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RESEARCH ARTICLE

ASSESSMENT OF THE THERMO-OXIDATION OF THREE CUCURBIT SEED OILS BY DIFFERENTIAL SCANNING CALORIMETRY

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Abstract

Differential scanning calorimetry (DSC) technique was used to assess the thermo-oxidation of oils of three cucurbit species named *Cucumis melo*, *Citulus lanatus* and *Cucumeropsis mannii*. The DSC oxidation was conducted under isothermal conditions at four different temperatures: 110, 120, 130 and 140 °C. Thermal oxidation by DSC performed in the presence of oxygen (50 ml/min) revealed three step oxidation of cucurbit seed oils. Oxidation temperature is high for *C. lanatus* oil (130 °C) compared with *C. melo* oil (125.1 °C) and *C. mannii* oil (121.0 °C). *C. lanatus* oil exhibited high value of oxidative induction time (T_0) at all isothermal temperatures and was consequently more thermo-stable than *C. melo* oil and *C. mannii* oil. The analyzed cucurbit oils were more stable than some edible oils (corn and sunflower oils) in comparison with their oxidative induction time. There was a good correlation ($R^2 \geq 0.91$) between oxidative induction time and isothermal temperatures which revealed that oxidative stability of oils can be accurately determined in short time by DSC.

Keywords: Cucurbit seed oil, differential scanning calorimetry, oxidation, induction time.

Introduction

The cucurbit kernels are widely used in many food preparations as thickener soup. These seed kernels are rich in fat (40 to 60 %) and exhibit good nutritive values (Samant and Rege, 1981; De Mello *et al*., 2001). According to Min and Boff (2001), oxidative stability of oil which was rich in linoleic and linolenic acids has been highly affected. Sunflower oil has approximately 70 % linoleic acid and is highly susceptible to lipid oxidation (Meydani *et al*., 1991).

A comparative study of physicochemical properties of three cucurbit specie oils (*C. mannii*, *C. lanatus* and *C. melo)* showed that linoleic acid was the major fatty acid (Gbogouri *et al*, 2011). To our knowledge, there is not much information on the thermo-oxidation effect on lipid fraction of cucurbit seed. Oxidative stability is one of the most important indicators for maintaining quality of edible oil during storage. The period in which the oxidation of edible oil takes place was called induction stage.

The time before a dramatic increase in the rate of lipid oxidation is the measure of the oxidative stability and is referred to as the induction time (Coppin and Pike, 2001). Today there are various methods to determine automatically oxidative stability of fats and oils: Active Oxygen Method (AOM), Racimat Method, Oxygen Stability Instrument (OSI), Differential Scanning Calorimetry (DSC), and Fourier Transform Infra Red (FTIR). Application of DSC as accelerated oil

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stability tests was used in isothermal mode for direct determination of the oxidative stability of oils.

To estimate the stability of oils and fats, the sample is usually subjected to an accelerated oxidation test under standardized conditions where heating is the most common means of accelerating the oxidation. The oxidation is an exothermic process and the evolved heat makes it possible to employ DSC for its study (Simon and Kolman, 2001). According to Tan and Che Man (2002), DSC is a useful tool to study the thermally-induced transitions or oxidation as a result of oils heating. Tan *et al.* (2002) conducted a comparative study to determine oxidative stability of vegetable oil by DSC and OSI methods. The result indicated that there is good correlation ($P < 0.01$) between the DSC induction time (DSC T_0) and OSI values. In Ivory Coast, the major source of commercial edible oil is palm. That is undoubtedly due to the failure to recognize the characteristics and sources of vegetable oil.

However, studies of the properties of other vegetable oils on the laboratory scale could allow them to be used as edible. As seen above, cucurbit seeds were an important source of oil (40 to 60 % of fat) that can be used in many food preparations. The aim of this paper is to evaluate rapidly the oxidative stability of three cucurbit seed oils using DSC technique.

