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Prediction of critical times for water-extracted avocado oil heated at high temperatures

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ABSTRACT

Vegetable oils are used in various cooking processes. However, when they are heated at high temperature and/or for a long period, chemical reactions can generate damaging substances for the health. The aim of the study was to predict the critical times at high temperatures of avocado oil. A four-level two-variable Central Composite Design was used to model the thermal oxidation of avocado oil extracted using the aqueous method. Temperature (120 – 180 °C) and time (11 – 209 min) were the independent variables. The response variable was the content in total polar compounds (TPC) with an upper limit defined at 25% (w/w). The composition and the oxidative status of fresh avocado oil were also investigated. The results obtained by a multiple regression analysis showed that data can be fitted with a second order polynomial equation ($R^2 = 0.98$, Adj. $R^2 = 0.97$) with all regression coefficients being significant ($p < 0.05$). The critical heating time ranged from 232 min to 214 min between 120 °C-140 °C and from 188 min to 4 min between 140 °C-180 °C. It was influenced by avocado oil composition. Thus, water-extracted avocado oil is not recommended for frying (140 °C – 180 °C) while it can be used for recipes involving long cooking time at moderate temperature (120 °C-140 °C).

Keywords: Avocado oil, heat treatment, polar compounds, Central Composite Design, upper limit heating time.

INTRODUCTION

Oil, an important plant material, has become an integral part of human diet. One major drawback in their handling and utilization is linked to their richness in unsaturated fatty acids making them prone to oxidative deteriorations especially during thermal treatment. Oxidation renders the oils less acceptable to consumers or for industrial

use as food ingredients (Angaye and Maluelosi, 2015).

Edible oils used as cooking medium at high temperatures are subject to thermo-oxidation, polymerization, and hydrolysis. The results are undesirable off-flavors and a decrease of the nutritional quality of the fried products (Nzikou et al., 2009). The stability of oils at elevated temperature depends on their fatty acid composition, their contents in natural antioxidants (tocopherols, phenolic compounds, sterols and carotenoids), and the presence of oxygen (Gordon and Magos, 1983; Kamal-Eldin and Appelqvist, 1996; Manzi et al., 1998; Choe and Min, 2006). Other components such as chlorophyll have prooxidant properties (Gutierrez-Rosales et al., 1992). Hence, proper control of processing conditions is required to delay the time when edible oils become useless (Shankar, 2014).

Many methods (peroxide value, conjugated dienes, para-anisidine value and TBARS value) are commonly used to monitor oxidation of foods (Abuzaytoun and Shahidi, 2006). But the quality indicator currently used in frying process is the content in total polar compounds (TPC). Directives establish its highest value in oils at 25% (Roman, 2012). TPC can thus be used to set up operating conditions that avoid the formation at high temperatures of inacceptable amounts of toxic compounds.

Avocado (*Persia americana*) oil is relatively new for culinary applications. Its production which has been developing in the early twenty-first century now reaches approximately 2000 tons/year that is a relatively small amount compared to other oils. In fact, only limited information is readily available on this product (Woolf et al., 2008). As avocado oil has not been considered as an important source of oil, few studies concern its properties for culinary application (Berasategi et al., 2012). Avocado oil possesses a similar fatty acid profile as olive oil. It contains around 76% monounsaturated fatty acids (oleic acid), 12% polyunsaturated fatty acids (linoleic and linolenic acids) and 12% saturated fats (palmitic and stearic acids). Avocado oil is rich in antioxidants, with α-

tocopherol (70-190 mg/kg of oil) representing the main antioxidant and to a lesser extent β-, γ- and δ-tocopherols. Carotenoids range between 1 - 3.5 mg/kg oil. Pulp-extracted avocado oil contains also chlorophyll between 11 - 19 mg/kg oil (Wong et al., 2010). Woolf et al. (2008) estimated the phytosterol content of avocado oil between 3.3 - 4.5 mg/g oil.

