm fruits was peeled, sliced and chopped to reduce the sample size using a Kiwi cutter. Samples of chopped mesocarp were oven-dried to reduce its moisture content to a range of about 5–6%. The sample was named as Uncooked-Chopped (UC) sample. Mustapa et al. (2009) reported that the UC sample palm fruits resulted in higher oil yield than cooked fruits. They also reported that the chopped samples gave higher extraction rate as compared to sliced samples. Lau et al. (2006) peeled off the palm mesocarp and dried in the oven at 60 °C until a constant weight was obtained. Prior to the extraction the dried mesocarp was ground into pieces of 1–3 mm length.

2.2. Sub-critical R134a extraction

The dynamic extraction is a superior SFE method when the solid sample has a high oil concentration (De Castro, Valcárcel, & Tena, 1994). Fresh liquid solvent at ambient temperature is charged and compressed to the desired pressure using high-pressure pump at the beginning of extraction. The fluid then flows through an extractor vessel containing the solute and the solvent. The fluid then leaves the vessel and is depressurised to atmospheric conditions, using a heated metering valve, or restrictor. At the same time, the extracted solute will precipitate or fall out of the fluid solution, to be collected and quantified gravimetrically, or by some conventional analytical technique. The solvent volume can either be measured using a gas totaliser or by measuring the flow rate of the solvent and the collected sample (Taylor, 1996).

Palm oil extraction experiments were carried out in a laboratory scale extraction apparatus shown schematically in Fig. 1. The apparatus consists of HPLC Pump (Model PU-980, Jasco, Japan) with a maximum capacity of 50 MPa (10,000 psi) which was used to deliver and regulate the R134a flow. Previous study by Mustapa et al. (2009) showed that the flow rate of sub-critical R134a resulted in a consistent oil solubility and shorter extraction time. Therefore, the R134a flow rate was maintained at 3 ml/min for all extraction conditions. For each extraction run, 1 ± 0.001 g of UC sample was placed in a 10-ml extractor (1.5 cm i.d. × 8 cm) and the extractor was placed in an oven (Model Memmert), which was used to control the operating temperature to within ±2 °C of the set point temperature for each run. The system pressure was controlled by a back-pressure regulator (Model 880–81, Jasco, Japan) with an

Fig. 1. Schematic diagram of the R134a extraction apparatus (1) R134a cylinder, (2) Chiller (set at –5 °C), (3) HPLC pump, (4) flow meter indicator, (5) pressure meter, (6) extraction cell, (7) oven (set the extraction temperature 40–80 °C), (8–9) temperature indicator, (10) heater (60–70 °C), (11) back-pressure regulator (BPR), (12) vial collector, (13) mass flow meter, (14–18) valve).
acurracy of ±1 bar that was maintained at 80 ± 1 °C, in order to prevent oil from clogging in the tubing. The oil dissolved in the subcritical R134a was collected in a sample trap (a 4-ml amber glass vial) by means of a heated valve that caused R134a to expand to the ambient pressure. The palm oil collected was determined gravimetrically using a balance (Model Mettler Toledo AL204). The outlet tubing was insulated with a Teflon insulator to prevent temperature drop solvent flowing from the extractor as well as from the oven.

2.3. Analysis of β-carotene concentration

The concentration of β-carotene was measured using a spectrophotometer (model UV–Vis, Shimadzu, Kyoto, Japan). Prior to analysis, sample extracts were melted at 60–70 °C and homogenised thoroughly. About 20 mg of extracts were diluted with 7 ml of hexane. The solution was transferred to a 1-cm quartz cuvette and the absorbance was read at 450 nm. This method was applied according to MPOB Test Method (2005). Some previous studies had determined the absorbance to be measured at 450 nm, which corresponded to the maximum absorption of carotenoids (Franca, Reber, Meireless, Machado, & Brunner, 1999; Hansen et al., 2001; Mendes et al., 1999; Subra et al., 1997). Solubility of carotene in R134a was calculated from the slope of the linear portion of the curves, which is similar to the approach used for calculating the solubility of oil in solvent (Ferreira, Nikolov, Doraiswamy, Meireless, & Petenate, 1999). On the other hand, the concentration of β-carotene content is expressed as ppm using the following formula:

\[ \text{Concentration of } \beta-\text{carotene} = \frac{V \times \frac{383}{1000W} \times (a_i - a_b)}{a} \]  

where \( a_i \) is absorbance of sample at 446 nm, \( a_b \) is the cuvette error, 383 is the extinction coefficient for carotenoids, \( V \) is the volume used for analysis, \( W \) is weight of sample in grams. Cuvette error was calculated based on three measurement repetitions.

