ORIGINAL ARTICLE

Effect of concentration of *Allium cepa* and *Pimpinella anisum* powders on the oxidative stability of oils extracted from peanuts cakes

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ABSTRACT

Background: Lipids are responsible for both the undesirable and desirable flavors of food; oxidation of lipids mainly results in the development of off-flavor and lipoxygenase-derived lipid-based volatiles that are responsible for flavor generation. These lipids can be found in animal, vegetable and marine foods sources. Among these vegetable lipids sources, peanuts are one of the main oleaginous used to prepare foods. **Aims:** This study aimed at assessing the effect of 0.5g, 1g, 2g and 4g of *Allium cepa* and *Pimpinella anisum* powders on the oxidative stability of lipids extracted from peanuts cakes. **Material and Methods:** The total phenolic content, flavonoid contents and the antioxidant properties of these spices were evaluated. In addition, lipids quality was assessed by chemical characterization of oils extracted from peanuts cakes. **Results:** Results revealed that *P. anisum* had the highest total phenolic (TPC = 61.66 mg GAE/g), flavonoid (FC = 34.95 mg CE/g) contents and DPPH free radical scavenging activities with values that ranged from 17.66 % to 89.18 %. The analysis of the oxidative state of oils extracted from peanuts cakes prepared with 0.5g, 1g, 2g and 4g of *Allium cepa* and *Pimpinella anisum* powders revealed that all oils samples with the exception of those extracted from cakes cooked with 2g and 4g of *P. anisum* powder had peroxide, P-anisidine, total oxidation, thiobarbituric acid and free fatty acid values in line with those recommended by the *Codex Alimentarius*. The principal component analysis (PCA) revealed that the free fatty acids, peroxide, P-anisidine, thiobarbituric acid values were more efficient to induce lipids oxidation in peanuts cakes. **Conclusions:** Preparing peanuts cakes with *Allium cepa* and *Pimpinella anisum* powders are more effective to limit lipids oxidation compared to peanuts cakes cooked without spices.

Keywords: *Allium cepa, Pimpinella anisum,* lipids quality, peanuts cakes, antioxidant, oxidative stability.

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1 Introduction

Oils and fat play an important role in the organoleptic quality of food since they bring about odor and essence, act as harbingers of odor and essence compounds, or transform the odor and essence of additional constituents [1]. Oils and fat can be found in animal, vegetable and marine foods sources [2]. Among these vegetable lipids sources, peanuts are one of the main oleaginous used to prepare foods [3]. Peanuts are sustainable crop using minimal natural resources for growing, in comparison with other nuts. Moreover, peanuts are able to fix nitrogen, and thus promote soil fertility. Peanuts are

composed mostly of 10 – 20 % carbohydrates, 16 – 36 % proteins and 36 – 54 % lipids. Peanuts consumption may lower the outbreak of inflammation and some diseases [4].

Peanuts go through many thermal processes such as grilling, cooking in boiled water, vapor cooking of roasted paste which can reduce their nutritional value. Been composed of about of fatty acids (81 % unsaturated) peanuts may likely undergo during culinary methods, lipid degradation reactions such as oxidation and decomposition reactions of oxidation products [5]. These reactions lead to the production of unpleasant substances and off-odors that modify the organoleptic and the marketability of foods [6]. The primary and secondary oxidation products of lipids are responsible for the formation of rancid odor [7], cardiovascular diseases and cancer [8].

Peanuts cakes constitute a food cooked with roasted groundnuts paste, tied in plantain leaves and subjected to steam cooking before consumption. During cooking of peanuts cakes, some spices are added [9]. In addition to their organoleptic and nutritional functions, these spices can provide to foods functional properties and pharmacological benefits [10]. Previous works such as those of Kurnia et al. [11] showed that *Allium cepa* is a rich source of secondary metabolites and several bioactivities. In addition, it is also known for its ability to reduce cholesterol level and to prevent cardiovascular diseases. It has also been proven that, red and yellow onion husks are potential sources of antioxidants [12]. Studies have also shown that *Pimpinella anisum* possesses phytochemical and antioxidant properties [13, 14]. Although numerous studies showed the phytochemical and antioxidant capacity of these spices, little work has been performed concerning the concentration effect of spice powders on the quality of oils extracted from peanuts cakes. This is because at a certain concentration (high) these spices can display prooxidative effects [15]. Therefore, the objective of this work was to evaluate concentration effect of *Allium cepa* and *Pimpinella anisum* powders on the oxidative stability of oils extracted from peanuts cakes.

2 Material and Methods

2.1 Spices and dried groundnuts seeds collection

Allium cepa (*A. cepa*), *Pimpinella anisum* (*P. anisum*) and dry peanuts were bought in Dschang market, located in the Western region of Cameroon. In order to lower the moisture content and to avoid destroying the phytochemical content and antioxidant properties, the spices were dried in a ventilated oven at 55 °C for two days and transformed to powder. The powder was obtained after using an electric blender (Royalty line; Model No: SME-600.6; Order No: 16 – RL – 942) at speed 3 to grind the dry spices.

2.2 Preparation of spices extracts

For this, 25g of *A. cepa* and 25g of *P. anisum* powder were soaked in 500 mL of water for two days at room temperature[16]. The extracts were later drained with filter paper Watman N°1. After draining and in order to remove the remaining water, the filtrates were put at 45 °C in a ventilated oven. The spice extracts obtain was kept in a freezer.