Mathematical modeling is an effective way of representing a particular process. It can help us to explore and understand the relationship between the process parameters and the quality of the products characterized by specific indicators. It can help to understand the quantitative behavior of a system (Shankar, 2014). To our knowledge, few studies are based on the modelling of edible oil oxidation at high temperatures (Ojanguren and Ayo, 2013; Shankar, 2014). Guillen and Uriarte (2011) have predicted the time at which linseed, sunflower, extra-virgin olive, grapeseed, soybean, sesame, rapeseed, peanut and hazelnut oils submitted to high temperature reached the limit of safety. The authors used the threshold value of TPC *versus* time and temperature, in a single-factor experimental design.

Nowadays, Response Surface Methodology (RSM) has gradually attracted the attention of many researchers. The method allows a reduction of the number of experiments and an understanding of the pattern in which the measured response is affected by changes in factors (Nuran, 2007). Its main advantage is that it allows the improvement of products from predicted properties thanks to prediction of interactive effects and effects due to collective contributions to the measured response. The most widely used RSM model is the Central Composite Rotatable Design (Alhassan et al., 2014).

This study aimed firstly at predicting critical oxidation times of crude avocado oil at high temperatures using a central composite design. The chemical properties of water-extracted avocado oil along with its oxidative status were also determined.

MATERIALS AND METHODS
Plant material

The cultivar Lula of *Persia americana* was provided by the National Research Institute of Agriculture and Development (IRAD), Foumbot Station, Cameroon. Aqueous extraction was conducted as described by Saha Foudjo et al. (2012). The operating conditions were: time (180 min), pH (4.5), temperature (45 °C) and water/pulp ratio (6).

Determination of avocado oil composition
Chlorophyll content

Chlorophyll content was determined as described in IUPAC (1995). The absorbance of 0.1 g of avocado oil per ml of hexane was measured at 630 nm, 670 nm and 710 nm. The chlorophyll content was then calculated using the absorption coefficient of pheophytin *a* (345.3 $M^{-1}.cm^{-1}$). It was expressed in g Eq pheophytin *a* per 100 g of oil.

Carotenoid content

Carotenoid content was determined as described by Ong et al. (1982). The absorbance of 6 mg of avocado oil per ml of iso-octane was measured at 446 nm. Carotenoid content was determined using the absorption coefficient of β-carotene (2610 $M^{-1}.cm^{-1}$) and expressed in g Eq β-carotene per 100 g of oil.

Tocopherol composition

The method was conducted according to Buttris and Diplock (1984) with the procedure described in Kabri et al. (2013). The avocado oil dissolved in n-hexane was analyzed using a HPLC (Ultimate@RS300, Dionex, France) coupled to a fluorescence detector (RF2000, Dionex, France). A silica column (Dionex advantage polar 2, 250 mm x 3 mm ID) maintained at 30°C was used. The signal was recorded at an excitation of 290 nm and an emission of 330 nm. Standard curves of tocopherols (α, β, γ, δ) were drawn for their quantification in avocado oil. The results were expressed as g/100 g of oil.

Squalene content and sterol composition

The procedure was conducted according to ISO 12228 (1999). 5α-cholestane was used as internal standard. After saponification, the sterols and squalene were analyzed using a gas chromatograph (Perkin Elmer Autosystem XL, Germany) equipped with a capillary column DB-5 (30 m of length, 0.32 mm ID, and 0.25 μm of thickness) and an ionization detector. The external standards were composed of: squalene (0.02 mg/ml), 5α-cholestane (0.02 mg/ml), brassicasterol (0.014 mg/ml), δ5-campesterol (0.012 mg/ml), stigmasterol (0.013 mg/mL) and β-sitosterol (0.019 mg/ml). The results were expressed in g/100 g of oil.

Fatty acid composition

The procedure was conducted according to AOCS (1989). A volume of 0.5 ml of pentadecanoic acid 0.5 mg/ml was used as internal standard. After alkaline methylation with BF_3 (13-15 %) at 100 °C for 5 min, the fatty acids were analyzed using a gas chromatograph (Perkin Elmer Auto system XL, Germany). The methylated forms of the following fatty acids were used as external standards at 0.5 mg/mg: myristic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. The results were expressed in g/100 g of oil and as percentages of total quantified fatty acids.