Materials and methods

Materials. Sun-dried cucurbit seeds of *Cucumeropsis mannii* Naudin, *Citrullus lanatus* var. *Citroides* (Thrumb.) Matsum & Nakai and *Cucumis melo* var. *agrestis*, were obtained from the Laboratory of genetic of the University of Abobo-Adjamé (Ivory Coast). Basic quality parameters of the seeds used in this study are to be free from pests, mycotoxins, poisonous seeds, mites and any bacterial-related diseases.

The seeds were crushed in a coffee grinder and were stored in hermetic bags at -20 °C until analysis. All the chemicals and solvents used were of analytical grade.

Oil extraction and chemical analysis. Seed oil of cucurbit species was extracted by method of Folch *et al.* (1957). Lipids index were analyzed using standard procedures: peroxide value using Standard 965.33 of the American Oil Chemists Society (1997), free fatty acids using Norme Européenne (1999) and iodine value using Norme Européenne (1999).

Fatty acid methyl esters (FAME) were obtained by transmethylation of lipid aliquots (100 mg) according to Ackman (1980). FAME were analyzed using gas chromatography on Perichrom™ 2000 system (Saulx-les-Chartreux, France), equipped with a flame ionisation detector (FID) and fused silica capillary column (50 m × 0.25 mm × 0.5 µm, BPX70 SGE Australia Pty Ltd).

Thermal oxidation by differential scanning calorimetry. Thermal oxidation of oils was measured using differential scanning calorimeter (DSC 204 F1 Phoenix®, Netzsch - Gerätebau GmbH, Germany) described previously (Oomah and Sitter, 2009). The calorimeter was calibrated according to standard procedures established by the manufacturer user book using indium and water. Approximately 8-10 mg of sample was weighed into open aluminium pans and while an empty open aluminium pan was used as reference.

Samples were heated to 50 °C to ensure melting of the lipids. Then they were heated from 50 °C to 350 °C at a scanning rate of 1 °C/min in the presence of purified oxygen (99.9 %) purged at 100 mL/min.

To determine the oxidative induction time (T_0), the method described by Tan *et al.* (2002) was used. Pans were heated at isothermal temperatures (110, 120, 130 and 140 °C) and purified oxygen (99.9 %) was passed through the sample at 50 mL/min. The T_0 of the oxidative reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm.

Statistical analysis. Results are expressed as the mean ± standard deviation of several values of samples with Kyplot (version 2.0 beta 15, ©1997-2001, Koichi Yoshioka) statistical software. The

data were statistically analyzed by one way analysis of variance (ANOVA). Means were compared by Turkey's test. Differences were considered statistically significant at *P < 0.05. All measures were done in triplicate.

Results and discussion

Chemical characteristics

The initial characteristics of cucurbit seed oils investigated appear in Tables 1a & 1b. *C. mannii* oil exhibited higher peroxide value (9.78 meq O_2/kg of oil) than *C. lanatus* oil (1.10 meq O_2/kg of oil) and *C. melo* oil (2.72 meq O_2/kg of oil). The iodine value indicated the degree of unsaturation of seed oils. There is no significant difference (*P > 0.05) in the iodine value of the three species of cucurbit seeds : 115.5 g of I_2/100 g of oil for *C. melo*, 111.8 g of I_2/100 g of oil for *C. mannii* and 113 g of I_2/100 g of oil for *C. lanatus*. The level of total free fatty acids (1.13%) of *C. melo* and *C mannii* were ca. two times lower than the values of *C. lanatus* (Table 1).