2.3 Phytochemical analysis

2.3.1 Total phenolic content (TPC)

The total phenol content of the different extracts was determined by the spectrophotometric method using the Folin-Ciocalteu reagent as described by Gao [17]. 0.01 mL of extract solution of concentration 5 mg/mL were introduced into a test tube. Subsequently, we added 1.39 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent. After three (3) min of rest, 0.4 mL of sodium carbonate (Na_2CO_3, 20 %) has been added to it. The tubes were then well vortexed and incubated for 20 min in a water bath at 40 °C and the absorbance was read against a blank at 765 nm using a BIOMATE brand spectrophotometer (Thermo Scientific™ 840208400/EMD). The calibration was performed using a freshly prepared aqueous solution of gallic acid (0.2 g/L) and the results were expressed as equivalent mg of gallic acid/g of extract.

2.3.2 Flavonoid's content (FC)

Marivona [18] method was used to determine the flavonoid content. 0.1 mL of extract was mixed with 1.4 mL of distilled water and subsequently 0.03 mL of a 5 % sodium nitrite (NaNO2) solution was added. After 5 min, 0.2 mL of a 10 % aluminum trichloride (AlCl3) solution was added. 0.2 mL of concentrated 10 % sodium hydroxide solution (NaOH) and 0.24 mL of distilled water were added to the mixture after five minutes of incubation. The whole was stirred using a vortex and the absorbance was measured at 510 nm. Results were expressed in milligrams catechin equivalent per g of extract.

2.4 Evaluation of the antioxidant activity

2.4.1 Ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity, of the different extracts, was evaluated by the method using DPPH° radical as described by Mensor et al. [19]. 900 µL of a solution of 2,2-diphenyl-1-picryl hydrazyl prepared in methanol was introduced into test tubes previously containing 100 µL of extract solution. The blanks consisted of 100 µL of extract solution and 900 µL of methanol. After 30 minutes of incubation in the dark and at room temperature, the absorbance was measured using a brand spectrophotometer at 517 nm. Methanol was

used to tare the spectrophotometer. The antiradical activity of the extracts (in %) was calculated according to formula (1):

$$A.Ar\ (\%) = [(Abs_{DPPH} - Abs_{sample}) \times 100/Abs_{DPPH}] \quad (1)$$

Where: A.Ar = Antiradical activity; Abs_{DPPH}= Absorbance of the DPPH solution; Abs_{sample}= Absorbance of the analyzed sample

The IC_{50} values (effective concentration equivalent to 50 % of DPPH° lost) were determined from the percentages of the antioxidant activity and the logarithm of the concentrations by plotting the regression lines of equation (2) and were expressed in mg/g.

$$\%AA = a \log (C) + b \quad (2)$$

2.4.2 Ferric Reducing Antioxidant Power (FRAP)

The method described by Oyaizu [20] was utilized to determine the Ferric Reducing Antioxidant Power of the extracts. In test tubes previously containing 0.5 mL of dissolved extract solution at a concentration of 1000 µg/mL, 1 mL of a potassium phosphate buffer solution (0.2 M, pH 6.6) and 1 mL of an aqueous solution of potassium hexacyanoferrate [$K_3Fe(CN)_6$] at 1 % were added. The mixture was then incubated for 30 minutes at 50 °C in a water bath and then 1 mL of a 10 % trichloroacetic acid solution was added. After centrifugation at 3000 tr for 10 min for 10 minutes, 1.5 mL of supernatant was removed and mixed with 1.5 mL of distilled water, followed by 0.1 mL of a methanolic solution of $FeCl_3$ at 0.1 %. The blank consisted of all reagents except the extract. The absorbance of the reaction mixture was read at 700 nm against this blank on a spectrophotometer. An increase in absorbance of the reaction mixture indicated an increase in reducing power.

2.4.3 Hydroxyl radical inhibition power ROH

The reaction between hydrogen peroxide and ferrous ions was used to determine the hydroxyl radical inhibition power of the extracts [21]. Extracts in potassium phosphate buffer (50 mM, pH 7.4) were analyzed at 0.2 and 0.5 mg/mL. 0.03 % hydrogen peroxide was prepared using deionized water. The analysis was performed on clear microplates containing 96 well. Potassium phosphate buffer (50 µL) was added to blank and control wells while extract (50 µL) at each concentration were added to other wells. 50 µL of 1.10-phenanthroline (3 mM), 50 µL of FeSO4.7H2O (3 mM) and 50 µL of hydrogen peroxide (0.03 %) made in water were also added respectively. In the blank control wells, 50 µL of water used instead of hydrogen peroxide. Plates were sealed with a plastic film and incubated at 37 °C at 150g for an hour and the absorbance was measured at 536 nm in a BioTek Epoch microplate reader (Fisher Scientific, Nepean, ON, Canada).

2.4.4 Oxygen Radical Absorbance Capacity (ORAC)

The methods described by Ratnasari et al. [22] was used to determine the peroxyl radical scavenging activity also known as oxygen radical absorbance capacity (ORAC). Potassium phosphate buffer of concentration 75 mM and a pH of 7.4 was used to prepare samples and standard. The standard concentrations were 5 – 100 µM and that of extracts was 0.2 mg/mL. 120 µL of fluorescein concentration 0.08 µM were added followed by 20 µL of diluted extract or standard in a 96 – well microplate. The plate was then sealed and incubated for 20 min at 37 °C in the FLx800 BioTek fluorimeter. After incubation, 60 µL of AAPH (150 mM) were added to every well and data were recorded after one minute interval. The ORAC values expressed in µM Trolox equivalents (TE)/g extract were then calculated using the net area under the curves.