Determination of the oxidative status of avocado oil
Peroxide value

Peroxide value was determined as described by Jian et al. (1992). 15.5 mg of avocado oil were dissolved with 9.9 ml chloroform/methanol 7:3 (v/v), 50 μl xylenol orange 10 mM and 50 μl iron (II) chloride 0.5%. After 5 min, the produced iron (III) complexed with xylenol orange to form a chromophore that absorbs at 560 nm. The absorbance was measured at 560 nm against a blank. A calibration curve of cumene hydroperoxide was used to determine the peroxide value expressed in mEq oxygen/kg oil.

Conjugated diene content

Conjugated dienes content were determined as described by IUPAC (1987). The absorbance of 0.8 mg of avocado oil dissolved in 1 ml of isooctane was measured

at 233 nm. The absorption coefficient of the hydroperoxide of linoleic acid: $2.525 \ 10^4$ $M^{-1}.cm^{-1}$ was used. Conjugated dienes were expressed as µmol/g of oil.

Para-anisidine value

Para-anisidine value was determined as described by ISO 6885 (2006 modified). The absorbance of a tube containing 0.25 g of avocado oil in 5 ml of isooctane and 1 ml of para-anisidine was measured at 350 nm against a blank after keeping it in a dark place for 8 min.

Malonadialdehyde determination

The procedure was conducted as described by Viau et al. (2016). After extraction and complexation with thiobarbituric acid (TBA), malonadialdehyde (MDA) was detected and quantified as MDA-TBA adduct with an UHPLC system (Dionex RS-LC Ultimate 3000) paired with an UV-vis detector. Signal acquisition was made at the wavelength of 535 nm. The data were expressed as MDA content in nmol/g oil with a calibration curve of MDA.

Total polar compound determination

Total polar compound content (TPC) was determined as described by Dobarganes et al. (2000). A chromatography column (10 mm x 150 mm) containing 5 g of silica gel 60 (Merck Millipore, Germany) was used to fractionate 0.5 g of avocado oil dissolved in 5 ml petroleum ether/diethyl ether 90:10 (v/v).The non-polar fraction was first eluted with 60 ml petroleum ether/diethyl ether 90:10 (v/v) and then the polar fraction with 50 ml diethyl ether. After evaporation, the 2 fractions were weighed. The TPC content was expressed as g/100 g of oil.

Heating process

For each experiment, 600 mg of avocado oil were heated in a beaker on a temperature-controlled magnetic stirrer. Temperature and duration of thermal treatment were determined by the modelling design calculation (table 1). After heating, each avocado oil sample was cooled down and its content (% w/w) in total polar compounds (TPC) determined.

Modelling Design

The study area defined for the independent variables: heating temperature and time (Table 1), was applied to a four-level two-variable Central Composite Design (CCD) (Myers and Montgomery, 2002). The TPC content was chosen as the response variable and the value of 25% was fixed as the upper limit to reduce health risks linked to the ingestion of thermally-oxidized oils. The independent variables were coded according to the following formula:

$$x_i = \frac{X_i - X_0}{\Delta X_i}, \quad i = 1, 2,$$

where x_i is the dimensionless coded value of X_i, X_o the value of X_i at the central point, and ΔX_i the step.

Sixteen experiments were randomly run. The following general second order polynomial equation was used to depict the thermal stability of water-extracted avocado oil:

$$Y = \beta o + \Sigma \ \beta_i \ x_i + \Sigma \ \beta_{ii} x^2_i + \Sigma \ \beta_{ij} \ x_i \ x_j + \varepsilon$$

where Y is the response variable (TPC content in %); β_o, β_i, β_{ij} and β_{ii} are the constant coefficients of the intercept, linear, quadratic and interaction terms respectively; x_i and x_j are coded independent variables and ε represents the residue.

Statistical analysis

The lack-of-fit test, ANOVA, Durbin-Watson test, significance of the regression coefficients and the coefficients of determination (R^2 and Adj. R^2) were calculated to assess the robustness and suitability of the fitted second-order polynomial equation. The significance level was $p < 0.05$. The regression analysis was conducted with the software Statgraphics Plus 5.1.

Table 1: Coded values and levels of independent variables used in the Central Composite Design used to model the effect of heat treatments on water-extracted avocado oil.

