European Journal of Nutrition & Food Safety

7(3): 144-154, 2017; Article no.EJNFS.2017.011
ISSN: 2347-5641

Performance of Green Tea Leaves Methanolic Extract in Stabilizing Refined, Bleached and Deodorized Palm Olein during Storage at Frying Temperature

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FTD and HMW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FTD, HMW, EA, GTB, MSLK, RBNP and ML managed the analyses of the study. Author FTD managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2017/34993

Received 22nd June 2017
Accepted 8th July 2017
Published 29th July 2017

Original Research Article

ABSTRACT

Aims: The present study was performed in order to test the effect of different concentrations of tea leaves methanolic extracts on the oxidative stability of palm olein subjected at frying temperature.
Study Design: Harvesting of fresh tea leaves, cleaning and drying, extraction of natural antioxidants, supplementation of refined palm olein with the extract and evaluation of its stability during storage at frying temperature (180 ± 5℃).
Place and Duration of Study: University of Dschang, Cameroon and Council for Scientific and Industrial Research-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India, from July 2015 to December 2015.
Methodology: The natural antioxidants were extracted by macerating dried green tea leaves in

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methanol. The concentrated extract was then added in refined palm olein without additives at concentrations 200, 600, 1000, 1400 and 1800 ppm respectively. Oil containing butylated hydroxytoluene (BHT) and oil without antioxidants served as positive and negative controls respectively. The oil samples were then stored in the oven at 180℃ for 6 consecutive days (4 hours heating/day). After every two days of heating, oil samples were collected and various chemical parameters (peroxide value, p-anisidine value, thiobarbituric acid value and iodine value were measured) and instrumental techniques (Gas-chromatography) were used to test the oil deterioration that permit the distinction of oxidative alteration level during storage.

Results: Quantitative measurements revealed significantly ($p<0.05$) higher rate of oxidative alteration level in Control and PO+BHT_{200ppm} (palm olein supplemented with butyl hydroxytoluene) during the storage compared to oil samples containing tea leaves extract as natural antioxidant. At all concentrations, tea extract was very efficient than butylated hydroxytoluene (BHT). It was also the best in delaying linoleic acid degradation during the process.

Conclusion: From these results, it can be concluded that green tea leaves are rich in natural antioxidants of good thermal stability, which can be exploited to delay the oxidation of palm olein during processing at high temperature and during storage.

Keywords: Green tea leaves; natural antioxidant; palm olein; frying temperature; oxidative stability.

1. INTRODUCTION

Frying of foods is one of the most common and popular practices in their preparation and manufacture. It normally takes about 5-10 min and involves high temperatures (175-190℃) [1]. It is a fast, convenient and energy-efficient cooking method that increases the palatability of foods and their nutritional value, due to fat absorption, crust formation, and pleasant flavors and odors [1]. During this process, the oil is subjected to physical and chemical changes which can facilitate its oxidative degradation in the presence of air and moisture. The chemical reactions including thermo-oxidation, hydrolysis, polymerization, isomerisation or cyclization take place in the frying oil, leading to the reduction of its quality, as well as the quality of the fried product [2]. Generally, oxidation reaction decrease the quality of oils and fried products by reduction their nutritional value (loss of essential fatty acids and essential amino acids; destruction of vitamins, and reduction of proteins digestibility of foods) [3]. It also alters the organoleptic properties of foods by changing their colour, texture, appearance and by producing rance odours and undesirable flavours. Besides affecting the nutritional and organoleptic properties of the products, during this process, some toxic compounds which are well recognized as harmful for human are also generated [4]. The rate of decomposition of the frying oil depends on several factors such as temperature and length of frying time; the type of food, the chemical composition of the oil and frying method [5].

In order to overcome the stability problem of frying oils, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *ter*-butyl hydroquinone (TBHQ) have been used as food preservatives for protecting the oil from oxidative damages. However, recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis [6]. Additionally, Chang et al*.* [7] demonstrated that BHA and BHT are quite volatile and easily decompose at high temperatures. In the same line, Hamama and Nawar [8] have proven that BHA and BHT loss half of their antioxidant activity after 45 and 55 min storage at 185℃. Due to this safety concerns and because nowadays consumers are quite cautious about the quality of their diet and its chemical additives, there is an increasing interest on the part of the food industry and preventive medicine to replace synthetic antioxidants with those of safer, more natural origins [9]. It is well accepted that plants are rich sources of antioxidants. Among plants, herbs and spices are prominent, because they contain a wide array of antioxidants including some vitamins, carotenoids, phenolic compounds etc, which render them (herbs and spices) as preservative agent in foods.

