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ISOLATION AND RECOVERY OF PHYTONUTRIENTS IN PALM BY ISOCRATED AND ISOBARIC FLASH CHROMATOGRAPHY

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ABSTRACT

The phytonutrients (carotenes, tocols, squalene and sterols) present in crude palm oil (CPO) are still intact after the CPO has been converted to palm oil methyl esters (POME), which is used as biodiesel. Isolation and recovery of these valuable phytonutrients from POME was carried out prior to the POME being burnt as fuel. This study reports on the isolation and recovery of palm phytonutrients using flash chromatography. Flash chromatography was conducted under isocratic and isobaric conditions in which the composition of the mobile phase as well as the pressure is made constant during the run. The elution of the phytonutrients from the stationary phase followed the order of: squalene, carotenes, tocols and last of all, sterols. Under the isocractic and isobaric conditions, four fractions were collected from the flash chromatograph. Squalene was concentrated by five-fold in fraction one while fraction two consists of ca. *95% carotenes. Fractions three and four consist of tocols and sterols respectively. The combination of mobile and stationary phase used in this study is found to be able to isolate and recover carotenes in high purity and yield.*

Keywords: carotenes, chromatography, flash, isobaric, isocratic.

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INTRODUCTION

Crude palm oil (CPO) contains *ca*. 1% unsaponifiable compounds which can be grouped as carotenes, tocols, squalene, sterols, *etc*. (Goh *et al*., 1985; Ng *et al*., 2004; 2006; Choo *et al*., 2005). These minor components are also known as phytonutrients due to their beneficial properties such as being antioxidant and anti-cancer (Goh *et al*., 1994; Nesaretnam *et al.,* 1992; 1995; Canfield *et al.,* 1995). Studies have shown that the palm phytonutrients, especially the carotenes and tocols, are able to reduce or combat the occurrence of cancer cells (Carlson *et al*., 2000; Goh *et al.,* 1994; Guthrie *et al.,* 1993; Nesaretnam *et al.,* 2000; 1995; Ziegler *et al.,* 1996a). In addition, their antioxidative property make these phytonutrients good ingredients in cosmetics and nutraceuticals formulations (Ziegler *et al.,* 1996a).

The phytonutrients originally present in CPO have been found to be intact after the CPO has been converted into palm oil methyl esters (POME) (Choo *et al.,* 1991; 2000). The mild process in which the interesterification and transesterification of CPO took place is able to bring about almost no changes in the concentration of the palm phytonutrients in the POME. Recent development in the production of POME for use as biodiesel has brought about the interest to extract and recover the phytonutrients from the said POME. As the phytonutrients are of high values, the extraction and recovery of

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phytonutrients from POME will be able to help making the production of palm biodiesel more viable.

Several attempts and processes have been made to extract the phytonutrients from POME prior to it being burnt as fuel. These include glass column chromatography, supercritical fluid technology, vacuum distillation (Puah *et al.*, 2008; 2006; 2005; Lau *et al.*, 2008; 2007; Ho, 2005; Latip *et al.*, 2000; 2001). Each of these processes has advantages and disadvantages. For example, glass column chromatography utilises vast amount of solvents and take a very long time to complete. In addition, it is customary to use different types of solvents during the run to desorb compounds of different polarity. This practice is not practical in pilot or production scale as the constant changing of solvents is not only labour consuming, but also takes a long time to stabilise the system again after a run. Supercritical fluid technology for the extraction of phytonutrients on the other hand, calls for operations at high pressure which involves high investment and operation cost.

This article reports on an isocratic and isobaric chromatographic method to isolate and recover the palm phytonutrients. Flash chromatography is generally associated with the extraction and purification in preparative or semi-preparative scale. It offers faster separation compared to other liquid chromatographic techniques such as glass column chromatography. Technically, flash chromatography is not much of difference from glass column chromatography with the exception that the desorption of compounds from stationary phase is facilitated by applying a small pressure which is generally less than 10 bar. In this study, pressure at less than 2 bar was applied to facilitate the desorption of the phytonutrients from the stationary phase. With this, the use of mobile phase and the time needed to complete a run is greatly reduced.

MATERIALS AND METHODS

Materials

All chemicals used were of chromatographic grade unless otherwise stated. Hexane, IPA and THF were purchased from Merck (Darmstadt, Germany).