Coded values	Independent variables	
	Temperature (°C)	Time (min)
-1.41	120	11
-1	130	40
0	155	110
+1	180	180
+1.41	190	209

RESULTS

Composition of avocado oil

The avocado oil used in this study contained monounsaturated fatty acids, 69% of the total quantified fatty acids, 3% polyunsaturated fatty acids and 28% saturated fatty acids (Table 2). It also contained phytosterols, vitamin E, chlorophyll, carotenoids and squalene (Table 2). Among the 4 forms of tocopherols identified, the α form was the main component (92.6%) and δ form had the lowest amount (0.1 ± 0.0 µg/g oil). No tocotrienols were detected. From the 4 forms of phytosterol identified, β-sitosterol was the most abundant (82.9%) and stigmasterol the less represented (20 ± 4 mg/100 g oil).

Oxidative status of freshly-extracted avocado oil

The results of the assessment of the oxidative status of avocado oil before heating process can be found in table 3. The results suggested that very small amounts of primary oxidative products (dienes and hydroperoxides) and secondary oxidative products (MDA) were generated during the extraction process. The polar compounds represented 8 ± 4% of the oil.

Modeling of polar compounds formation and prediction of critical heating times of water-extracted avocado oil

The TPC content of water-extracted avocado oil submitted to heat treatment according to the 16 experiments of the Central Composite Design (CCD) varied between 10% and 60%. The lowest TPC content was observed for a heating time of 11 min and a temperature of 155 °C. The highest TPC was obtained after 180 min at 180 °C. To test if CCD could describe the relationship between polar compounds formation and both temperature and time, the lack-of-fit test was calculated. The lack-of-fit test was not significant ($p = 0.05$), which demonstrates that the multiple regression equation properly explained the relationship between the formation of polar compounds and both temperature and time (Table 3). The coefficient of determination, R^2 (0.98), very close to 1, denoted a high correlation between observed and predicted values. The coefficient of determination was almost similar to the adjusted R^2 (0.97) showing that the model accurately described the relationship that binds the TPC content to the factors temperature and time. The curvature observed in the response surface and contour plots was due to the quadratic terms of temperature and time (Figure 1). The shape of the response surface plot confirmed the suitability of a second order model. The set of points of the homoscedasticity of residues (Figure 2) was distributed almost uniformly around the horizontal axis, which indicates that the residual variance was constant. The Durbin-Watson test was not significant ($t = 2.36$, $p = 0.34$), the residues were therefore not correlated. In sum, the hypotheses formulated on the residuals for a linear regression model were respected. The analysis of variance (Table 3) showed that all regression terms were highly significant ($p < 0.01$). The following polynomial second order equation was derived:

$$\text{TPC} = 14.60 + 8.49\, x_1 + 14.77\, x_2 + 3.86\, x_1^2 + 10.05\, x_2^2 + 6.22\, x_1 x_2$$

Where x_1 and x_2 are respectively the coded values of time and temperature. TPC is the total polar compound content (%).

According to the parameters of the above equation and the response surface plot, the total polar compound content during heating of avocado oil increases when either temperature and/or time increases. Based on the equation coefficients, temperature has a higher effect than time during thermal oxidation (14.77 > 8.49). Figure 1 showed that the total polar compound content was nearly constant between 120 – 140 °C. Indeed, according to the well-known effect of temperature on chemical reactions and as currently observed for thermos-oxidation of edible oil, an increase of the temperature used for cooking (> 140 °C) adversely affected the oxidative stability of avocado oil. When the heating temperature did not exceed 140 °C, TPC were formed at very slow rates and their level remained low and under the limit (25%) even after a heating time longer than 3 h 30 min. When temperature was higher than 140 °C, TPC increased sharply with heating time.

The results shown in table 4 evidenced that the upper limit for heating time decreased as the temperature increased. It remained fairly constant between 120 °C to 140 °C. This is in agreement with the graph analysis done above (Figure 1).

Table 2: Chemical composition of the freshly-extracted avocado oil.

