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Quality protein maize (QPM) seeds grown in Côte d'Ivoire: A source of high value edible oil

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The search for new sources of oil with improved properties has focused our attention on the characteristics of oils extracted from white and yellow quality protein maize (QPM) seeds, two hybrids of classic maize (*Zea mays* L.). Physicochemical parameters of extracted oils were respectively as follow: refractive index (1.47 ± 0.00), free fatty acids (FFA) (1.4 ± 0.00 %), peroxide value (2.00 ± 0.00 and 1.33 ± 0.60 meq O_2 /kg), iodine value (136.20 ± 1.22 and 137.60 ± 1.22 g I_2 /100 g), and saponification value (203.83 ± 1.62 and 205.70 ± 1.62 mg KOH/g). Biochemical and nutritive analysis have revealed the following assets: unsaponifiable matter (1.43 ± 0.21 and 1.70 ± 0.10 %), phosphorus (0.10 ± 0.02 mg/g), carotenoids (0.86 ± 0.01 and 1.06 ± 0.01 mg/g), vitamin A (0.45 ± 0.01 and 0.63 ± 0.01 mg/g) and vitamin E (0.32 ± 0.01 and 0.39 ± 0.01 mg/g). White and yellow QPM oilseeds showed higher content of linoleic acid (~ 60.2 % of total fatty acids). All these interesting characteristics should arouse attention for the usage of white and yellow QPM oilseeds as alternative to traditional corn oil in food and pharmaceutical industries.

Key words: *Zea mays*, quality protein maize, seed oils characterization, vitamin E, linoleic acid.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop and major source of energy, protein and other nutrients for human and livestock in the world (Osagie and Eka, 1998; Jompuk et al., 2011). Maize grain accounts for ~15 to 56% of the total daily calories in diets of people in ~25 developing countries, particularly in Africa and Latin America (Prasanna et al., 2001). This vegetable crop exists in different forms with respect to colour of plant and ear and is consumed in several ways: sun dried, cooked, fermented, roasted, pounded or crushed (Ganiyu et al., 2010). With regards to biochemical composition, ordinary or classic maize has low protein quality. Indeed, maize proteins have poor nutri-

tional value for humans because of reduced content of essential amino acids such as lysine which average (~2%) is less than one-half of the concentration recommended for human nutrition (FAO, 1992; Prasanna et al., 2001). To ameliorate the poor nutritional protein value of maize grains, quality protein maize (QPM) has been developed by combining the genetic systems of the gene mutant *opaque-2* ($\delta 2$) and genetic endosperm modifiers (Bello et al., 2012). Products obtained from processing of both ordinary and quality protein maize include starch, high-fructose corn syrup, livestock feed and corn oil (Kolawole and Titilayo, 2012).

Ordinary maize seeds contain ~3 to 5% oil. Crude oil

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polar and polar lipid components (Moreau et al., 1999). The important properties of corn oil include its low level of extracted from this seeds contains triacylglycerol (TAG) as major component (96 to 97%) and other minor non saturated fatty acids, its high levels of polyunsaturated (essential) fatty acids and its relatively high content in bioactive compounds such as unsaponifiable matter, carotenoids, phytosterols and α -tocopherol (Gunstone, 2002). Although the levels of linoleic acid in US corn oil average ~60%, its levels in corn oil produced outside the US are closer to 50%, with most of the difference being accounted for by higher amounts of oleic acid (Strecker et al., 1996). Corn oil has been recognized as containing the highest levels of unsaponifiables (1.3 to 2.3%) of all the commercial vegetable oils and the three main chemical components of this lipid fraction are phytosterols, tocopherols and squalene (Moreau et al., 2001). Corn germ oil contains two phytosterol lipid classes, free phytosterols and phytosterol fatty acyl esters. The major phytosterol in corn germ oil are β -sitosterol > campesterol > stigmasterol (Ham et al., 2000). A study demonstrated that, compared to canola and soybean oils, corn oil produced the lowest levels of oxidation products and retained the highest levels of tocopherols, during five days at continuous frying temperatures (Strecker et al., 1990). The levels of carotenoids in commercial corn oil are relatively low, partly due to their low concentrations in the germ (2 to 4%) and partly due to their removal during bleaching (Weber, 1987). The major food uses of corn oil are cooking/salad oil, margarines and spread. Corn oil has long been a popular cooking oil because of its mild flavor, its oxidative stability and its reputation as healthy edible oil (Gunstone, 2002).