2.5 Processing of peanuts cakes

Dry peanuts were selected (to eliminate bad seeds and physical contaminants) and roasted at 190 ± 10 °C for 10 min in a pot placed on an electric heater. In order not to get burn, the peanuts seeds were stirred continuously with a kitchen ladle. The roasted peanut seeds were then ground using a "Moulinex" brand mixer (Royalty line; Model No: SME – 600.6; Order No: 16 – RL – 942) to obtain a homogenous paste. 100g of paste were mixed with 50 mL of warm water (50 ± 2 °C) as well as 0.5; 1; 2 and 4g of *A. cepa*, and *P.anisum* powders, using a kitchen ladle in order to obtain a homogeneous mixture. The mixture obtained was wrapped in plantain leaves. The tied pastes (supplemented individually with each spice powder at different concentrations and without spice) were individually cooked for one hour and 30 minutes inside a pot at a temperature of 93.5 °C. The bottom of the pot was protected with plantain leaves to avoid water to enter into the cakes and also for it not to burn. The pot used was washed after each cooking. The processing method and cooking time of peanuts cakes was selected based on a survey carried out among women producers of peanut cakes. At the end of each cooking, lipids of each cake supplemented with spice powders were extracted after the cakes was allow to cool at room temperature.

2.6 Oil extraction

The methods described by Bligh and Dyer [23] was used to extract the lipids of the different peanut's cakes. This method is based on the use of organic solvent to extract all polar and non-polar lipids. For 100 g of cake sample introduced into a glass blender, 100 mL of chloroform and 200 mL of methanol were added and the whole was ground for two minutes. After grinding, 100 mL of chloroform was added to the ground material and the whole was mixed for 30 seconds. The mixture was screened and filtered. Total extraction was ensured by adding chloroform to the retentate, respecting the final solvent ratio of 2:1 (V:V) of chloroform: methanol. The mixture was decanted in a funnel until separation into two phases. The organic phase was collected in a weighed flask after adding anhydrous sodium sulfate to eliminate all traces of humidity. The solvent was then evaporated in a rotary evaporator at 50 °C. The oil samples were collected in dark bottles and stored in places with low temperatures (-18 °C).

2.7 Lipid's quality assessment of oils extracted from peanuts cakes

The lipids quality of oils extracted from peanuts cake was evaluated by determining the rate of primary oxidation product (peroxide value) as describe by the IDF standard method [24]; the rate of formation of 2 carbonyls alkenals and 2,4-decadienals (P-anisidine value) [25], the total oxidation value using Shahidi and Wanasundara [26] method. Draper and Hadley [27] method was used to determine the rate of formation of malondialdehyde (TBARS); the degree of unsaturation of peanuts cake oil (iodine value) using the AOCS official method Cd 8-53 [28] and free fatty acid value using the official method [28].

2.8 Statistical analysis

Results were subjected to analysis of variance with Student Newman-Keuls Multiple Comparison Test, to determine the significance of the data expressed as mean ± standard deviations. GraphPad Instat version 5.0 software was used to bring out values that was significantly different at a probability level of $p < 0.05$. The Principal Component Analysis (PCA) and correlations were applied to find the existence of links between the primary and the secondary oxidation products analyzed. Samples with similar characteristics were group using XLSTAT 2014 software.

3 Results

3.1 Total phenolic content (TPC) and flavonoids content (FC)

According to Table 1, the total phenolic and flavonoids contents were higher in *P. anisum* extract than in *A cepa* extract powder.

Table 1. Total phenolic (TPC) and flavonoids content (FC) of *A cepa* and *P. anisum* powders

Samples	TPC (mg GAE/g)	FC (mg CE/g)
A. cepa	45.71 ± 0.00^{b}	28.36 ± 0.01^{b}
P. anisum	61.66 ± 0.08^{a}	34.95 ± 0.05^{a}

Values with different superscripts differ significantly at $p < 0.05$

3.2 Antioxidant activity of extracts

3.2.1 Ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical

Figure 1 presents the ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical. In a nutshell, at 25 µg/mL, *P. anisum* extract presented the lowest scavenged DPPH radical (17.66 %) compared to that of *A. cepa* (26.95 %) while at 50; 100; and 200 µg/mL *P. anisum* activity was greater than that of *A. cepa* with values from 42.27 %, 87.10 % and 89.18 % respectively. *A. cepa* at 50 µg/mL (38.02 %); 100 µg/mL (40.52 %) and at 200 µg/mL (41.82 %) presented the smallest ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical.

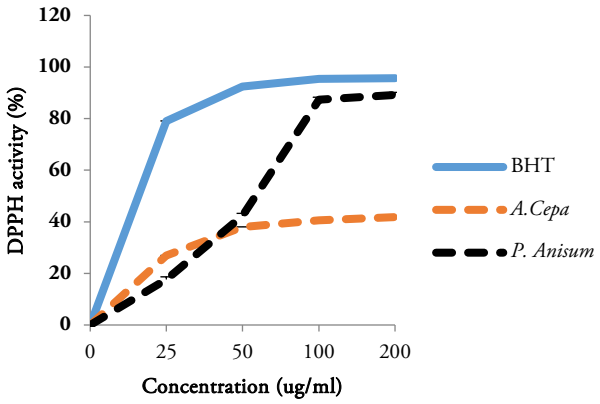

Figure 1. Ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical of the aqueous extract of *A cepa* and *P. anisum* extracts

3.2.2 Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power production of *A. cepa* and *P. anisum* extracts at various concentration in comparison to that of BHT is illustrated in Figure 2. It can be observed that *P. anisum* extract showed higher FRAP activity ($p < 0.05$) than *A. cepa*. The FRAP activity of *P. anisum* at concentration 50 µg/mL was similar ($p > 0.05$) to that of *A. cepa*.