SAPONIFIABLE MATTER

Classes	Fatty acids	Contents (10^{-2} g/100 g)	Percentages (%)	Total (%)
Saturated fatty acids	myristic acid	2 ± 0	0 ± 0	28 ± 1
	palmitic acid	2280 ± 32	26 ± 1	
	stearic acid	168 ± 7	2 ± 0	
monounsaturated fatty acids	palmitoleic acid	2277 ± 21	25 ± 1	69 ± 0
	oleic acid	3967 ± 158	44 ± 1	
polyunsaturated fatty acids	linoleic acid	185 ± 7	2 ± 0	3 ± 0
	linolenic acid	74 ± 3	1 ± 0	
Total		9039	100	100

UNSAPONIFIABLE MATTER

Constituents	Sub-constituents	Content
Chlorophyll (10^{-4} g/100 g oil)		9 ± 2
Carotenoid (10^{-4} g/100 g oil)		4 ± 0
Squalene (10^{-3} g/100 g oil)		133 ± 7
Tocopherols (10^{-5} g/100 g oil)	α	263 ± 1
	β	3 ± 0
	γ	17 ± 0
	δ	1 ± 0
Sterols (10^{-3} g/100 g oil)	Brassicasterol	22 ± 9
	Δ5-campesterol	63 ± 2
	stigmasterol	20 ± 4
	β-sitosterol	506 ± 39

Table 3: Indicators of oxidation status of the freshly-extracted avocado oil.

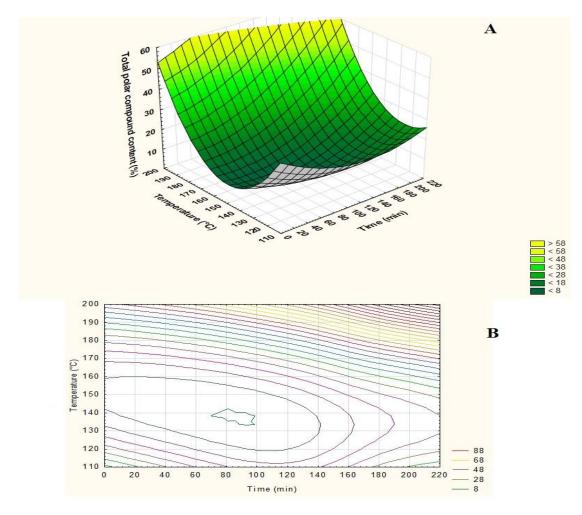| Parameters	Content
Peroxide value (meq oxygen/kg)	4 ± 0
Para-anisidine value	4 ± 2
MDA content (nmol/g)	3 ± 0
Conjugated diene content (μmol/g)	3 ± 0
Total polar compound content (%)	8 ± 4

Table 4: Significativity of parameters and adequation of the Central Composite Design during heat treatments of water-extracted avocado oil.

Parameter	Sum of squares	Degree of freedom	Mean squares	F-ratio	p-value
β_1	577.21	1	577.21	209.89	0.0000*
β_2	1744.97	1	1744.97	634.53	0.0000*
β_{11}	119.27	1	119.27	43.37	0.0000*
β_{22}	809.11	1	809.11	56.390	0.0000*
β_{12}	155.03	1	155.03	294.22	0.0000*
Lack-of-fit test	35.58	3	11.86	4.31	0.0500
Pure error	19.24	7	2.75		

* Significant level ($p < 0, 05$); F-ratio: ratio of Fisher.

Table 5: Predicted upper limit heating times ($t_{25\%}$) of water-extracted avocado oil at different temperatures with a upper limit for total polar compound content defined at 25%.

Temperature (°C)	Predicted upper limit heating time at 25% (min)
120	232
130	230
140	214
150	188
160	151
170	97
180	4

Figure 1: Plots showing the effect of temperature and time on the total polar compounds content of water-extracted avocado oil after heat treatments: (**A**) response plot and (**B**) contour plot.
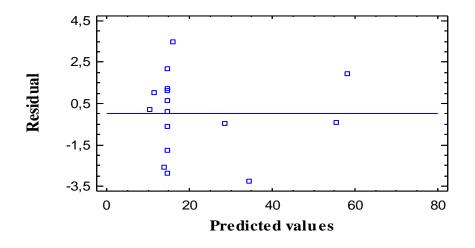

Figure 2: Homoscedasticity of residues for the modeling of avocado oil oxidation.