Tea leaves are good sources of antioxidants that have been reported among herbs for use as antioxidants in fat and oil systems [10,11]. In many developping countries, old green tea leaves are generally considered as waste, and always eleminated from the tree in order to facilitate the development of young leaves, which are used for tea drink production [12]. These old

leaves, instead to be throwed, could be exploited for other purposes. In one study, Womeni et al. [12] have demonstrated the effects of methanolic extracts of old green tea leaves on the oxidative stability of palm olein during an accelerated storage of 30 days at 70℃. Results of these investigations revealed tea leaves to be a good source of natural antioxidants, mainly phenolic compounds with a higher antioxidant activity. They also demonstrated that this extract has good thermal stability and is efficient in delaying palm olein oxidation during accelerated storage. In the same way, we found necessary to also know the potential effect of this extract on palm olein at frying temperature.

The present study was then performed in order to evaluate the effect of different concentrations of tea leaves methanolic extracts on the oxidative stability of palm olein subjected at frying temperature. Palm olein has been used in this study because it is the widely used industrial frying oil [13].

2. MATERIALS AND METHODS

2.1 Materials

The reagents and chemicals used in this study were of analytical grade. They were procured from HiMedia Laboratories Pvt. Ltd, Sd Fine Chemicals, Mumbai, India and Sigma-Aldrich, St; Louis, USA. Concerning the standards fatty acids methyl esters, they were provided by Sigma-Aldrich, St. Louis, USA.

The refined palm olein without additive used in this study was purchased at the SCS/RAFCA Palm Oil Industry Company Ltd, based at Bafoussam, West Cameroon. The fresh green tea leaves (*Camellia sinensis*) were harvested in Djuttitsa, West Cameroon, in April 2013.

2.2 Methods

2.2.1 Extraction of tea leaves antioxidants

The extraction of antioxidants was made according to the method described by Womeni et al. [12].The fresh mature leaves were cleaned and dried in the oven at 50℃ for 48 h. After this, they were grinded to pass through a 1 mm diameter sieve. 100 g of that powder was macerated at room temperature in 800 ml of methanol with regular shaking, during 48 h. After filtration using the N° 1 Wattman paper, the residues were again macerated in 400 ml of methanol, this in order to maximize the extraction of phenolic antioxidants. The obtained filtrate was mixed to the previous one, before elimination of the solvent on a rotatory evaporator at 40℃ under reduced pressure. The concentrated extract was stored in the refrigerator at 4℃ for further analysis.

2.2.2 Samples preparation

The samples were prepared according to the method described by Iqbal et al. [14]. The concentrated methanolic extract was dissolved in 1 ml of solvent (methanol) and individually added in 100 g of preheated palm olein (50℃ during 3 h) at five differents concentrations (200, 600, 1000,1400 and 1800 mg/kg or ppm). Butylated hydroxytoluene used at its recommended concentration (200 mg/kg or ppm) [15] served as positive control in order to compare the preservative property of the extract. Palm olein without antioxidant, also prepared as previously described served as negative control. It is important to note that the amount of methanol added was evaporated to a value similar or less than 10 mg/kg or 50 mg/kg, which are the recommended concentrations of methanol to be added in food as recommended by the regulations [16-18]. After this, oils samples were used for Schaal oven test.

2.2.3 Schaal oven test

The method decribed by Sultana et al. [19] was used with slight modifications. The prepared oil samples were stored in an electric hot air oven at 180℃ for 6 consecutive days (4 h heating per day). Oil samples were collected after every two days and kept in the refrigerator at 4℃ for future analysis. Their stability toward oxidation was evaluated by measuring primary and secondary oxidation products. The parameters evaluated are cited below. Additionally, the modifications in the fatty acid profile of each oil sample during the storage were also investigated.

2.2.4 Measurement of oxidation parameters

Peroxide value of each oil sample was determined according to the spectro-photometrical IDF standard method, 74A: 1991 [20]; The secondary oxidation products were measured using thiobarbituric acid and *p*-anisidine tests, as described by [21] and the AOCS official method guide CD 18-90 [22] respectively. The iodine value was also

evaluated by the AOCS official method, but CD 1-25 [22]. The total oxidation of different treatments was calculated from their peroxide and *p*-anisidine values, through the following equation: TOTOX (Total oxidation) = 2PV + AV, as reported by Shahidi and Wanasundara [23].

