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Supercritical fluid extraction of avocado oil

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ABSTRACT

Avocado oil was extracted by means of supercritical carbon dioxide using four different extraction conditions and by hexane. The extracted oils were compared with regards to the colour, acid values, and unsaponifiable matter content. The two extraction methods were shown to be statistically equivalent with regards to the acid value but the colour and unsaponifiable matter content was significantly different.

INTRODUCTION

Crude avocado oil is a viscous liquid with strong brown-green pigmentation. After refining, the oil colour turns light yellow with a greenish tint. The green pigmentation is due to the chlorophylls a and b existing in the skin at high concentration, and which also is present in the pulp (Jacobsberg, 1988). Southwell, Harris and Swetman (1990) reported that even oil, extracted from peeled fruit, contains a distinct green coloration because of the presence of chlorophyll. Werman and Neeman (1987: 229) determined that crude avocado oil, produced by centrifugation, contained 41.3 ppm chlorophyll while 69.2 ppm was extracted by hexane. High chlorophyll content will however cause oil stability problems, because chlorophyll serves as a photosensitiser in oxidative processes (Jacobsberg, 1988, Werman & Neeman, 1986 and Werman & Neeman, 1987).

Werman, Mokady and Neeman (1996) reported that a lipid fraction is found in the extract from the seed, which is undesirable in edible avocado oil. This lipid fraction in the unsaponifiable fraction from the seed causes growth inhibition and abnormalities of lipid metabolism in growing rats.

The oil from the Fuerte avocado contains between 94% and 98% triglycerides and between 2% and 6% complex lipids (Jacobsberg, 1988). Morin (1996: 245) reported that the lipid fraction varies over a larger range, from 1% to 12% in the extracted oil. These complex lipids are insoluble in an aqueous medium after saponification of the oil. This fraction is known as the unsaponifiable fraction of the oil. The unsaponifiable fraction contains sought after properties in formulations for the cosmetic and pharmaceutical industries, thanks to its high skin penetration coefficient and the specific biological action of its sterols. Edible uses of the oil itself are rather limited because of the competition with olive oil. However, as a by-product from unsaponifiable extraction, it might become more economically attractive. It is thus important that any extraction technique should be effective in the extraction of the unsaponifiable fraction (Jacobsberg, 1988).

EXPERIMENTAL

Avocado fruit, cultivar Fuerte of unknown origin, was purchased from a local market. The fruit was allowed to ripen before processing. Dried avocado slices were prepared by oven drying destoned, unpeeled, fresh fruit at 80°C for 24 hours. Dried avocado was ground to less than 2 mm by means of a Kenwood food processor (Model PFP 32) and stored in a freezer at 4ºC until analysis.

Solvent extractions were performed on a 10.00 g dried sample using hexane (Merck – AR) and a Soxhlet extractor for 8 hours. Solvent was removed by vacuum evaporation and exposure to heat in a drying oven at 100ºC until constant mass.

SF extractions were performed on 4.000 g avocado samples in a 10 ml extractor. Extractions were performed for 2 hours with a fluid flow rate of 4.5 ml/min, measured at the pump head. Extractions were performed at 350 and 532 atm pressures and temperatures of 37ºC and 81ºC. Prior to any analysis the extracted oil was, for 30 min, subjected to vacuum evaporation in order to remove any water and dissolved carbon dioxide.

Colour comparisons of the avocado oil, extracted by supercritical carbon dioxide and Soxhlet, were performed on an UV spectrophotometer (Ultrospec 3000) by absorption meas-

Table 1. Spectroscopic quantification of the colour of avocado oil extracted with hexane and supercritical carbon dioxide at the following conditions: 81ºC/532, 81ºC/350, 37ºC/532 and 37ºC/350 atm

Extraction condition	Absorption value	Visual quantification
37ºC/350 atm	0.325	Straw-yellow
37ºC/532 atm	0.410	Straw-yellow with a greenish tint
81ºC/350 atm	0.526	Straw-yellow with a strong greenish tint
81ºC/532 atm	0.765	Green with a yellow tint
Hexane	0.876	Dark green