3. Results and discussion

3.1. Extraction yield of palm oil

The oil yield is defined as the weight of total extracted oil (g) per weight of sample feed (g) on wet weight basis at the given extraction temperature and pressure. The oil yields were calculated using Eq. 2:

\[ \text{Yield (g oil/g sample feed)} = \left( \frac{\text{Extracted oil (g)}}{\text{Sample feed (g)}} \right) \times 100 \]  

Palm oil yields and degree of extraction for palm oil processing at various operating conditions are presented in Table 1. The degree of extraction was defined as weight of oil yield (g) per weight of total oil extractable, which was obtained from Soxhlet extraction (g).

From Table 1, it is observed that the highest total palm oil yield was obtained at 100 bar and 80 °C, whereas the lowest was at 60 bar and 40 °C. The maximum palm oil yield achieved was 66.06%, and the highest degree of extraction of palm oil using sub-critical R134a was 93.34%. Both the maximum yield and degree of extraction were obtained at 100 bar and 80 °C. This was attributed to the increase in the solvent density with increased pressure, and the increase in vapour pressure of the solute with increasing temperature. Increasing pressure from 60 to 100 bar at constant temperature causes the oil yield to increase. On the other hand, increasing temperature from 40 to 80 °C at constant pressure also causes the oil yield to increase. Combination of both factors of pressure and temperature contributed to the high oil yield at the maximum pressure and temperature.

Table 1 also presents the degree of extraction, which indicates the achievable oil yield using R134a solvent, over the oil yield derived from Soxhlet extraction. The degree of extraction demonstrates the extraction capability of R134a solvent. It is observed that the degree of extraction increased with pressure and temperature. The variation of degree of extraction with pressure and temperature shows that the solvent was unable to dissolve oil in the sample completely. This is because the extractable oil from samples depended on the solvent density (hence, the solvating power).

Cao and Ito (2003) performed supercritical extraction of grape seed oil and found that 6% of grape seed oil was extracted, as compared to 10% total oil yield obtained using Soxhlet extraction. On the other hand, it is observed from Table 1 that the extraction of palm oil using sub-critical R134a can exceed 90% of extractable oil, indicating that extraction using R134a as comparable to the oil yield by Soxhlet extraction. This proves the suitability of R134a to be used for extraction.

3.2. Solubility of β-carotene

The analysis of β-carotene was performed at the final extraction yield to investigate the total extractability of the component. The typical curve for carotene fractions is shown in Fig. 2. From the figure, it is observed that, at both pressures of 60 and 80 bar, the linear portion of the curve during the first stage of extraction lay on a single line. The overlapping region for carotenes occurred between about 20 to 200 min of extraction time. This duration coincided with the constant extraction rate for palm oil. This trend indicated that the extraction of palm oil and carotenoids were controlled by the external mass transfer mechanism.

From the experimental work, it was observed that, from about 20 to 120 min of extraction time, the initial fractions were yellowish, whereas the latter fractions appeared as orange extracts. This phenomenon was also observed by Markom et al. (2001). They stated that more β-carotene was extracted as the experiment proceeded. Franca et al. (1999) who carried out the extraction of carotenoids from Mauritia flexuosa concluded that the largest amount of carotenes was extracted in the diffusion-controlled period. Therefore, it is necessary to conduct the experiments until the end of the extraction period. In order to observe the course of carotene extraction as well as to calculate its solubility, the concentration in each fraction was collected at regular time intervals.