2.2.5 Effect of tea leaves extract on fatty acid profile of palm olein at frying temperature

2.2.5.1 Preparation of fatty acids methyl esters

Two drops of each oil sample were individually mixed with a methanolic solution of sulfuric acid (2%), and the fatty acids methyl esters were obtained by transesterification [24]. The Fatty acids methyl esters were trapped using ethyl acetate before to be washed with water in order to emilinate the acid. After this step, the FAMEs, diluted in ethyl acetate were dried using anhydrous sodium sulphate and concentrated on a rotatory evaporator. Chloroform was used to collect these FAMEs, which were further analyzed by Gas-chromatography coupled to a flamme ionization detector. Analysis was performed in triplicates.

2.2.5.2 Gas chromatography

The analysis of the fatty acid methyl esters was made on an Agilent gas chromatograph (Agilent Technologies, Palo Alto, CA, USA, N° of serie 7890A), coupled to a flame ionization detector, and using a DB-225 capillary column (30 m x 0.25 µm of film thickness). Initially, the temperature of the column was maintained at 160℃ for 2 min. After, it increases to 220℃ (5℃/min) and was finally maintained at 220℃ for 10 min. The mobile phase was nitrogen, and its flow rate was 1.5 ml/min. The temperature of the detector and injector were respectively 250 and 230℃. The fatty acids were identified by comparing their retention times to that of standards fatty acids methyl esters, analyzed under the same conditions.

2.3 Statistical Analysis

Results obtained in this study were subjected to one-way analysis of variance (ANOVA) with Dunnet and Student-Newman-Keuls tests using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. When the probability was less than 5%, the difference was significant.

3. RESULTS AND DISCUSSION

3.1 Peroxide Value

Peroxide value (PV) is a widely used measure of primary oxidation stage of lipid, indicating the amount of peroxides formed in fats and oils when they oxidize [25]. Changes in Peroxide values of stabilized and control palm olein samples are showed in Table 1. There was a significant increase ($p<0.05$) in peroxide values of the entire samples containing antioxidants over the heating period, indicating the noticeable phenomenon of oxidation. Similar trend was observed in control sample from day 0 to 4, but, from the fourth to sixth day, a significant decrease in PV was registered. The increase in peroxide value observed in all the samples can be attributed to the formation of hydroperoxydes, which are the primary oxidation products of oils and fats. This accumulation was slow in palm olein sample containing BHT as antioxidant, while it was rapid in oils supplemented with the extract. From this, we cannot directly conclude on the best stability of palm olein stabilized with BHT compared to thoses containing tea leaves extract as antioxidant, because, at high temperature, low or high peroxide values can indicate the good stability of oil or its high decomposition. Generally, when oils are heated at high temperature (>100℃), hydroperoxides are formed and spontaneously converted into secondary oxidation products, mainly carbonyls and aldehydic compounds [26]. So, in such conditions, before giving any conclusion of the statut of the oil, the secondary oxidation products must be quantified, through the para-anisidine test or the thiobarbituric acid test, which are specific to these oxidation products. The decrease in peroxide value observed in the control sample from the 4^{th} storage day might be attributed to rapid transformation of hydroperoxide into aldehydes (2,-alkenals, 2, 4-dienals), ketones, alkoxyl and hydroxyl radicals, by homolysis of the peroxide bonds [26]. It is clearly observed in this sample that, from the fourth storage day, the rate of production of hydroperoxides was lower than that of their decomposition into secondary oxidation products. This means that the control sample was significantly altered compared to oil samples supplemented with antioxidants. So, the absence of preservative in the control sample can explain these changes. However, the fact that from the fourth to the sixth day the PV of oil supplemented with antioxidants was still increasing can be attributed to the presence of antioxidants, and to

the lower decomposition rate of hydroperoxides compared to their formation. So, the observed phenomenon in oil supplemented with antioxidants might be due to the ability of the antioxidants present to delay palm olein peroxidation by donating their hydrogen atom for stabilization of free radicals. In fact, Womeni et al. [12] have proven the good phenolic content, and the good antioxidant and thermal stability of the methanolic extract of green tea leaves, as well as its powerful potential to inhibit palm olein adulteration during accelerated storage of 30 days at 70℃. As previously mentioned, before giving any conclusion on the potent ability of the tea extracts in delaying palm olein oxidation during storage at frying temperature, it is important to quantify secondary oxidation products in these oil samples. However, similar observations have been made by Che Man and Tan [27], during the deep-fat frying of potatoe chips using palm olein containing extracts of oleoresin rosemary and sage as natural antioxidants, in comparison to BHT, BHA and oil without antioxidant.