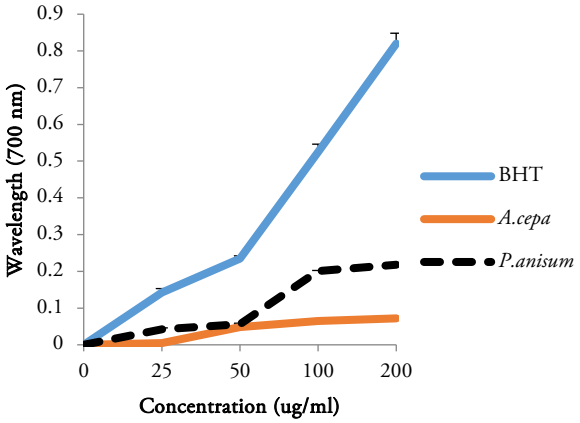

Figure 2. FRAP of *A cepa* and *P. anisum* aqueous extracts

3.2.3 Oxygen Radical Absorbance Capacity (ORAC)

The oxygen radical absorbance capacity (ORAC) of *A. cepa* and *P. anisum* extracts is shown in figure 3. In a nutshell *P. anisum* (405.50 µMTE/g) presented an ORAC value greater than that of *A. cepa* (34.53 µMTE/g).

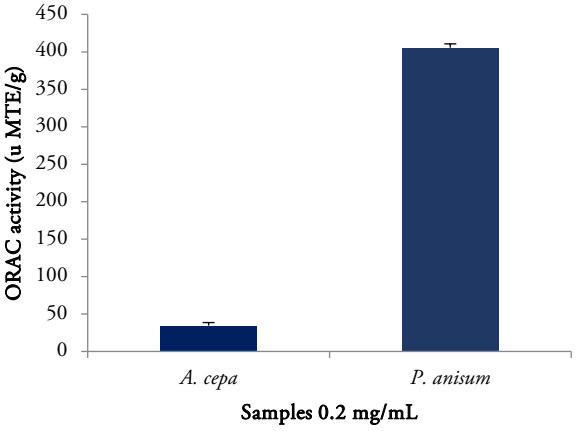

Figure 3. ORAC of *A. cepa* and *P. anisum* extracts

3.2.4 Hydroxyl radical scavenging assays (ROH)

As shown on Figure 4, ROH of *P. anisum* (8.3 %) was higher than that of *A. cepa* (4.4 %).

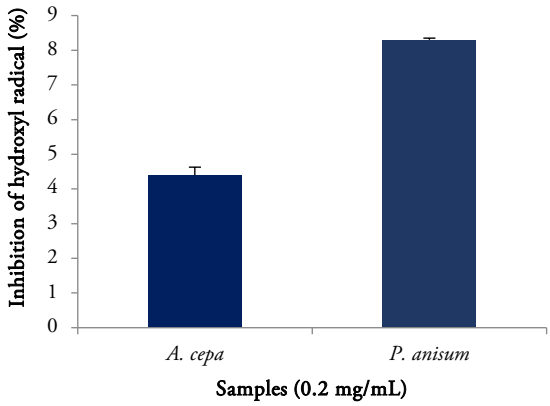

Figure 4. Hydroxyl radical scavenging assays (ROH) of the aqueous extract *A. cepa* and *P. anisum* extracts

3.3 Effect of *A. cepa* and *P. anisum* on the oxidative stability of lipids extracted from peanuts cakes

3.3.1 Effect of *A. cepa* and *P. anisum* on the formation of primary oxidation products (peroxide value)

Table 2 presents the peroxide value (PV) of peanuts cakes oils prepared with zero (OGC0) spices and the addition of 0.5g, 1g, 2g and 4g *of A. cepa* and *P. anisum* powders. Lipids from cakes prepared with *A. cepa* (OAC) and *P. anisum* (OPA) powders showed a significant decrease ($p < 0.05$) in the formation of primary oxidation products compared to that of sample OGC0. For cakes cooked with *A. cepa* powder the lowest PV was obtained with 1g (OAC: 2.59 $meqO_2/Kg$); while for cakes cooked with *P. anisum* powder the lowest PV was also observed with cake cooked with 1g (OPA: 3.20 $meqO_2/Kg$). In contrast, oil extracted from sample OGC0 had the highest PV (18.39 $meqO_2/Kg$).

3.3.2 Effect of *A. cepa* and *P. anisum* on rate of formation of 2 carbonyls alkenals and 2.4-decadienals (P-anisidine value)

Table 2 shows the effects of *A. cepa* and *P. anisum* on the rate of formation of carbonyls 2-alkenals and 2.4-decadienals (P-

anisidine value) of peanuts cakes oils prepared adding 0.5g, 1g, 2g and 4g of these spices. Regarding this table, all peanuts' cakes oils prepared with *A. cepa* and *P. anisum* had significantly smaller ($p < 0.05$) P-anisidine value compare to the P-anisidine value of sample OGC0 (49.49). Oil from peanuts cake prepared with 2g of *A. cepa* (OAC: 6.45) presented the lowest P-anisidine value.