Table 1a. *Initial chemical characteristics of cucurbit seed oils*

Oil type	Peroxide value meq O_2/kg of oil	Free fatty acid (%)	Iodine value (g of I_2/100 g of oil)
Citrullus lanatus	1.10 ± 0.03^a	1.81 ± 0.12^b	113.00 ± 1.00^a
Cucumeropsis mannii	9.78 ± 0.10^c	1.13 ± 0.00^a	112.00 ± 2.00^a
Cucumis melo	2.72 ± 0.04^b	1.13 ± 0.00^a	115.50 ± 0.30^a

Mean ± standard deviation of three determinations. In the same column, mean values followed by the same letter are not significantly different (P >0.05)

Table 1b. *Main fatty acid composition of cucurbit seed oils (% total fatty acids)*

Fatty acids	*Cucumis melo*	*Cucumeropsis mannii*	*Citrullus lanatus*
16:0	10.66 ± 0.08^a	16.33 ± 0.29^a	11.87 ± 0.18^a
18:0	9.02 ± 0.01^a	13.89 ± 0.22^a	9.35 ± 0.16^a
C18:1n 9	9.12 ± 0.03^a	14.54 ± 0.13^a	14.43 ± 0.11^a
18:2n 6	70.76 ± 0.02^a	54.81 ± 0.13^a	63.94 ± 0.23^a
18:3n 3	0.46 ± 0.01^a	0.42 ± 0.02^a	0.43 ± 0.01^a
SFA	19.68 ± 0.04^a	30.22 ± 0.50^a	20.97 ± 0.24^a
MUFA	9.02 ± 0.01^a	14.54 ± 0.13^a	14.50 ± 0.15^a
PUFA	71.22 ± 0.02^a	55.23 ± 0.14^a	64.54 ± 0.11^a

Mean ± standard deviation of three determinations. In the same row, mean values followed by the same letter are not significantly different (*P > 0.05).

The acidity of these oils varied from 2.30 mg of NaOH/g oil to 4.30 mg of NaOH/g oil. The fatty acid group composition (Table 1b) of the oil indicated that *C. melo* oil contained the high content of polyunsaturated fatty acids (PUFA) (71.22 %) and the low percentage of saturated fatty

acid (SFA) (19.68 %) and monounsaturated fatty acids (9.02 %). *C mannii* oil has the lower percentage of PUFA (55.23 %) and the higher percentage of SFA (30.22 %) than *C. melo* oil (64.54 % and 20.97 %, respectively). *C. lanatus* oil contained 64.54 % of PUFA and 20.97 % of SFA. Linoleic acid (C18:2n 6) was largely predominant, 70.76% for *C. melo*, 63.94% for *C. lanatus* and 54.84% for *C. mannii*.

DSC isothermal oxidation

Oxidation of oil is due to the formation of free radicals in the early stages of the oxidation process. In this respect, oxidative stability is defined as the resistance of the formation of radicals and is expressed as the period of time during which radicals are formed slowly before a sharp linear increase is observed (Thomsen *et al.*, 2000).

Oxidative stability is an important parameter in evaluating the quality of oils and fats as it gives a good estimate of their susceptibility to oxidative degeneration, the main cause of their alteration (Aparicio *et al.*, 1999). The sharp of DSC oxidation curves of cucurbit seed oils (Fig. 1) from 50 to 350 °C, was similar and revealed at least three exothermic effects. Table 2 summarized the thermo-oxidative parameters. According to Oomah and Sitter (2009), these peaks could be considered as an indication of the level of cross-linking. The onset temperatures of the investigated cucurbit oils ranged from 112 °C to 124.5 °C and corresponded to the start of the oxidation process. *C. lanatus* oil had the high onset temperature (124.5 °C) than *C. mannii* oil (112.6 °C) and *C. melo* oil (116.5°C). DSC oxidation of *C. mannii* oil started at 112.6-145.0 °C followed by *C. melo* oil (116.5-153.5°C) and by *C. lanatus* oil (127.5-154 °C), and peaked at 145.0 °C, 153.5 °C and 154.0 °C, respectively.

Significant differences ($P<0.05$) were observed in onset and oxidation temperatures for *C. lanatus* and the two other oils. That indicates the thermostability of *C. lanatus* compared to *C. mannii* and *C. melo* oils. This difference could be attributed to their initial chemical characteristics (peroxide values) and the presence of natural antioxidant in the cucurbit oils.