3.2 *p*-Anisidine Value

p-Anisidine value, which reveals the presence of secondary fatty acid oxidation products, mainly 2-alkenals and 2, 4-dienals is a good and fiable indicator of lipid oxidation [28]. Less stable hydroperoxides undergo further breakdown to form aldehydes and ketones, which are secondary oxidation products imparting rance odors in oils. The changes in *p*-anisidine value of palm olein supplemented with natural antioxidants in comparison to the control are presented in Table 1. It appears clear that during the storage, there was an increase in the *p*-anisidine value in all the samples. Control and PO+BHT_{200ppm} have exhibited similar ($p>0.05$) *p*-anisidine values, but significantly higher than those of palm olein containing tea leaves extract as antioxidant. This means that secondary oxidation in palm olein samples stabilized with natural antioxidants was significantly retarded compared to the other samples. The activity of the extract seems to be concentration-dependant, because, high concentrated samples have exhibited lowest *p*-anisidine values compared to the less concentrated ones. The highest *p*-anisidine value of control compared to the other samples might be attributed to the absence of antioxidant in that oil. Hence, free radicals formed at the initiation stage of oxidation are not stabilized, leading to the rapid formation of hydroperoxides followed by their fast decomposition into secondary oxidation products, mainly 2-alkenals and 2, 4-dienals. The same phenomenon in OP+BHT_{200ppm} could be the consequence of the thermal instability of BHT, due to its decomposition and volatilization under the effect of heat, leaving the oil unprotected. This result confirm those obtained by Womeni et al. [12] where, BHT was less stable in palm olein at 110℃ than tea leaves methanolic extracts, using the Rancimat test. Similar results have also been obtained by Abd El Ghany et al. [29]. According to Chang et al. [7] and Hamama and Nawar [8], synthetic antioxidants (BHA and BHT) are quite volatile and decompose rapidly at high processing temperature. The low *p*-anisidine value in palm olein samples supplemented with the natural extract might be the consequence of the action and the thermal stability of antioxidant constitutives of this extract. From these results, it is now clearly understood that the lowest peroxide value of PO+BHT_{200ppm} was due to the rapid conversion of peroxides formed into secondary oxidation products, while palm olein containing extracts were relatively stable. The obtained results showing, that tea leaves methanolic extracts can significantly reduce the secondary oxidation products formations in palm olein during storage at frying temperature are in accordance with those of Raza et al. [30] who found methanolic extract of *Althea rosea*, *Chemopodium album*, *Cichorium intybus* and *Fumaria indica* to being efficient in limiting the formation of secondary oxidation products in sunflower oil during storage at frying temperature for 60 min.

3.3 TOTOX Value

Changes in TOTOX value of oil samples during the storage at frying temperature is presented in Table 1. Total oxidation was increasing with the storage time. PO+BHT_{200ppm} and Control have exhibited a significantly higher ($p<0.05$) TOTOX values than oil samples supplemented with natural antioxidants. The protective effect of the extract was concentration-dependent. It is observed that the total oxidation was decreasing with the rise of the concentration of extract. The lowest oxidation state of palm olein samples stabilized with the extract of *Camellia sinensis* might be attributed to the presence in antioxidant compounds in the later. In many, studies, *Camellia sinensis* extracts have been proven to being rich in polyphenols, which are known to being responsible of the major antioxidant activity of plant extracts [10,31]. The efficiency of this

extract in limiting oxidation of vegetable oils has also already been reported [10,11]. The highest oxidation state of Control could be due to the absence of antioxidants. in this sample However, that of PO+BHT_{200ppm} might be attributed to the thermal instability of BHT at elevated temperature. The thermal instability of BHT at high temperature has already been mentioned by Chang et al. [7]. Our natural plant extract might contain antioxidants with a good thermal stability than BHT. This observation is supported by the finding of Iqbal and Bhanger [32] who showed that the methanolic extracts of garlic is stable at 185℃ for 80 min while, BHA losse half of its antioxidant activity after 45 min at the same temperature. Our results are in agreement with those of Che Man and Tan [27] and Raza et al. [30] who showed some plants extracts to being able to limit oil oxidation during frying of potatoe chips and storage of oil samples at frying temperatures respectively.