3.3.3 Effect of *A. cepa* and *P. anisum* on the thiobarbituric acid value (TBARS)

Table 2 summarizes the effect of 0.5g, 1g, 2g and 4g *A. cepa* and *P. anisum* powder on the TBARS value of oil extracted from peanuts cakes. At first glance, oils from cakes prepared with the addition of 0.5g, 1g, 2g and 4g of *A. cepa* and *P. anisum* powder showed the formation of malondialdehyde (MDA) significantly lesser ($p < 0.05$) than that of the control (OGC0: 2.14 meq MDA/Kg). With *A. cepa* powder, peanut cake prepared with 1g presented an oil with the lowest TBARS value (OAC: 0.64 meq MDA/Kg) and with *P. anisum*, it was that of 4g that has the lowest TBARS value (OPA: 1.19 meq MDA/Kg).

Table 2. Peroxide, P-anisidine, Thiobarbituric acid, Iodine, Free fatty acids and Total oxidation values from peanuts cake prepared adding 0.5g, 1g, 2g and 4g of *A. cepa* and *P. anisum* powders

Peroxide values (meq O2/Kg)				
Samples	**$PV_{0.5g}$**	**PV_{1g}**	**PV_{2g}**	**PV_{4g}**
- OAC	3.18 ± 1.55^{bC}	2.59 ± 0.03^{cD}	8.31 ± 0.00^{bA}	6.03 ± 0.87^{bB}
- OPA	3.94 ± 0.19^{bB}	3.20 ± 0.39^{bB}	7.79 ± 0.38^{bA}	6.75 ± 2.39^{bA}
- OGC0	18.39 ± 1.25^{aA}	18.39 ± 1.25^{aA}	18.39 ± 1.25^{aA}	18.39 ± 1.25^{aA}
P-Anisidine values				
	P-anisidine $_{0.5g}$	**P-anisidine $_{1g}$**	**P-anisidine $_{2g}$**	**P-anisidine $_{4g}$**
- OAC	16.64 ± 0.00^{bA}	9.17 ± 1.14^{bC}	6.45 ± 0.00^{cD}	13.93 ± 2.57^{cB}
- OPA	16.77 ± 0.00^{bC}	12.51 ± 1.53^{cD}	29.57 ± 2.54^{bB}	40.53 ± 0.00^{aA}
- OGC0	49.49 ± 2.41^{aA}	49.49 ± 2.41^{aA}	49.49 ± 2.41^{aA}	49.49 ± 2.41^{bA}
TBARS values (meq MDA/Kg)				
	$TBA_{0.5g}$	**TBA_{1g}**	**TBA_{2g}**	**TBA_{4g}**
- OAC	1.86 ± 0.04^{aA}	0.64 ± 0.14^{cC}	1.58 ± 0.13^{bB}	1.83 ± 0.11^{bA}
- OPA	1.64 ± 0.06^{bA}	1.37 ± 0.20^{bA}	1.49 ± 0.01^{bA}	1.19 ± 0.00^{bA}
- OGC0	2.14 ± 0.04^{aA}	2.14 ± 0.04^{aA}	2.14 ± 0.04^{aA}	2.14 ± 0.04^{aA}
Iodine values (gI2/100g)				
	$IV_{0.5g}$	**IV_{1g}**	**IV_{2g}**	**IV_{4g}**
- OAC	51.00 ± 1.20^{bD}	55.10 ± 0.58^{aB}	53.49 ± 2.37^{bC}	58.83 ± 1.22^{aA}
- OPA	54.14 ± 1.19^{aB}	53.06 ± 0.00^{bB}	59.93 ± 3.18^{aA}	54.17 ± 0.55^{bB}
- OGC0	49.00 ± 0.77^{cA}	49.00 ± 0.77^{cA}	49.00 ± 0.77^{cA}	49.00 ± 0.77^{cA}
Acid value (% oleic acids)				
	$IV_{0.5g}$	**IV_{1g}**	**IV_{2g}**	**IV_{4g}**
- OAC	0.56 ± 0.00^{cA}	0.55 ± 0.00^{bA}	0.45 ± 0.03^{cB}	0.56 ± 0.00^{cA}
- OPA	0.84 ± 0.00^{bA}	0.55 ± 0.00^{bB}	0.83 ± 0.01^{bA}	0.82 ± 0.00^{bA}
- OGC0	1.55 ± 0.19^{aA}	1.55 ± 0.19^{aA}	1.55 ± 0.19^{aA}	1.55 ± 0.19^{aA}
Totox values				
	Totox $_{0.5g}$	**Totox $_{1g}$**	**Totox $_{2g}$**	**Totox $_{4g}$**
- OAC	23.02 ± 0.31^{bC}	14.36 ± 1.22^{cD}	29.07 ± 0.00^{cA}	26.00 ± 1.74^{cB}
- OPA	24.66 ± 1.01^{bC}	18.93 ± 2.32^{bD}	45.16 ± 3.30^{bB}	54.03 ± 4.78^{bA}
- OGC0	86.29 ± 4.92^{aA}	86.29 ± 4.92^{aA}	86.29 ± 4.92^{aA}	86.29 ± 4.92^{aA}

Values with different superscripts in the same column in minor letters and in the same line in capital letters differ significantly at $p < 0.05$.OAC: Peanuts cake oil prepared with addition of *A. cepa*; OPA: Peanuts cake oil prepared with addition of *P. anisum*; OGC0: Peanuts cake oil prepared zero spice.