In addition to the extensive literature on the physicochemical characterization of the traditional corn oil, recent studies on "high oil" and "high oleic acid oil" maize hybrids have been reported. Generally, high oil maize seed contains about twice the amount of regular seeds and high oleic acid corn oil contains ~40 to 45% of oleic acid (Leto and Ulrich, 2001). Except scientific data concerning the lipid content of QPM seeds, there is no report to the best of our knowledge on the physicochemical and nutritive assessment of oils extracted from these maize hybrids (Osei et al., 1999; Oliveira et al., 2006). Therefore we have focused our attention on the QPM seeds (white and yellow varieties) vulgarized and grown in Côte d'Ivoire. So, the aim of this work was to investigate these QPM oilseeds properties in order to explore and discuss their nutritional and Industrial potentiality.

MATERIALS AND METHODS

White and yellow QPM seeds were collected in June 2012 from the experimental plot of land of National Program of Rice growing (PNR) in Yamoussoukro city (Côte d'Ivoire). Seeds were rinsed thoroughly with distilled water to remove dirt and dried at 40°C for

24 h in an electric oven (Memmert, Germany) according to Ali et al. (2008).

Chemicals

Analytical high performance liquid chromatography (HPLC) grade solvents, standards and reagents were used to perform analysis. Solvents (n-hexane, chloroform, acetic acid, diethyl-ether, ethanol, methanol and n-heptane) were from Merck (Germany). Standards such as fatty acids (palmitic acid, oleic acid, linoleic acid and linolenic acid), β -carotene, retinol (vitamin A), α -tocopherol acetate and erucic acid were from Sigma-Aldrich (Germany). Wijs reagent was from Prolabo (France).

Oilseeds extraction

Oils were extracted from 50 g crushed seeds (Laboratory crusher, Culatti, France) with 300 ml of n-hexane (40 to 60°C) in a Soxhlet extractor. Then the solvent was removed (vacuum-packed) at 40°C with a rotary evaporator (Heidolph, Hei-Vap, Germany). The extracted lipid was weighed to determine the oil content of the seed. Crude oils were stored at 4°C in air tight brown sterile glass bottles (Ejikeme et al., 2010) until further use for physicochemical and biochemical analysis.

Physicochemical analysis

Specific gravity and refractive index

Specific gravity and refractive index of oilseeds were determined at 25°C following the IUPAC (1979) method by using a pycnometer and a refractometer (Abbe, Optic Ivymen, Spain), respectively.

Specific extinction

The determination of specific extinction of oilseeds was carried out following the method described by Anwar et al. (2007). Oil samples were diluted (1%) with hexane and absorbance was measured at 232 and 270 nm using an ultraviolet visible (UV-Vis) spectrophotometer (T80+, PG Instruments, England).

Photometric colour index

The photometric colour index (pci) was determined according to the method described by Pike (2003). The oil sample (1 g) was weighed and dissolved in 20 ml of hexane. The absorbance was read at the following wavelengths: 400, 550, 620 and 670 nm using a spectrophotometer (T80+, PG Instruments, England). The solvent was used as blank. Photometric colour index was calculated as shown in Equation 1.

$$pci = 1.29 \times (A_{400}) + 69.70 \times (A_{500}) + 41.20 \times (A_{620}) - 56.41 \times (A_{670}) \quad (1)$$

Where, A is the absorbance.

Viscosity

Viscosity of oilseeds was determined at different temperatures (20 to 80°C) by using a viscometer apparatus (SVM 3000, Anton Paar GmbH, Austria) equipped with a syringe filled with 1 ml of oilseed

sample. Values of viscosities were automatically recorded after temperature programming.

pH, acid, peroxide, iodine and saponification values

pH value of oil samples was determined at 25°C according to Afane et al. (1997) by using a pH-meter (Hanna, Hi 8915 ATC, Spain). Oil sample (2 ml) was dissolved in 15 ml of n-hexane. The pH-meter electrode was standardized with buffer solutions (pH 4.0 and 7.0) and then immersed into the sample to record pH value.

Acid, peroxide, iodine and saponification values were determined following the AOAC (1997) methods.

Biochemical analysis

Moisture, impurities, total fatty matter and total saponifiable matter

Moisture, impurities, total fatty matter and total saponifiable matter contents were determined according to the MPOB (2005) test methods.

Unsaponifiable matter and carotenoids content

Unsaponifiable matter content of oil samples was determined following the IUPAC (1979) method. Carotenoids content of oilseeds was determined by using colorimetric method (Wolf, 1968). For this, 100 mg of oilseed sample was dissolved in 2 ml of hexane. The absorbance was measured at 450 nm using a spectrophotometer (T80+, PG instruments, England). A standard curve of β -carotene (1 mg/ml) was used as reference.