3.4 Thiobarbituric Acid Value

The TBA test has been widely used as an objective measure of secondary oxidation products of oils. It relates to the level of malondialdehyde formed during oxidation of lipids [32]. It was assumed that accumulation of these products during the storage of oil affects its quality and is responsible for development of rancid odors and off-flavor of the oil. Changes in TBA value of palm olein samples supplemented with antioxidants in comparison to the control are illustrated in Table 2. TBA value of all the samples was increasing during the storage at frying temperature. As previously observed with the *p*-anisidine value, Control and PO+BHT_{200ppm} have exhibited the highest secondary oxidation than oil samples containing natural antioxidants. From days 0 to 4, the TBA values of oil samples enriched with natural antioxidant were stable and similar ($p<0.05$). After the fourth day, there was a significant increase in TBA value of all the treatments. This increment was less in high concentrated oil samples. These results are in agreement with those of *p*-anisidine value, because even there, Control and PO+BHT_{200ppm} have exhibited the highest secondary oxidation state than oil containing Tea leaves extract. This means that malondialdehyde is rapidly formed in these oil samples than the other ones. This could also be due to the absence of antioxidants in control and to the thermal instability of BHT at high temperature. So, oils become unprotected and their oxidation rate increase. The lowest TBA values of oils enriched with *Camellia sinensis* extract confirm the good preservative effect of tea extract and its good thermal stability, as shown by Chen et al. [11]. This extract, at all concentrations, has significantly reduced the formation of malondialdehyde in palm olein. Polyphenolic compounds present in this extract might be responsible of the observed effect. This result is in accordance with the finding of Che Man and Tan [27], who have reported that the extract of oleoresin rosemary and sage were more efficient than BHT and BHA in limiting malondialdehyde formation in palm olein during frying of potatoe chips.

3.5 Iodine Value

The iodine values of stabilized and control palm olein samples over an incubation period of 6 days at 180℃ is shown in Table 2. It is observed that the iodine value decrease gradually during the storage. The rate of decrement in control was higher than that of oil samples containing natural or synthetic antioxidants. Among the oil samples containing the antioxidants, PO+BHT_{200ppm} has exhibited the highest decrement in iodine value. A decrease in iodine value of oils and fats is generally attributed to the destruction of fatty acid double bonds caused by oxidation processes [33]. This suggest unsaturated fatty acids of control and PO+BHT_{200ppm} to being more destroyed by oxidation processes than those of oil samples enriched with tea *Camellia sinensis* extract. The observed effect of the extract could be due to its good stability and efficiency towards the neutralization of free radicals formed in oil during the storage, by a hydrogen donating effect. This could be related to the presence and action of phenolic compounds in the extract. Control was more oxidized, this may be because of the absence of antioxidant, while, the low iodine value of PO+BHT_{200ppm} could be attributed as previously mentioned to the less stability of BHT at high temperature processing as previously mentioned in the litterature by Chang et al. [7]. It appears clear from this that tea leaves extracts is most effective in preventing fatty acid double bonds alteration in palm olein than BHT during a storage of 6 days at frying temperature.Our findings are in accordance with those reported by Che Man and Tan [27]. These authors demonstrated that the extracts of oleoresin rosemary and sage at concentrations 200 ppm significantly limited the decrease in iodine value of palm olein during frying of potatoe chips compare to BHT, BHA and control. However, they were not in agreement with those of Raza et al. [30] who have shown that BHT was

most efficient in preventing deterioration of unsaturated fatty acids of sunflower oils than the extracts of *Althea rosea*, *Chemopodium album*, *Cichorium intybus* and *Fumaria indica* during an accelerated storage of 60 min at frying temperature. The processing time might explain the depictable differences.