Table 2. Free fatty acid values of avocado oil extracted with hexane and supercritical carbon dioxide at the following conditions: 81ºC/532, 81ºC/350, 37ºC/532 and 37ºC/350 atm

Extraction condition	Free fatty acid value (mg KOH/g)
37ºC/350 atm	1.9 ± 0.1
37ºC/532 atm	2.0 ± 0.1
81ºC/350 atm	1.8 ± 0.1
81ºC/532 atm	1.9 ± 0.1
Hexane	1.9 ± 0.1

Table 3. The unsaponifiable content and the saponification value of avocado oil fractions obtained with three, 20 min, consecutive extractions at 81ºC and 532 atm

Sample number	Unsaponifiable content (% m/m)
Fraction 20 min	13.9 ± 0.2
Fraction 40 min	9.6 ± 0.2
Fraction 60 min	5.6 ± 0.2

urements at 670.5 nm of 1.000 g extracted oil diluted to 10.00 ml in carbon tetra chloride (Merck – AR).

The acid values and the percentage unsaponifiable content was determined, using IUPAC methods 2.201 and 2.401 (Paquot & Hautfenne, 1987: 88).

RESULTS AND DISCUSSION

Sample preparation

In order to aid the grinding and extraction processes, the skin with its higher fibre content was included in the sample. The results should therefore not be interpreted only as a reflection of the oil content of only the pulp.

The kernel of the avocado contains only a small percentage of oil (less than 1% (m/m)). The inclusion of the kernel into the sample will introduce up to 25% (m/m) of matter with low oil content and together with the reported detrimental effect on the metabolism of rats, as a result of compounds in the kernel, it was not included in the sample.

Colour of the extracted oils

The colour of the avocado oil extracted by SFE at the four different SFE operating conditions was compared to that extracted by hexane. Comparison of the colour was done, qualitatively by visual inspection of the extracts and quantitatively as absorption measurements at 670.5 nm. The colour of the oil obtained at 350 atm and 37ºC was evaluated to be straw-yellow while the oil extracted at 532 atm and 37ºC was evaluated to be straw-yellow with a greenish tint. The extract obtained at 350 atm and 81ºC was straw-yellow with a strong greenish tint while that obtained at 532 atm and 81ºC was green with a yellow tint. The oil extracted by hexane was a darker green than the oils extracted by supercritical carbon dioxide.

Quantitative absorption measurements of the extracts were made in order to quantify the visual classification of the oils. The green colour in the oils was determined by thin layer chromatography, as a result of the co-extraction of chlorophyll from the matrix. The increased absorption values measured were ascribed to different amounts of chlorophyll extracted. The results of the spectroscopic analysis are summarised in Table 1 and include a visual quantification system with regard to absorption values and colour of these avocado oil samples.

The SF-extracted oil at 350 atm and 37ºC yield an absorption value of 0.325 which is indicative of a straw-yellow colour while the solvent extracted oil yield a value of 0.876 absorption units and is indicative of a dark green colour. The difference in absorption values indicates that less chlorophyll was co-extracted during all SF ex-

tractions than with solvent extraction.

The co-extraction of chlorophyll will influence the extraction yield. The chlorophyll content should thus be subtracted from the total mass of oil extracted, before comparison can be made with regard to the efficiency of triglycerides extraction. Oil containing chlorophyll, is considered to be of lower quality than oil without chlorophyll. The chlorophyll will cause oxidation of avocado oil and should thus be removed by a purification process. Once chlorophyll is removed, the oil can no longer be classified as virgin oil.

The specific application the avocado oil is required for will influence the operating conditions at which the supercritical extraction must be conducted. If the percentage oil is to be compared with hexane extraction, high-temperature and high-pressure should be used. If the extracted oil is to be used as virgin oil, low-temperature and pressure should be applied.

The acid value

The acid value or free fatty acid content (FFA) of the oil is a measure of the extent to which hydrolysis has liberated the fatty acids from their ester linkage with the parent glyceride molecule. The possible influence that the different supercritical extraction conditions might have on the FFA content of the oil was investigated and compared to that obtained by solvent extraction. The results are presented in Table 2.