According to Smith et *al*. (2007), peanut and corn oils which had small peroxide values had the greatest oxidative stabilities compared to high oleic sunflower and soybean oils. The DSC oxidation temperatures of the present cucurbit oils were slightly below the temperatures reported for edible oils (130-180 °C) and peaked at 145-154 °C, depending on species (Fig. 1 and Table 2).

Table 2. *Thermo-oxidation temperatures (°C) of cucurbit seed oils*

Oil type	Onset (°C)	Oxidation temperature	peak 1	Peak2	Peak 3
C. melo	116.5 ± 1.8^{a}	125.1 ± 0.5^{a}	153.5 ± 0.6^{b}	200.2 ± 0.2^{a}	282.2 ± 2.3^{a}
C. mannii	112.6 ± 0.6^{a}	121.0 ± 0.7^{a}	145.0 ± 1.1^{a}	220.9 ± 0.4^{b}	286.0 ± 3.1^{a}
C. lanatus	124.5 ± 0.7^{b}	130.0 ± 1.0^{b}	154.0 ± 0.3^{b}	201.5 ± 0.3^{a}	284.4 ± 2.0^{a}

Mean ± standard deviation of three determinations. In the same column, mean values followed by the different letters are significantly different ($P <0.05$).

The thermoxidation temperatures (Onset, oxidation and peak 1 temperatures) of investigated cucurbit seed oils looked alike values of *Echinacea* seed oils reported by Oomah *et al.* (2006). *C. lanatus* and *C. melo* oils showed comparable peak 1, peak 2 and peak 3 temperatures each. Oxidation temperature and peak 1 temperature of *C. mannii* oil were lower and significantly different ($P<0.05$) than oxidation and peak 1 temperatures of *C. lanatus* and *C. melo* oils. Peak 3 temperatures of the three cucurbit seed oils ranged from 282 to 286 °C and were not significantly different ($P<0.05$). *C. mannii* oil, which had the lowest onset, oxidation and peak 1 temperatures, exhibited the highest peak 2 and peak 3 temperatures.

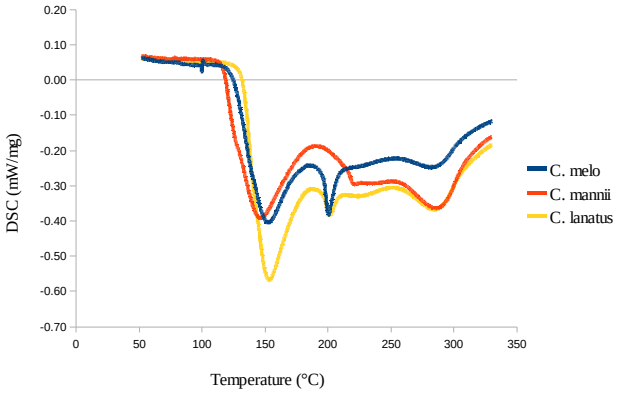

Figure 1. *Differential scanning calorimetry of the thermo-oxidation profiles of cucurbit seed oils.*

Oxidation induction time

Induction period is a preparatory stage in which the chemical compounds needed for the full development of oxidation are formed. This period was considered as a relative measure of oxidative stability. During the induction period, alkyl radicals are formed and undergo reaction with oxygen molecules to form hydroperoxides and peroxide radicals during the propagation phase. Termination is done via association of two radicals to form a stable adduct (Brand-Williams *et al.*, 1995).

The oxidative stability of oils can be analyzed by extrapolated the oxidative induction time (T_0) using DSC (Tan *et al.*, 2002). Fig. 2 shows the DSC oxidation curves of cucurbit seeds oil at 100 °C. Straight line (Fig. 2, curve 1) was observed with the stream of nitrogen (99.99 %) flowing at 50 mL/min).