DISCUSSION

The oxidative status of the oil after extraction, namely the presence of oxidation-derived compounds, can affect the rate of the oxidation (Min et Jung, 1989). The peroxide value was well below the admissible value for an edible oil of good quality that is 20 meq oxygen/kg (O'Connor et al., 2007). Content of conjugated dienes content was in the same order that the value recommended for virgin olive oil in the Codex Alimentarius (2001) that is 3.5 µmol/g. MDA content was lower than that of olive oil (2 mmol/g), reported by Romojaro et al. (2013). MDA content was also found to be lower compared to fresh rapeseed, sunflower, kiwiseed and tuna oils (0.6 to 29 µmol/kg) reported by Viau et al. (2016). Para-anisidine value (4.5) was also below the recommended values, 20 (arbitrary unit), by GOED for omega-3 oils (GOED, 2012). The very low level of oxidative indicators suggest that the 8% of the TPC did not come from oxidative products but from polar lipids naturally present in crude unrefined vegetable oils such as free fatty acids and membrane lipids (phospho- and glycolipids), slightly polar (amphiphilic) lipids such as monoacylglycerols, diacylglycerols (Dulf et al., 2013) and other polar compounds such as phenolics. Thus, the avocado oil produced with the aqueous extraction method was of good quality based on the oxidative indicators.

Time and temperature are prominent but controllable influencing factors on thermo-oxidation of edible oils. For instance, time was also found as a key factor in the prediction of limit frying time of sunflower oil (Farhoost and Tavassoli-Hafrani, 2011). These authors observed a linear relationship between total polar compound content and time in agreement with Guillén and Uriarte (2011) working on 17 edible oils among them virgin olive oil, sunflower and linseed oils. Guillén and Uriarte (2011) also observed that the more the oil is rich in oleic acid the less it produces polar compounds while the reverse was found for oils rich in linoleic and linolenic acids. Two-third of fatty acids of the avocado oil used for this study was monounsaturated fatty acids. Thus, based on its fatty acid profile it was only moderately susceptible to oxidation. Additionally, it contained appreciable amounts of natural antioxidants such as tocopherols, carotenoids and sterols.

The limit heating time, for which a TPC content of the avocado oil was predicted to reach 25%, was found to decrease from about 3 hours to 4 min when the temperature increased from 150 °C to 180 °C. Beresategi et al. (2012) showed that cold-pressed avocado oil and virgin olive oil had the same thermal stability at 180 °C. These authors reported a lower phytosterol content (339.6 mg/100 g) and a higher content of tocopherols (245 µg/g) compared to that of this study. Phytosterols might inhibit the polymerization process during oxidation (Gordon and Magos, 1983). Tocopherols (284×10^{-5} g/100 g) compete with unsaturated fatty acids for peroxyl radicals and are converted to tocopheroxyl radicals, more stable that their lipid analogues and inhibit the propagation of oxidation (Kamal-Eldin and Appelavist, 1996). Tocopherols can be regenerated by squalene (0.1 g/100 g) (Manzi et al., 1998). Moreover, carotenoids present in avocado oil (4×10^{-4} g/100 g) play an antioxidant role by scavenging singlet oxygen and radicals and inactivating sensitizers (Chloe and Min, 2006). In contrast, chlorophyll (9×10^{-4} g/100 g oil) and its derivatives present in the oil are known to act as sensitizers to promote oxidation (Gutierrez-Rosales et al., 1992).

Conclusion

The results presented in this study showed that avocado oil produced with aqueous extraction method contained antioxidant and prooxidant compounds. Moreover, the freshly-produced avocado oil was of good quality based on the oxidative indicators. The four-level two-variable Central Composite Design was suitable and robust enough to predict each upper limit heating time of avocado oil at high temperatures. For the temperature range chosen (120 °C –

180 °C), the upper limit heating time varies between 232 min to 4 min. TPC content evolved in 2 phases between 120 °C-180 °C: a rapid increase between 140 °C-180 °C and a stationary phase between 120 °C-140 °C. Thus, water-extracted avocado oil is not recommended for cooking at high temperatures (140 °C – 180 °C) such as frying while it can be used for recipes involving long cooking time at moderate temperature (120 °C-140 °C).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

BUSF collected the sample, performed experiments and wrote the manuscript. GK, EF and CG supervised the experiments and revised critically for important intellectual content. All authors read and approved the final manuscript.

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