Vitamin A content

Vitamin A content was determined by using colorimetric method (Dungan et al., 1964). For this, oil sample (100 mg) was dissolved in 2 ml of chloroform. To 1 ml of aliquot, 4 ml of a trifluoroacetic-chloroform (1:3, v/v) solution was added. The absorbance was measured at 620 nm using a spectrophotometer (T80+, PG instruments, England). A standard curve of retinol (1 mg/ml) was used as reference.

Phosphorus content

Phosphorus content of oil samples was determined following the IUPAC (1979) colorimetric method. The test oil portion (5 g) was burned to ashes in the presence of magnesium oxide. The ashes obtained were dissolved in diluted nitric acid solution (65 %). Absorbance was then measured at 460 nm using a spectrophotometer (T80+, PG instruments, England) after adding an aqueous ammonium vanadate solution. A standard curve of phosphorus (1 mg/ml) was used as reference.

Vitamin E content

Oilseed samples were previously prepared as described by Gimeno et al. (2000). The oil sample (1 g) was diluted in 10 ml of hexane. Thereafter, 200 μ l of this mixture was transferred into a screw-capped tube where 800 μ l of methanol were added. After being vortex-mixed and centrifuged (3000 rpm for 5 min), the samples were filtered through a 0.45 μ m pore size filter and the overlay was

used for high performance liquid chromatography (HPLC) analysis.

Separation by HPLC was carried out using an acquity liquid chromatography system (Waters, USA) equipped with an optical detector TUV system and a BEH C₁₈ column (150 X 0.25 mm i.d., 1.7 μ m particle size) (Waters, USA). The injection volume was 10 μ l. The mobile phase was methanol-water (98:2, v/v) and the elution was performed at a flow rate of 2 ml/min. The analytical column was kept at 45°C. α -tocopherol of oil samples was identified by comparing its retention time with this of authentic standard. Quantification of α -tocopherol identified in oil samples was done by using a standard curve (concentration versus peak area) of α -tocopherol acetate. All the data obtained were stored and processed by Empower software (Waters, USA).

Fatty acid composition

The fatty acids were converted to their methyl esters (FAMES) as described by the European Communities (1991). Approximately 0.1 g of oil sample was mixed with 2 ml of n-heptane and 0.2 ml of a methanolic solution of potassium hydroxide (2N). The whole mixture was shaken up for 30 s and allowed to settle for 5 min. The top layer containing the FAMES was used for gas chromatography (GC) analysis.

FAMES solution (1 μ l) containing the internal standard (erucic acid) was injected into a gas chromatograph (Shimadzu, GC 14 A, Japan) equipped with a flame ionization detector (FID) and a capillary column TRD1 (60 m X 0.25 mm i.d. X 0.25 μ m film thickness). The carrier gas was nitrogen and the flow rate was adjusted to 23 ml/min. Temperatures of detector and injector were 250°C. The initial column temperature was fixed to 100°C and programmed to increase by 5°C per min intervals until 220°C and kept for 10 min at this temperature. The fatty acid methyl esters peaks were identified by comparing their retention times with those of standards. After adjusting areas with the internal standard (erucic acid), the yield of each fatty acid was calculated as follow: area of the fatty acid/areas of total fatty acids in the oil sample \times 100 (%).

Statistical analysis

In the present experiment, each test for the sample was analyzed in triplicate. Data were expressed as means \pm standard deviation (SD). Differences between means were analysed by analysis of variance (one way ANOVA) using StatPlus 2008 (Analystsoft Inc) software. Statistical significant difference was stated at $p < 0.05$.

RESULTS

Oil yield

The oil content of white QPM seeds was $5.36 \pm 0.02\%$ and that of yellow QPM seed was $5.44 \pm 0.04\%$.

Physicochemical characteristics

There was no significant difference ($p > 0.05$) between most of physicochemical parameters of the two seed oils except for specific extinction at 232 nm (3.01 ± 0.00 and 3.45 ± 0.00), photometric colour index (101.81 ± 0.01 and 158.78 ± 0.01) viscosity (53.50 ± 0.20 and 59.87 ± 0.30 mPas), activation energy (19.37 ± 0.00 and 17.90 ± 0.00

Table 1. Physicochemical properties of white and yellow QPM oilseeds.