3.3.4 Effect of *A. cepa* and *P. anisum* on the oils unsaturation level (Iodine value (IV))

As shown in Table 2, unsaturated fat levels (as measured by Iodine value) of peanuts cakes oils prepared with 0.5g, 1g, 2g and 4g *A. cepa* with *P. anisum* increased when compared to that of sample OGC0 (49.00 gI_2/100g) oil. With oil samples extracted from cakes cooked *A. cepa* powders, sample OAC_{4g} had the highest IV (58.82 gI_2/100g) while for cakes prepared with *P. anisum* powders, the highest iodine value was observed with that of 2g (59.93 gI_2/100g).

3.3.5 Effect of *A. cepa* and *P. anisum* on the acid value (AV)

Determination of acid value (AV) of oils extracted from peanuts cakes cooked with 0.5g, 1g, 2g and 4g of *A. cepa* and *P. anisum* powders (expressed in % oleic acid) revealed that oil extracted from sample OGC0 (1.55 % oleic acid) presented a significantly high free fatty acids level ($p < 0.05$) compared to oils extracted from samples that were supplemented with *A. cepa* and *P. anisum* powders (Table 2). For oil samples extracted from cakes cooked with *A. cepa* powder, the lowest AV was observed with oil of the cake prepared with 2g (OAC: 0.45 % oleic acid). For those cooked with *P. anisum* powder, the oil extracted from the cake cooked with 1g (OPA: 0.55 % oleic acid) had the lowest AV.

3.3.6 Effect of *A. cepa* and *P. anisum* on the total oxidation value (Totox)

Table 2 shows that the oil extracted from sample OGC0 (86.29) was significantly ($p < 0.05$) oxidized compared to oils extracted from cakes cooked with *A. cepa* and *P. anisum* powders at different concentrations. The lowest Totox value with cake prepared with *A.cepa* powder was observed with the oil extracted from that cooked with 1g (OAC: 14.36); with *P. anisum* powder peanuts cake prepared with 1g had oil with the smallest Totox (OPA: 18.93).

3.3.7 Correlation between the AV, the formation of primary (Peroxide value) and secondary oxidation (P-anisidine; TBARS products), the unsaturated level (IV) and Totox values of peanuts cakes oils

Table 3 presents the correlation coefficient (r) between the acid value (AV), the formation of primary (PV) and secondary oxidation (P-anisidine; thiobarbituric acids values) products, the unsaturated level (iodine value) and totox values of peanuts cakes oils prepared adding *A. cepa* and *P. anisum* powders. According to this table, the peroxide values of peanuts cakes oils prepared with the addition of *A. cepa* and *P. anisum* powders were positively and significantly correlated with the thiobarbituric acid values (r = 0.5549), P-anisidine values (r = 0.4833), acids values (r = 0.7920), total oxidation values (r = 0.7567) and negatively and significantly correlated with the iodine values (r = -35.49. In addition, the P-anisidine value was significantly and positively correlated with the free fatty acids value (r = 0.6989) and the total oxidation value (r = 0.9380). The thiobarbituric acid value was significantly and positively correlated to the acids value (r = 0.4794), to the totox value (r = 0.3364) and negatively and significantly correlated with the iodine value (r = -31.06). The acids value was significantly and positively correlated to the total oxidation value (r = 0.8354).

In addition to correlations, the principal component analysis (PCA) made it possible to establish a map of the variables

Table 3. Correlation between the acids, peroxide, P-anisidine, thiobarbituric acid, iodine and total oxidation values of oils

Variables	PV	P-anisidine	TBARS	IV	AV	TOTOX
PV	1					
P-anisidine	**0.4833**	1				
TBARS	**0.5549**	0.1563	1			
IV	**-0.3549**	-0.2130	**-0.3106**	1		
AV	**0.7920**	**0.6989**	**0.4792**	**-0.4226**	1	
Totox	**0.7567**	**0.9380**	**0.3364**	-0.2996	**0.8354**	1

Values with different superscripts in the same column in minor letters and in the same line in capital letters differ significantly at $p < 0.05$.OAC: Peanuts cake oil prepared with addition of *A. cepa*; OPA: Peanuts cake oil prepared with addition of *P. anisum*; OGC0: Peanuts cake oil prepared zero spice.

according to the different quantities of *A. cepa* and *P. anisum* powders used individually in the preparation of the peanut's cakes (Figure 5). The eigenvalues being characteristic of the principal components were extracted so as to explain the maximum of possible variations. It appears on Figure 5 that five variables F1, F2, F3, F4 and F5 with respective eigenvalues of 3.68; 1.07; 0.73; 0.34 and 0.16 described 100% variations of cumulative variability of 61.44 %; 79.28 %; 91.49 %; 97.26 % and 100 % respectively.