Phytonutrients Concentrate

Concentrate rich in carotenes, tocols, squalene and sterols were obtained from a process as described by Choo *et al*. (1991). The phytonutrients concentrate obtained contained *ca.* 0.2% squalene, 2.6% carotenes, 1.0% tocols and 0.8% sterols.

Flash Chromatography

Isolation and recovery of palm phytonutrients in groups of carotenes, tocols, squalene and sterols was carried out using a Biotage flash chromatography system. The 1.3 g phytonutrients concentrate was dissolved in minimal hexane and added to the top of a 7.5 cm length, 4 cm internal diameter silica column. The mobile phase used was hexane: IPA (99.5:0.5) with pressure less than 2 bar. Flow rate of mobile phase was 18 ml min^{-1}.

Analyses of Total Carotenes

Total carotenes content was determined by the method as described in *MPOB Test Method*. Absorbance at 446 nm was measured.

Analyses of Tocols

Analyses of tocols was carried out using a HPLC coupled with a fluorescence detector (λ_{ex}: 295 nm; λ_{em}: 325 nm). A Lichrosorb 60A Silica analytical column, 4.6 mm i.d. x 250 mm length was used. Mobile phase was hexane: THF: IPA (1000:60:4) at 1 ml min^{-1}.

Analyses of Squalene and Sterols

Squalene and sterols were analysed using GC in a method as described by Lau *et al.* (2008). A Hewlett Packard 5890 Series II Plus gas-liquid chromatograph was used. The column used as an SGE 15 m x 0.32 mm i.d. BPX5 0.25 μm capillary column (SGE, Melbourne, Australia). Initial oven temperature was set at 100°C for 1 min and increased to 400°C at the rate of 10°C min^{-1}. The injector and detector temperatures were set at 370°C. The oven equilibrium time was 3 min under a pressure of 6.60 psi. The carrier gas (helium) was set at flow velocity ranges from 1.99 – 2 ml min^{-1} cm^{-1} s^{-1}. The range of split ratio between the compressed air and H_2 gas was 0.0 – 1.

RESULTS AND DISCUSSIONS

Four fractions were collected from the outlet of the flash chromatograph. The fractions collected consisted of different colours by visual observation. The first fraction was pale yellow, second fraction

was deep orange while the third fraction was yellow. The last fraction was pale yellow to colourless. Each of these fraction was analysed for the carotenes, tocols, squalene and sterols contents.

Based on an earlier observation by TLC, squalene was the first of the palm phytonutrients to be eluted, followed by carotenes, tocols and last of all, the sterols. This observation is also true in flash chromatography separation. *Figure 1* depicts the concentration of the palm phytonutrients in each of the fraction collected while *Figure 2* depicts the recovery of these phytonutrients.

Squalene was concentrated by five-fold from its content in the starting material in Fraction 1 where the majority of it is recovered (96%). The material balance showed that the remaining squalene was found in Fraction 2. The mobile and stationary phase used in this study is a good combination to isolate and recover carotenes from palm phytonutrients concentrate as the carotenes were isolated and recovered in high purity (*ca.* 95%) and yield (*ca.* 96%). The carotenes were concentrated by more than thirty-fold. The total carotenes content was analysed by UV spectrophotometry method as described in the *MPOB Test Method* (2005). Although there are 11 types of individual carotenes present in palm oil, the UV absorbance at 446 nm was measured as the major carotene in palm oil is β-carotene, which absorbs maximum UV at 446 nm. As such, the total carotenes in palm is referred to as total β-carotenes. Analyses of individual carotenes in palm however, can be carried out using HPLC coupled with a photodiode array detector (Tay and Choo, 2000).