Curve 1 indicated clearly no exothermic curve, while exothermic oxidation curve (Fig. 2, curve 2) was observed when oil samples were run under oxygen atmosphere (99.99 %) flowing at 50 mL/min. The oxidation process is a principally exothermic reaction which occurs between the oil and oxygen.

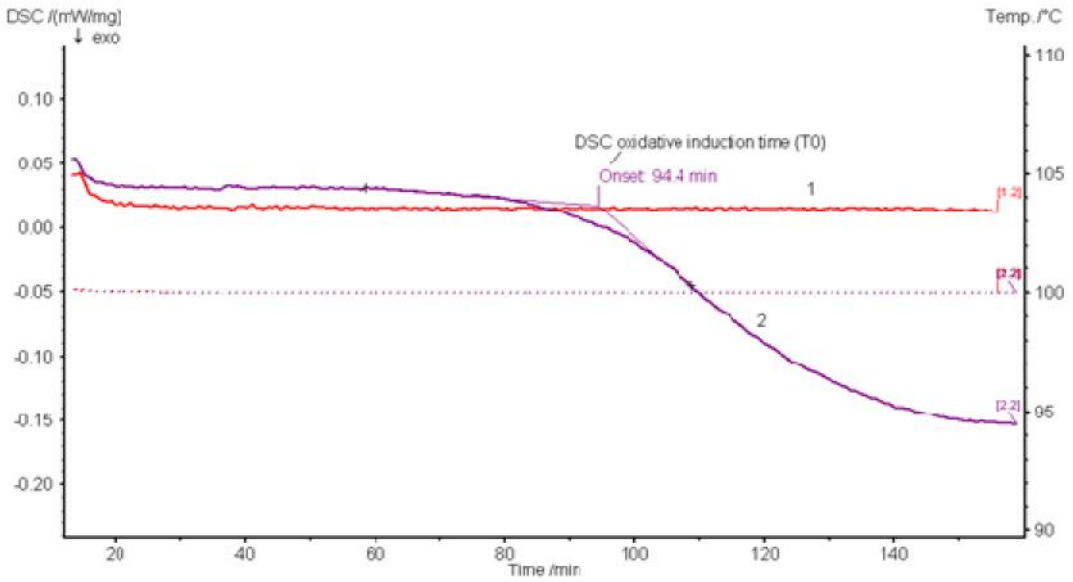

Figure 2. *Differential scanning calorimetry oxidation curve of cucurbit seeds oil. (1) Isothermal curve at 100 °C with nitrogen (99.99 %) flowing at 50 mL/min; and (2) Isothermal curve at 100 °C with oxygen (99.99 %) flowing at 50 mL/min.*

The induction time of oils

Fig. 3 describe the induction period of oils plotted as the function of isothermal temperatures ranged from 110 °C to 140 °C. Each DSC isothermal temperature has a significant effect on DSC T_0 measurements. An increase of isothermal temperature allowed the decrease of T_0 of all oils. At all isothermal temperatures, the DSC oxidative induction time (T_0) of *C. lanatus* oil was high

followed by *C. mannii* and *C. melo* oils. It was indicated that *C. lanatus* oil was more thermally stable than *C. mannii* and *C. melo* oils. For example at 110 °C, *C. lanatus* shows oxidative stability up to 270 min, higher than *C. mannii* (224.9 min) and *C. melo* (174.3 min).

The oxidative stability of *C. lanatus* may be due to the higher amount of tocopherol (Badifou, 1991) and the low content of unsaturated fatty acids in the comparison to the other oils (Table 1, see above). It is generally known that oils with high degree of unsaturated fatty acid are more susceptible to lipid oxidation. The regression equations of logarithm isothermal temperature (T) values against DSC T_0 values were established and given in Table 3.

There is an excellent correlation between oxidative induction time (T_0) and DSC isothermal temperature. The coefficient of determination R^2 of the analyzed oils were above 0.91, showing good linear regression, which revealed that the oxidative stability of oils can be accurately determined in a short time by DSC.