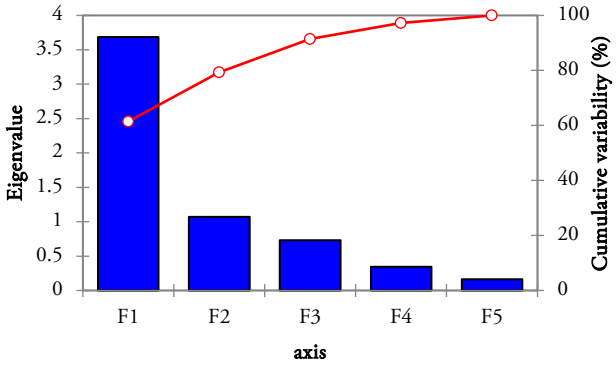

Figure 5. Scree plot of principal component analysis

As shown on Figure 6, the principal component analysis (PCA) diagrams of the variables can gather the different factors. This latter confirms the relationship presented in Table 3. However, the interest is that the factors not only make it possible to observe the relationship, but also to directly visualize the proximities between the parameters that have been evaluated in this study. In addition, the analysis of the quantities of *A. cepa* and *P. anisum* powders used for the preparation of peanuts cakes by ascending hierarchical classification (AHC) made it possible to define three classes also represented in Figure 6. The first class was composed of cakes cooked without spice powders (OGC0) and that prepared with 4g of *P. anisum* powder. The second class was composed of cakes prepared with 0.5g, 1g, 2g and 4g of *A. cepa* powder and cakes supplemented with 0.5g and 1g of *P. anisum* powder. While, the third class was that of groundnut cake cooked with 2g of *P. anisum* powder.

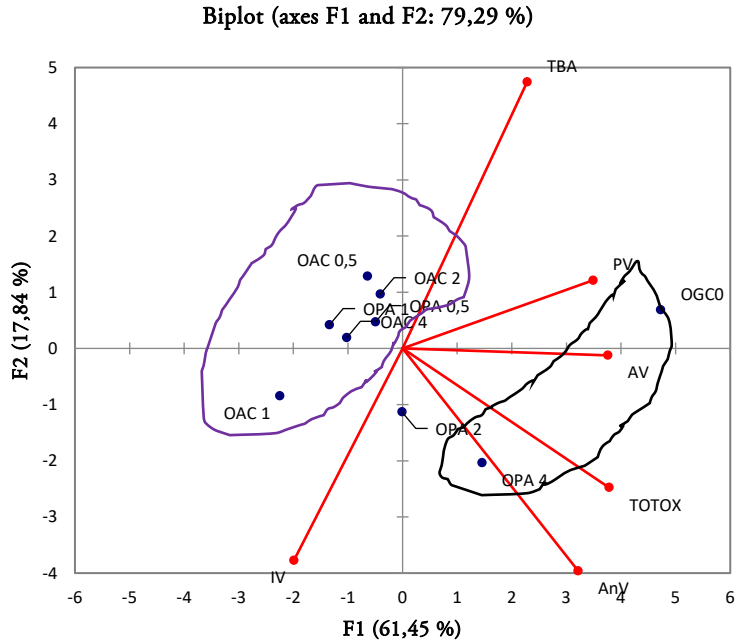

Figure 6. PCA diagrams of the variables relating to the effect of the addition of *A. cepa* and *P. anisum* powders at different quantities on the peroxide, anisidine, thiobarbituric acid, iodine, acid and totoxvalues of oils extracted from peanuts cakes

4 Discussion

The phytochemical analysis revealed that the TPC and FC obtained in this study with *A. cepa* were greater than those obtained by Maguipa et al. [29] respectively 29.92 mg GAE/g extract and 1.94 mg CE/g. In addition, the TPC result of this study was also greater than that of Ola–Mudathir et al. [30] (3.41 mg GAE/g extract) with methanolic extract. However, the FC of *A cepa* obtained in this study was lower than that of Ola–Mudathir et al. [30] (604.63 mg CE/g). Regards to the TPC obtained with *P. anisum* the results were higher than those of Topčagić et al. [14] who obtained 38.46 and 42.75 mg GAE/g extract respectively with aqueous and methanolic extract. Also, the TPC and the FC obtained in this study with *P. anisum* were similar to those found in the literature [13]. Ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical was correlated to the total phenolic contents because the extract that had the highest TPC had the highest antioxidant activity against DPPH radical. Phenols are therefore responsible for the scavenging activity of plants [31,32]. However, El-Missiry and El Gindy [33] proved that the antioxidant capacity in plants was based on their richness in phenolic compounds and mainly to their hydroxyl groups. The antioxidant activity against DPPH radical of *A cepa* of our work was greater than that of Maguipa et al. [29]. Regards to FRAP, the activities observed with *P. anisum* can be explained by the existence of flavonoids that can serve to the reduction of iron (III) to iron (II) [34]. The ORAC test evaluate the ability of polyphenols to hand out proton to peroxide radicals [21]. In this work, the ORAC value of *P. anisum* extract was higher than that obtained by Topčagić et al. [14] (0.40 µMTE/g). On the other hand, the ORAC value obtained with *A. cepa* was lower than that of Chernukha et al. [12] and Asokkumar et al. [35] with red *A. cepa* variety respectively (540.02 µMTE/g; 709.17 µ MTE/g). ROH cause enormous biological damage and lipid oxidation and are potent cytotoxic agents, able to assault biological molecules in membranes [36]. The high rate of ROH scavenging observed with *P. anisum*, may be as a result of it high level of TPC than that of *A. cepa* because phenols are responsible for the scavenging activity of plants [31, 32]. The variation in the level of the total phenolics and flavonoids contents may be due to genotypic and environmental differences such as location, climate, temperature, soil fertility, pest exposure within species, diseases, time of taking samples, parts of plant tested, determination methods and the type of solvent use for extractions [37].