Tocols which were recovered in Fraction 3 of the flash chromatography run was concentrated by only about three-fold from its original content in the starting material. The recovery of tocols in this particular fraction was 84%. Analyses of tocols was carried out by HPLC in a method as previously described. There are five individual tocols found to be present in palm, namely, α-tocopherol,

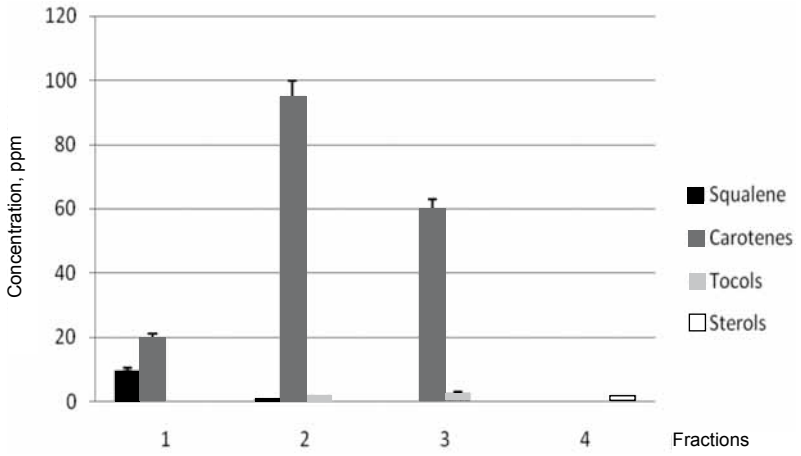

Figure 1. Concentration of squalene, carotenes, tocols and sterols in fractions collected from flash chromatograph.

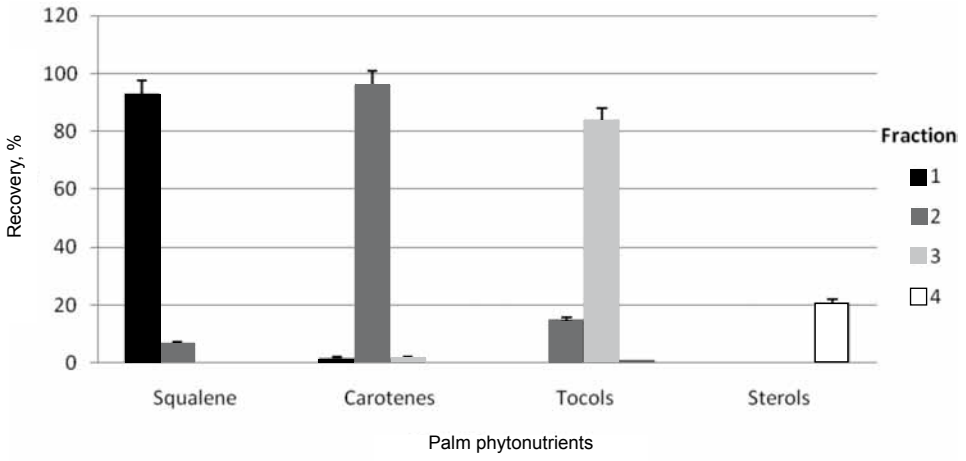

Figure 2. Recovery of palm phytonutrients in fractions from flash chromatograph.

α-tocotrienol, γ-tocopherol, γ-tocotrienol and δ-tocotrienol. There is a report on a newly identified tocol, α-tocomonoenol in palm (Ng *et al.*, 2004). The tocols content in this study however, is reported as the total of the five tocols previously described as α-tocomonoenol was not detected to be present in the phytonutrients concentrate in this study.

The last fraction collected from the flash chromatography consisted of *ca.* 2% sterols, which was concentrated by about 2.5-fold from its original concentration in the starting material. The recovery of sterols in this particular fraction is quite poor, *ca*. 2%. It is believed that most of the sterols were still retained in the stationary phase. Sterols, being the most polar compared to other phytonutrients present in the palm phytonutrients concentrate are more strongly retained in the stationary phase. The polarity of the mobile phase used is not able to desorb the sterols from the stationary phase.

The isocratic and isobaric conditions used in this study is able to yield fraction with carotenes in high purity and high yield. Although other palm phytonutrients such as squalene and tocols were concentrated by many folds compared to their concentrations in the starting material, they do not show prospect as promising as the carotenes. The desorption of sterols by normal phase flash chromatography still needs to be improved to further concentrate them into higher concentration.

CONCLUSION

Isobaric and isocratic flash chromatography can be used to isolate and recover the carotenes, tocols, squalene and sterols from a concentrate rich in phytonutrients that is obtained from the production of CPO methyl esters. Furthermore, the time needed for the separation is much reduced compared to the conventional glass column chromatography.

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