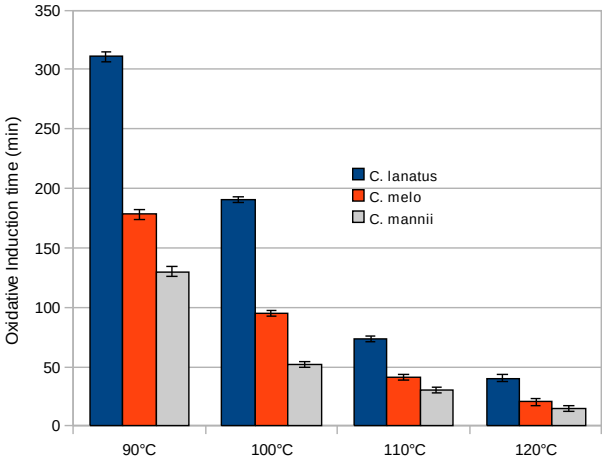

Figure 3. *Oxidative induction time (T_0) plotted as a function of four isothermal temperatures*

Table 3. *Relationship between DSC oxidative induction time (T_0) and isothermal temperatures (T)*

Oil type	Regression equation	Coefficient of determination (R^2)
C. lanatus	$T_0 = -2343.44 \log_{10} T + 5024.02$	0.91
C. mannii	$T_0 = -1969.49 \log_{10} T + 4223.19$	0.93
C. melo	$T_0 = -1490.47 \log_{10} T + 3200.54$	0.93

There is an increase of 10°C in isothermal temperature from 110 to 140 °C, DSC T_0 decreased approximately to half of its previous reading (Table 4). This is consistent with the Q_{10} low for the relationship between temperature and the rate of chemical reaction.

Similar results were reported on eleven edible oils (Tan *et al.*, 2002). In the comparison of DSC T_0 (Table 4) of cucurbit seed oils to various edible oils studied by Tan *et al.* (2002), we can notice a very good oxidative stability of cucurbit seed oils.

C. lanatus shows higher DSC T_0 than corn, canola and sunflower oils at all DSC isothermal temperature *C. mannii* and *C. melo* oils are more stable than corn and sunflower oils.

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Table 4. *Oxidation time (T_0) by differential scanning calorimetry of cucurbit seed oils compared by the other oils*

Oil type	DSC T_0 (min)			
	110 °C	**120 °C**	**130 °C**	**140 °C**
C. lanatus	270.0 ± 1.5[c]	113.8 ± 2.0[c]	52.1 ± 1.2[c]	21.0 ± 0.4[b]
C. mannii	224.9 ± 1.0[b]	100.8 ± 1.2[b]	44.7 ± 0.4[b]	16.8 ± 0.2[a]
C. melo	174.3 ± 1.1[a]	81.5 ± 1.1[a]	38.1± 0.5[a]	17.1 ± 0.6[a]
RBDPOo[1]*	515.19A	287.42A	160.42A	82.36A
Corn*	166.55C	83.57F	47.74F	21.45E
Canola*	259.96B	126.41E	61.67E	37.41D
Sunflower*	131.88D	70.50G	33.34H	19.98F

Mean ± standard deviation of three determinations. In the same column, mean values followed by the different letters (a-b) are significantly (P <0.05) different.

ND, not detected

[1]RBDOPo, refined-bleached-deodorised palm plein

*Tan, Che Man, Selemat & Yusoff (2002). Means within each column with different letters (A-G) are significantly (P < 0.05) different.

Conclusion

The present study shows that the DSC technique is an efficient tool for the evaluation of the thermo-oxidation of seed oils. *C. lanatus* oil is very thermostable oil compared to *C. melo* and *C. mannii*, and to some edible oils (corn, sunflower and canola oils).

Considering the oxidative stability, cucurbit seed oils, particularly *C. lanatus* oil may find some applications in frying and cooking processes.

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