With regards to the study of chemical analysis of oils extracted from peanuts cakes, the low PV of peanuts cakes oils prepared adding *A. cepa* and *P. anisum* can be explained by the existence of antioxidants that would have delayed or blocked the appearance of primary oxidation products (hydroperoxides) by quenching the initiation and propagation steps of auto-oxidation reactions [38]. All PV of peanuts cakes oils prepared with *A. cepa* and *P. anisum* at different quantities was under the standard (10 meq O_2/kg)[39]. However, elevated P-anisidine values of peanuts cakes oils of sample OGC0, can be explained by a rapid formation primary oxidation product, due to lack of spices and their conversion into of carbonyl 2-alkenals and 2,4-decadienals under the effect of temperature [40]. The low P-anisidine obtained with *A. cepa* and *P. anisum* can be justified by the effect of antioxidants present that are capable to block free fatty acid formation [37]. With the exception of P-anisidine of oils from cakes prepared adding 2g, 4g of *P. anisum* and that of sample OGC0 all the other oil samples presented P-anisidine lower than 20 which was the recommended value [41]. The high level of malondialdehyde observed with peanuts cake oil from sample OGC0 would be the consequence of the absence of spice in this sample because they are rich sources of antioxidant. The decrease in malondialdehyde level observed with oils extracted from cake prepared with *A. cepa* and *P. anisum* was a results of antioxidant compounds in *A. cepa* and *P. anisum* powders that have delayed or blocked the appearance of primary oxidation products and their conversion into malondialdehyde (MDA) by giving up their hydrogen atom thus enabling them to maintain the peanuts cakes oils quality [38]. All peanuts' cakes prepared with the addition of *A. cepa* and *P. anisum* spice powders, showed MDA levels below the standard which, according to Codex Alimentarius [39] must be less than 2 meqMDA/kg. The low alteration of the double bonds of oils extracted from cakes cooked with *A. cepa* and *P. anisum* powders can be the results of antioxidants (polyphenols and flavonoids) [24] components that have delayed or blocked the destruction of double bonds. The significant decrease ($p < 0.05$) in acid values of oils extracted from cakes supplemented spices powders could be explained either by a very low hydrolytic activity, by a transformation of fatty acids free radicals initially present into hydroperoxides or either by stabilization by the antioxidants present which would had trap the free radicals by blocking the initiation phase of oxidation, by complexing the catalysts, by reacting with oxygen or by deflecting the effects of light or radiation from the food which would have preserve the free fatty acids released [42]. The absence of spices in OGC0 would be linked to the strong alteration of the latter or to the prooxidant effect [15] of the spices in oils extracted from cakes cooked with 2g and 4g of *P. anisum* powder. Regards to this observation, the variation in phytochemical and antioxidant activities are dose dependent because *P. anisum* powder had the highest TPC, FC and antioxidant activities; and cakes that was prepared with high concentrations of *P.anisum* powder (2 and 4g) had poor lipids quality. With the exception of oils extracted from cakes prepared with the addition of 2g of *A. cepa* powder, 2g and 4g of *P. anisum* powder and that of oil extracted from sample OGC0 all the others oil samples

presented Totox values less or equal to the recommended value which was 26 ppm [41]. Results of this study show a significant and positive correlation between the peroxide with total oxidation; between P-anisidine and free fatty acids values are in line with those of Kobyliński [43]. In order to bring out peanuts cakes that had good oil quality, the parameters used for PCA were peroxide value (PV), p-anisidine value (p-anisidine), thiobarbituric acid value (TBARS), iodine value (IV), acid value (AV) and the total oxidation value (Totox). Results revealed that, totox was positively and highly correlated to F1 (0.9365); TBARS to F2 (0.6332) and F4 (0.3194); the iodine value to F3 (0.7068) and the acid value to F5 (0.3453). With PCA the first class showing that AV, PV, P-anisidine, TBARS and Totox of oils extracted from cakes prepared without spice powder (OGC0) and that prepared with 4g of *P. anisum* powder are linked would be due of the transformation of the free fatty acids released during the hydrolysis of diacylglycerols and triacylglycerols into primary oxidation products which are hydroperoxides [44]. Studies have showed that, lipids oxidation deteriorates the quality of foods by the development of off–flavor, rancidity, degradation in texture, changing of color, reduction of shelf life and during storage, lipids oxidation releases products, which made foods unfit for human consumption. These reactions on foods leads to the loss of nutritive value and eventually declining consumer confidence in the product. Moreover, intake of such food products which contain oxidized lipid constituents can modify proteins, DNA, tumor initiation and membrane structure in biological system [45].

Limitations of the study: It would be of high interest to identify the chemical composition of the extracts in order to determine the structure, the nature, and the names of compounds responsible for the antioxidant activity using high performance liquid chromatography method (HPLC).

5 Conclusion

This study showed that the used of spices especially *A. cepa* and *P. anisum* powders in the preparation of peanuts cakes limited the oxidative state of oil extracted from foods by reducing the destruction of double bonds of fatty acids, the production of primary oxidation products and the transformation of this latter into malondialdehyde, 2-alkenals and 2,4- decadienals which are secondary oxidation products. The principal component analysis confirmed that good results were obtained with cakes prepared with 0.5g, 1g, 2g and 4g of *A. cepa* powder and cakes cooked with 0.5g and 1g of *P. anisum* powder. Therefore, consuming peanuts cakes prepared with these two spices can help to prevent cancer and cardiovascular diseases.

In the near future, we plan to perform sensory analysis to assess the acceptability and scalability of peanut cake prepared with powders of *A. cepa* and *P. anisum* at different concentrations.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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