Parameter	Oilseed	
	White QPM	Yellow QPM
Specific gravity at 20°C	0.92 ± 0.01 ^a	0.91 ± 0.01 ^a
Refractive index at 20°C	1.47 ± 0.00 ^a	1.47 ± 0.00 ^a
Specific extinction at 232 nm	3.01 ± 0.00 ^a	3.45 ± 0.00 ^b
Specific extinction at 270 nm	1.59 ± 0.00 ^a	1.55 ± 0.00 ^a
Photometric colour index	101.81 ± 0.01 ^a	158.78 ± 0.01 ^b
Viscosity at 20°C (mPas)	53.50 ± 0.20 ^a	59.87 ± 0.30 ^b
Activation energy (kJ/mol)	19.37 ± 0.00 ^a	17.90 ± 0.00 ^b
pH at 25°C	5.67 ± 0.02 ^a	5.73 ± 0.02 ^a
Acid value (mg KOH/g)	2.80 ± 0.00 ^a	2.80 ± 0.00 ^a
Free fatty acids (% oleic acid)	1.40 ± 0.00 ^a	1.40 ± 0.00 ^a
Peroxide value (meq O ₂ /kg)	2.00 ± 0.00 ^a	1.33 ± 0.60 ^b
Iodine value (g I ₂ /100 g)	136.20 ± 1.22 ^a	137.60 ± 1.22 ^a
Saponification value (mg KOH/g)	203.83 ± 1.62 ^a	205.70 ± 1.62 ^a

Data represents mean ± SD. Each experiment was performed in triplicate. Different lowercase letters in the same line indicate significant difference at $p < 0.05$.

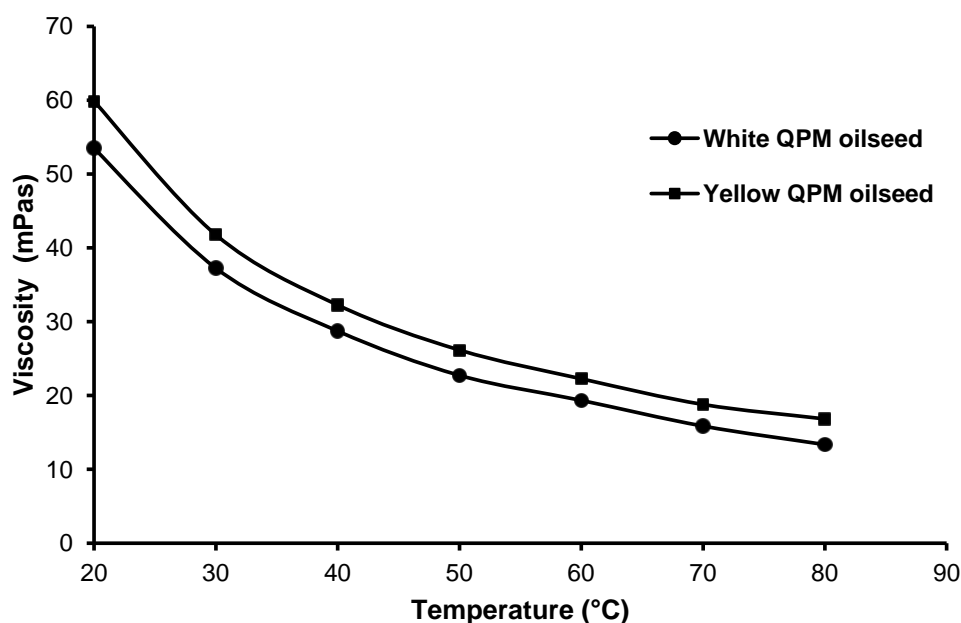


Figure 1. Effect of temperature on white and yellow QPM oilseeds viscosity. Experiments were performed by measuring viscosity of crude oilseeds in the temperature range varying from 20 to 80 °C.

kJ/mol) and peroxide values (2.00 ± 0.00 and 1.33 ± 0.60 meq O₂/kg), respectively (Table 1).

The values of specific gravity were 0.91 ± 0.01 while those of refractive index were $\sim 1.47 \pm 0.00$. Quality parameters such as free fatty acids (FFA) and specific extinction at 270 nm were closed to 1.4 ± 0.00 % and 1.6 ± 0.00 , respectively. The saponification values of the two oilseeds were ~ 204 to 206 ± 1.62 mg KOH/g and their io-

dine values were ~ 36 to 138 ± 1.22 g I₂/100 g (Table 1)

The effect of temperature on viscosity of studied oilseeds (Figure 1) shows that viscosity decreased from 59 to 13 mPas when temperature increased from 20 to 80°C. The Arrhenius plot of these oilseeds (Figure 2), derived from the exponentially curves of viscosities (Figure 1), indicates values of activation energy of 19.37 ± 0.00 and 17.90 ± 0.00 kJ/mol for white and yellow QPM