Low-concentration of carotene sample at the beginning of extraction may also lead to the same yield value at different conditions. Further increase in the mass of R134a used (as well as extraction time) tended to increase the amount of extracted carotene at 60 bar, greater than the amount extracted at 80 bar. This
The solubility of β-carotene in R134a at various pressures and temperatures is presented in Fig. 3. It is observed that the solubility of β-carotene generally increased with the increase in extraction pressure. However, it is interesting to note that at all temperatures studied; the solubility of β-carotene decreased with pressure from 45 to 80 bar. Further pressure increase to 100 bar resulted in a solubility increase for β-carotene at 60 °C, apparently to its highest level.

The previous observation may be due to the competing effects of solubility at 80 bar between β-carotene and other more soluble components present in the sample. Thus, a high solubility of the co-extracted components with carotenoids tended to reduce the solubility of β-carotene. However, further increase in pressure to 100 bar at all temperatures had increased the β-carotene solubility. The increase might be due to high solubility of β-carotene compared to other components co-existing in carotenoids. Puah et al. (2005) noted that the presence of other components being co-extracted with carotene could result in low solubility of β-carotene.

In terms of temperature effect, it was found that the solubility of β-carotene increased with temperature from 40 to 80 °C at pressure below 80 bar. However, a crossover of solubility phenomena was observed at pressures above 80 bar, between 60 and 80 °C. This result is shown in Fig. 3. As pressure increased to more than 80 bar, the solubility of β-carotene at 80 °C was observed to decrease to lower than at 60 °C. Under these conditions, there are competing effects between the decrease in R134a density and increase in β-carotene vapour pressure when temperature increase was expected to play a role in determining the solubility of β-carotene.

However, the R134a density decrease predominates compared to the vapour pressure effect. This phenomenon reduces the solubility of β-carotene at 80 °C. In this case, the increase in β-carotene vapour pressure has little influence on the solubility. On the other hand, the maximum solubility achieved at 60 °C was due to the R134a density (hence solvating power) being higher than the density at 80 °C. This result was consistent with the general relationship governing density–temperature–pressure, where density generally decreases with increasing temperature at constant pressure.

The capability to selectively extract compounds such as β-carotene (in this study) highlights the unique feature of SFE technology. Note from Fig. 3 that the highest solubility is obtained at 100 bar and 60 °C. This indicates that high β-carotene selectivity in R134a occurred at the highest pressure and moderate temperature. Low solubility of carotenoids at the highest temperature (80 °C) may be due to some degradation of β-carotene at that point. On the other hand, low solute vapour pressure at 40 °C may have prevented the solutes from leaving the solid matrix into the fluid, thereby resulting in lower β-carotene solubility.

Markom et al. (2001) on the other hand reported that, at 200 bar and temperatures of 40–60 °C small amounts of β-carotene were extracted. Franca and Meirelles (1997) performed oil extraction from pressed palm oil fibres using supercritical CO2 to recover carotenones. They found that the total amount of carotenones remained constant during the course of extraction at 300 bar, but showed a significant increase at 200 and 250 bar. Hansen et al. (2001) investigated the effects of temperature on the solubility of β-carotene in SC-CO2 and in sub-critical R134a. They reported that the solubility of β-carotene in R134a increased with pressure and decreased with temperature. Our findings show that the solubility of β-carotene decreased with pressure at 80 bar, contrary to the findings of Hansen et al. (2001). They also noted that the solubility in R134a as rather low, and that the solubility deviations between the two solvents were relatively small. On the other hand, Lau et al. (2006), who investigated the extraction of palm oil from palm mesocarp using supercritical carbon dioxide, reported the carotenone content increased gradually when increasing the operating temperature and pressure from 40 to 80 °C and 14 to 30 MPa.

The solubility found in this study was slightly different from some of the findings reported on the solubility of β-carotene in supercritical CO2 (Johannsen & Brunner, 1997; Mendes et al., 1999; Sakaki, 1992; Subra et al., 1997 and Sovová et al., 2001). They reported that β-carotene solubilities increased with pressure at constant temperature for experiments conducted at a pressure range of between 120 and 350 bar and a temperature range of between 40° to 80 °C. Fig. 4 compares the solubilities derived from several works. Note that the solubilities achieved in this work are an order of magnitude lower than those found in other studies. However, Saldaha et al. (2010) who investigated the extraction of lycopene and β-carotene from tomato skin + pulp at 400 bar and 40 and 70 °C, using SC-CO2, SC-CO2,5% canola oil and SC-CO2,5% ethanol, reported that the solubility of β-carotene tended to increase with increasing temperature, and was in the range of 6.2 ± 0.9 x 10–7 to 18 ± 4 x 10–7 mole fraction.