Statistical comparison of the results, at the 95% confidence level, showed no significant difference between the different methods. From these results it was concluded that neither supercritical carbon dioxide, the temperature, nor the pressure resulted in hydrolysis and liberation of the fatty acids from their parent glyceride.

Determination of the FFA content of the different oils did, however, indicate two very important experimental conditions that must be met before any comparisons should be drawn. Firstly, the oil extracted with supercritical carbon dioxide contains dissolved carbon dioxide. This carbon dioxide must be removed prior to analysis as it influences the acidity of the oil sample. As an example it was determined that without removal of the carbon dioxide, the FFA values were 4.2 mg KOH/g and when carbon dioxide was removed the FFA decreased to 1.8 mg KOH/g.

The second important experimental condition that must be considered, involves the degree of oxidation of the oil in the original dried avocado material. Oxidation products of the longer chain fatty acids, include volatile organic acids such as acetic acid. If a starting material in an advanced oxidised state is extracted by supercritical carbon dioxide, these volatile acids will be co-extracted and thus influence the acid content of the oil. Volatile acids are, however, removed from the oil during the extraction and evaporation processes when hexane is used. The temperature required to remove carbon dioxide from the supercritical extracted oil, is as a rule, normally lower than that required to remove the hexane from the oil.

The unsaponifiable matter

Because of the importance of the unsaponifiable fraction for utilisation in the cosmetic and pharmaceutical industries, it is imperative that this fraction must also be extracted during supercritical fluid extraction.

The extractability of the unsaponifiable content was investigated by extracting three consecutive 20 min fractions from the same avocado material. These extracts were analysed to evaluate the influence of non-exhaustive extractions on the saponification and unsaponifiable content of the avocado oil. The results are summarised in Table 3.

The unsaponifiable content decreases during SFE and the composition of the avocado oil, obtained at different extraction times, will thus differ.

By removing the first fraction of avocado oil during the extraction process, unsaponifiable enriched oil can be produced. The unsaponifiable fraction from the avocado fruit is regarded as the valuable fraction of the oil. Conducting an extraction in such a manner that this fraction is enriched will enhance the economic viability of avocado oil extraction by supercritical carbon dioxide.

The increased concentration of the unsaponifiable matter at the onset of the extraction is regarded as an indication that these compounds might have higher solubility in the carbon dioxide than the triglycerides. This implies that it might be possible to further enrich or fractionally extract the unsaponifiable matter.

CONCLUSION

It was found that unmodified supercritical carbon dioxide was better than hexane as a solvent for the extraction of avocado oil. Chlorophyll serves as a photosensitiser in oxidative processes of avocado oil and should thus be removed to enhance the stability of the oil. In addition to the lower chlorophyll content, SFE also resulted in shorter extraction times and with the absence of organic solvents, SFE is preferred to hexane extraction.

The free fatty acid values, extracted at four different supercritical conditions and that extracted by hexane were compared (Table 2). The free fatty acid values were determined to be statistically equivalent. This equivalence between the extracted oils showed that neither the extraction methods, nor the temperature and pressure combinations, resulted in differences.

The effect of carbon dioxide, entrained in the extracted oil, was quantified and it was shown that it influences the determination of the free fatty acid value. Entrained carbon dioxide will cause an apparent high free fatty acid value of the oil. This high free fatty acid value is, however, not a true reflection of the oxidation of the oil or the extracted components but includes the acidic effect of entrained carbon dioxide. It is thus important to remove the carbon dioxide prior to analysis of the free fatty acid value.

The colour of the hexane-extracted oil was evaluated to be darker green than any of the oils obtained by SFE. The green colour of the oil was ascribed to the co-extraction of chlorophyll, which should be removed by a purification step when avocado oil is industrially extracted. The necessity for an additional purification step will increase the production cost and will result in losses of oil during purification. Application of SFE in such a manner that chlorophyll is not co-extracted will result in avocado oil that does not require purification and will thus enhance the economic viability of SFE as an industrial process.

The concentration of the unsaponifiable matter in avocado oil decreases as SFE proceeds (Table 3). Combination of the first fractions from different extractions will enrich the unsaponifiable concentration of the oil and enhance the economic viability of an industrial SFE process.

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