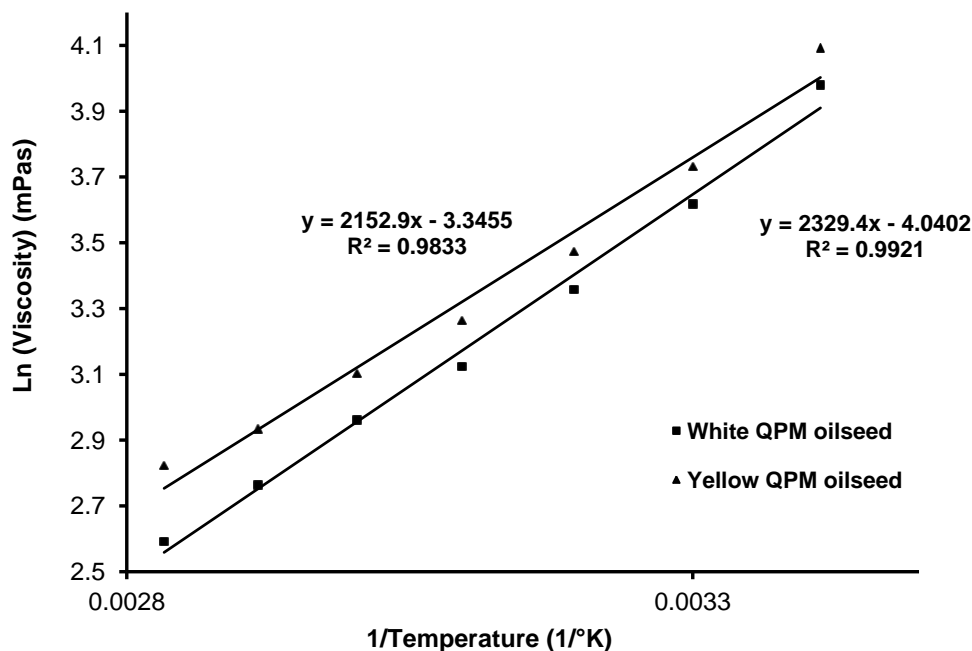


Figure 2. Arrhenius plot of white and yellow QPM oilseeds. Experiments were performed by applying linear regression analysis to the logarithmic form of Arrhenius equation.

Table 2. Biochemical and nutritive properties of white and yellow QPM oilseeds.

Parameter	Oilseed	
	White QPM	Yellow QPM
Moisture (%)	0.28 ± 0.01 ^a	0.26 ± 0.01 ^a
Impurities (%)	0.014 ± 0.00 ^a	0.013 ± 0.00 ^a
Unsaponifiable matter (%)	1.43 ± 0.21 ^a	1.70 ± 0.10 ^b
Total fatty matter (%)	99.70 ± 0.02 ^a	99.72 ± 0.01 ^a
Total saponifiable matter (%)	98.27 ± 0.20 ^a	98.03 ± 0.09 ^a
Phosphorus (mg/g)	0.10 ± 0.02 ^a	0.11 ± 0.02 ^a
Carotenoids (mg/g)	0.86 ± 0.01 ^a	1.06 ± 0.01 ^b
Vitamin A (mg/g)	0.45 ± 0.01 ^a	0.63 ± 0.01 ^b
Vitamin E (mg/g)	0.32 ± 0.01 ^a	0.39 ± 0.01 ^b
Palmitic acid (C _{16:0}) (%)	10.80 ± 0.01 ^a	10.64 ± 0.01 ^a
Oleic acid (C _{18:1}) (%)	27.20 ± 0.01 ^a	27.25 ± 0.01 ^a
Linoleic acid (C _{18:2}) (%)	60.15 ± 0.01 ^a	60.33 ± 0.01 ^a
Linolenic acid (C _{18:3}) (%)	1.30 ± 0.01 ^a	1.33 ± 0.01 ^a

Data represents mean ± SD. Each experiment was performed in triplicate. Different lowercase letters in the same line indicate significant difference at $p < 0.05$.

oilseeds respectively.

Biochemical and nutritive characteristics

The parameters which show significant difference ($p < 0.05$) were unsaponifiable matter (1.43 ± 0.21 and $1.70 \pm 0.10\%$), carotenoids (0.86 ± 0.01 and 1.06 ± 0.01 mg/g),

vitamin A (0.45 ± 0.01 and 0.63 ± 0.01 mg/g) and vitamin E (0.32 ± 0.01 and 0.39 ± 0.01 mg/g), respectively (Table 2). The chromatographic profile of α -tocopherol (vitamin E) in white and yellow QPM oilseeds is given in Figure 3.

Intrinsic biochemical parameters such as moisture, total fatty matter and total saponifiable matter were respectively closed to 0.27 ± 0.01 , 99.7 ± 0.01 and $98 \pm 0.09\%$.