The maximum carotene amount was obtained at 100 bar and 60 °C while the smallest was at 80 bar and 40 °C. The discrepancies between the results of this work and those of other studies were probably due to the different characteristics of CO2 and R134a solvents apart from the lower pressures used in this work compared to the pressures used with CO2 solvent. The polarity of R134a
molecules may not be adequate in dissolving the non-polar molecules of β-carotene. This factor was also expected to contribute to the differences in carotene solubilities. Hansen et al. (2001) also stated that the low solubility of carotene in R134a as compared to CO2 was because R134a is a polar compound while carotene is a non-polar compound. Finally, the different types of sample used may also have caused the solubility discrepancies. Johannsen and Brunner (1997) and Puah et al. (2005) noted that different materials or different methods used for the solubility measurement as well as the purity of β-carotene could also lead to solubility differences. Saldaha et al. (2010) also reported that the β-carotene solubility obtained from multi-component measurement was lower than the solubility obtained from binary measurement. They attributed the difference to the type of sample matrix as well as the components interactions, which can change over time throughout the dynamic extraction period. On the other hand, Lau et al. (2006) reported carotenes have low solubility due to high molecular weight as well as low vapour pressure.

The analysis on β-carotene was performed during the final extraction yield to investigate the total extractability of the component. It was observed that the concentration of β-carotene increased with increase in extraction pressure as shown in Fig. 5. However, it is interesting to note that at all pressures studied, the concentration of β-carotene decreased markedly at 80 bar, with temperature of 60 °C giving the lowest β-carotene concentration. This happened possibly due to the high solubility of undesired components which were co-extracted with carotenoids at 80 bar, thereby resulting in lower β-carotene concentration. However, the β-carotene concentration tended to rise again after the pressure was increased to 100 bar at all the temperatures studied. The increase that was observed after 80 bar may possibly be due to the higher solubility of β-carotene as compared to the solubility of other components co-existing in carotenoids. The variation of β-carotene concentration demonstrated the system’s selective extraction capability, which is a special characteristic of SFE technology. The highest concentration was obtained at 100 bar and 60 °C. This means that β-carotene extraction was selectively maximised at the highest pressure and moderate temperature. Low solubility of carotenoids at the highest temperature (80 °C) may probably be due to a small degradation of β-carotene as well as its low solubility in R134a at this temperature. On the other hand, at 40 °C the solutes have apparently lower tendency to escape from the solid matrix to the fluid phase due to the low solute vapour pressure. This may have led to the low β-carotene concentration at 40 and 80 °C.

3.3. Statistical analysis (ANOVA)

Statistical analysis (ANOVA) of β-carotene solubility in sub-critical R134a was performed in Excel 2007. It was found that the effect of both temperature (p-value = 0.075) and pressure (p-value = 0.596) at 95% confidence level were negligible. However, temperature effect on β-carotene solubility was slightly more significant compared to the effect of pressure. Thus, it can be concluded that the effect of temperature on β-carotene vapour pressure was more important in influencing the solubility of β-carotene in sub-critical R134a.

4. Conclusion

The yield of palm oil extracted from palm mesocarp using R134a at sub-critical conditions increased with increasing pressure and temperature. The maximum yield of 66.06% was achieved at 100 bar and 80 °C. The extraction of palm oil and the recovery of β-carotene using sub-critical R134a are feasible since the extracted β-carotene concentration was higher in this case, as compared to the concentration obtained from conventional processing. The highest concentration of β-carotene of 780 ppm was obtained at 100 bar and 60 °C and the lowest β-carotene concentration of 370 ppm was obtained at 80 bar and 40 °C. The results show that simultaneous extraction of palm oil and recovery of carotenoids using R134a is feasible.

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