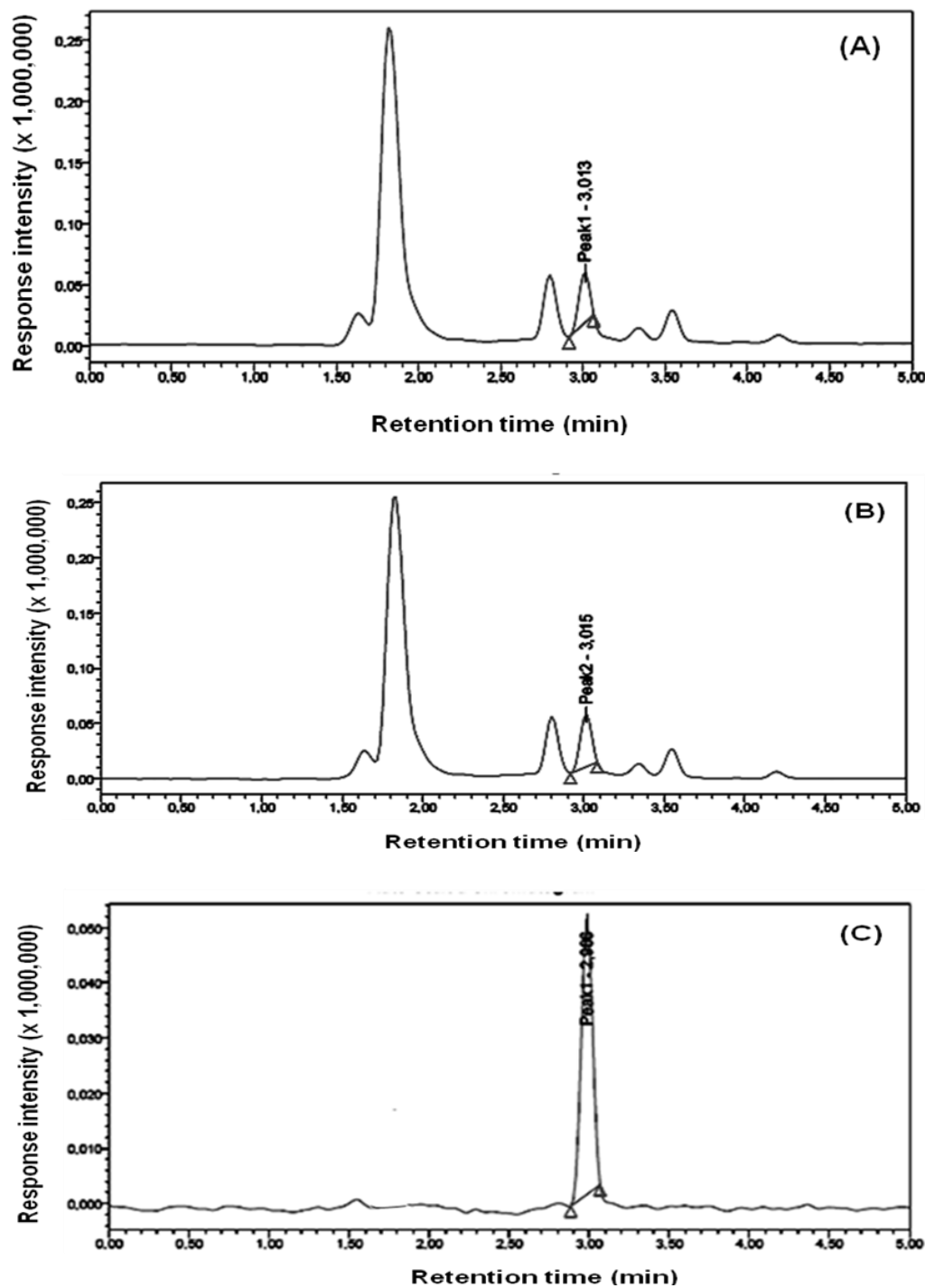


Figure 3. High performance liquid chromatograms of α -tocopherol constituent of white and yellow QPM oilseeds. Experiments were performed by HPLC analysis of α -tocopherol at 292 nm. (A) Chromatogram of white QPM oilseed, (B) Chromatogram of yellow QPM oilseed, (C) Chromatogram of α -tocopherol standard.

The values of impurities content of the studied oilseeds were lower and $\sim 0.013\%$. The mean value of phosphorus content in these oilseeds was $0.10 \pm 0.02\%$ (Table 2).

Chromatographic profiles of fatty acids composition and

their relative amounts in white and yellow QPM oilseeds are given in Figure 4 and Table 2, respectively. Fatty acid proportions of the studied oilseeds highlighted the presence of three main compounds namely palmitic, oleic

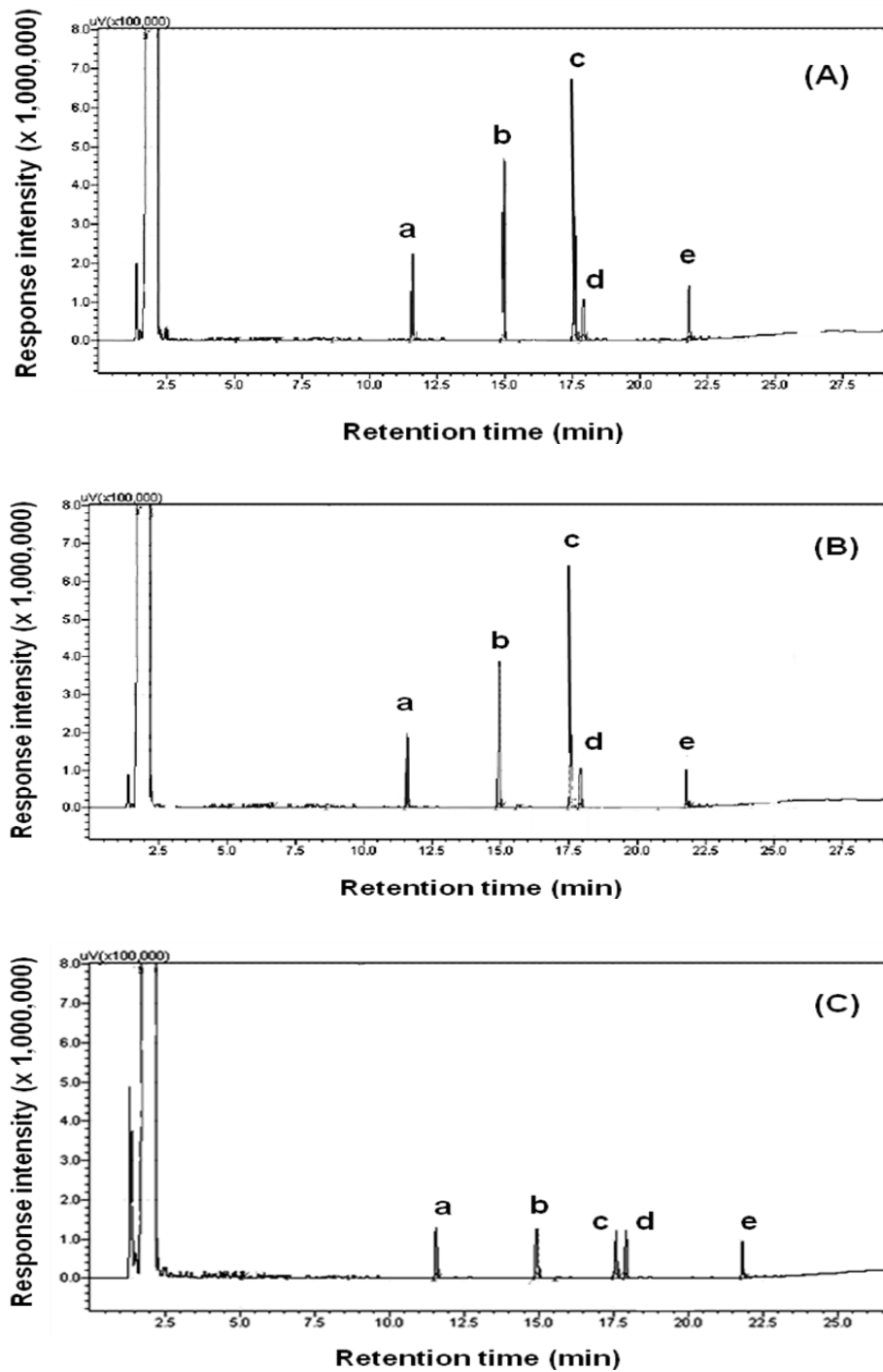


Figure 4. Gas chromatograms of fatty acids constituents of white and yellow QPM oilseeds. Experiments were performed by gas chromatographic analysis (GC-FID) of fatty acids methyl esters derived from white and yellow QPM oilseeds. **(A)** Chromatogram of white QPM oilseed, **(B)** Chromatogram of yellow QPM oilseed, **(C)** Chromatogram of fatty acids standards. **(a)** palmitic acid, **(b)** oleic acid, **(c)** linoleic acid, **(d)** linolenic acid, **(e)** erucic acid (internal standard).

and linoleic acids (Figure 4). On average, these three fatty acids were $\sim 98.15 \pm 0.03\%$ and $98.22 \pm 0.03\%$ of

total fatty acids in white and yellow QPM oilseeds, respectively (Table 2).

Polyunsaturated fatty acids (PUFA) of the studied oilseeds were essentially made up of linoleic acid ($60.20 \pm 0.01\%$) while palmitic acid was the saturated fatty acid (SFA) detected in the mean proportion of $10.7 \pm 0.01\%$. These oilseeds were also composed of monounsaturated fatty acids (MUFA) that was essentially oleic acid in the proportion of $\sim 27.2 \pm 0.01\%$ (Table 2).

DISCUSSION

The oil content of white and yellow QPM seeds is slightly upper than that (3-5%) of ordinary or classic maize (Moreau et al., 1999). These variations between oil yields in seeds could be attributed to their cultivation climate, ripening stage, harvesting time and the extraction method employed (Egbekun and Ehieze, 1997).

The specific gravity refractive indexes of white and yellow QPM oilseeds are within the range of those reported for most conventional edible oils (Rossell, 1991; Codex alimentarius, 1993). The determination of specific extinction at 232 and 270 nm is a good measure of oxidative state of oils in terms of evaluation of conjugated diene and conjugated triene products contents (Anwar et al., 2007). In view of specific extinction value at 270 nm (1.872) of sunflower seed oil, white and yellow QPM oilseeds show more oxidative stability (MPOB, 2005). The viscosity values of both of the oilseeds were in the range (50 to 100 mPas) of most vegetable oils (Besbes et al., 2004). These results, as well as the relatively low values of activation energy, corroborate the fluid state of the studied oils at ambient temperature and this physical characteristic could be suitable in food industries to provide texture and softness to products (Dubois et al., 2007).

The present study indicates that white and yellow QPM seed oils contain lower FFA and so, they can be recommended for salads seasoning and can be stored for longer period without deterioration (Anwar et al., 2007; Matos et al., 2009). The relatively low peroxide values of these oilseeds indicate that they are less liable to oxidative rancidity at ambient temperature (DeMan, 1992). The iodine values of white and yellow QPM oilseeds are higher than 127 to 133 g I₂/100 g of classic corn oil (Strecker et al., 1996). In addition, the iodine values of the studied oilseeds were approximately the same as those of other oils such as soybean (120 to 143 g I₂/100 g) and sunflower (110 to 143 g I₂/100 g) oils (Codex alimentarius, 1993). In view of the results above, white and yellow QPM oilseeds could be categorized as semi-drying oils which consist predominately in polyunsaturated fatty acids (Anhwange et al., 2010). The studied oilseeds could also be recommended for soap making and in the manufacture of lather shaving creams with regard due to their higher saponification values than that (187-193) of classic corn oil (Strecker et al., 1996).

White and yellow QPM oilseeds could have more tech-

nological ability with regard to their impurities contents which are lower than that (0.024%) of palm oil (MPOB, 2005). Also, the unsaponifiable matter contents of these oilseeds are similar to that of classic corn oil but higher than those reported for other high value oils such as cotton seed oil (0.52%), peanut oil (0.33%) and palm kernel oil (0.22%) (Kapseu and Parmentier, 1997). This lipid fraction is a good source of stabilizers used in food industry (Gunstone, 2002). As concern carotenoid constituents, known as precursor of vitamin A in human body, their level in white and QPM oilseeds are compared favourably with that (400 to 1000 ppm) of crude palm oil (Jalani et al., 1997). Vitamin A contents of white and yellow QPM oilseeds were lower than that reported (1 mg/g) for palm oil (Codex alimentarius, 1993). The consumption of these oilseeds could cover infant (0 to 6 months) needs, which are estimated at 0.375 mg per day for vitamin A (FAO, 2001). In addition, white and yellow QPM oilseeds show more oxidative stability and nutritional benefits with regards to their highest level in α -tocopherol (vitamin E) than that (0.28 mg/g) of classic corn oil (Goffman and Bohme, 2001). In view to the linoleic acid content, white and yellow QPM oilseeds could be considered as more balanced "linoleic oils" than traditional maize seed oil which linoleic acid content is ~ 50 to 57% (Dubois et al., 2007). In addition, these studied oilseeds could be used as alternative of US corn oil which linoleic acid content is $\sim 60\%$ (Strecker et al., 1996). Therefore, the higher content of linoleic observed in the studied oilseeds may be beneficial for reducing cardiovascular disease risk (Das, 2006).

It could be concluded in view of the present investigation results that white and yellow QPM oilseeds are more advantageous in human nutrition than traditional maize oilseeds. Indeed, physicochemical properties of these oilseeds show a low content in peroxide value and a highest iodine value. As concern biochemical and nutritive properties, white and yellow QPM oilseeds are good sources of carotenoids, vitamin A and vitamin E. In addition, the relatively higher content of linoleic acid confers to these oils, good edible and dietetic values. White and yellow QPM oilseeds could be used in scientific research as solution for improvement of traditional maize oilseeds.

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