3.6 Effects of the Extract on the Fatty Acid Profile of Palm Olein

The fatty acid composition of refined palm olein without additives used in this study is presented in Fig. 1 and the changes in fatty acid composition of palm olein samples are given in Table 3. It was found that there was not important modifications in myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic (C18:1) acids profile during the storage at frying temperature. However, a significant decrease in linoleic acid percent of oil samples was observed (Table 2). At the beginning, the linoleic acid percent of unheated palm olein was 10.55% (Table 2). After 6 days of heating, these values decreased until 9.65, 9.75, 9.84, 9.91, 9.98, 10.05 and 10.10% respectively for PO, PO+BHT_{200ppm}, PO+$Ca.S_{200ppm}$, PO+$Ca.S_{600ppm}$, PO+$Ca.S_{1000ppm}$, PO+$Ca.S_{1400ppm}$ and PO+$Ca.S_{1800ppm}$ (Table 2). This decrement might be the consequence of the transformation of unsaturated fatty acids of oil into primary and secondary oxidation products. From Table 2, It clearly appears that the linoleic acid percent in palm olein samples containing our natural extracts was significantly higher than that of the control and PO+BHT_{200ppm} at the 6^{th} day, showing that linoleic acid was most preserved by our natural extract (at all the concentrations) from the oxidative alterations. The effect of this extract was concentration-dependent. The less variations observed in the percent of myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic (C18:1) acids profile during the storage, might be due to their resistance toward oxidation, because of their high fusion point. The lowest percent in linoleic acid noted in control and PO+BHT_{200ppm} could be the results of the lack of antioxidant in control and the complete loss of the BHT activity at high temperature.These results are in accordance with those found by Che man and Tan [27], who have reported similar decrement in linoleic acid of palm olein containing oleoresin rosemary and sage extracts as natural antioxidants during frying of potatoe chips. They also showed that these natural extracts were most efficient in retarding linoleic acid oxidation destruction than BHA at the last frying day.

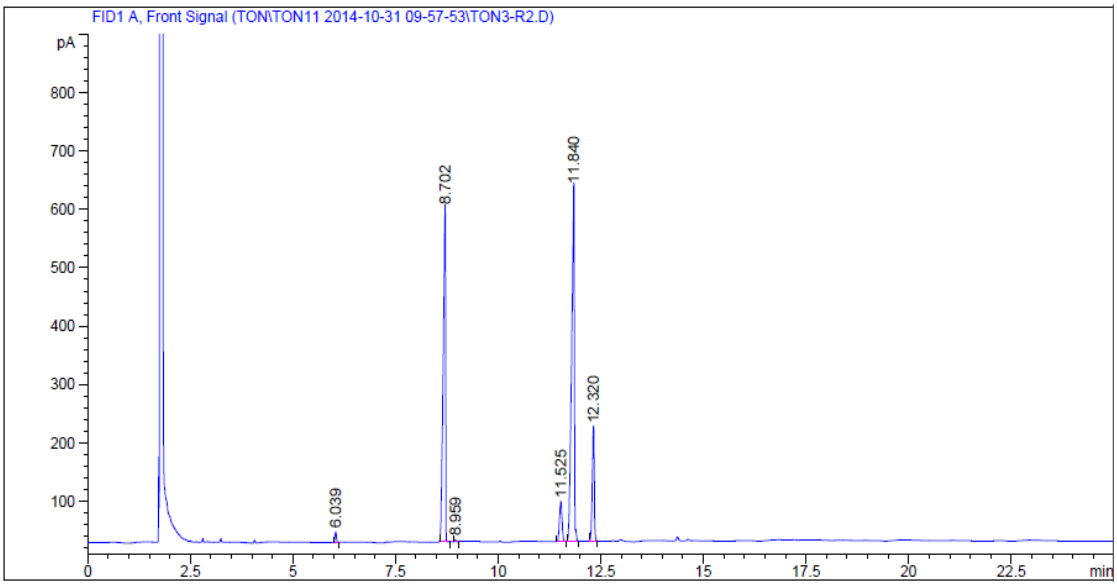

Fig. 1. Gas chromatography-flame ionization detection chromatogram of the fatty acid composition of fresh refined palm olein [RT(retention time)=6.030: Myristic acid (C14:0) (0.75%); RT=8.702: Palmitic acid (C16:0) (37.65%); RT=8.959: Palmitoleic acid (C16:1) (0.15%); RT=11.525: Stearic acid (4.78) (4.75%); RT=11.840: Oleic acid (18:1) (46.09%); and RT=12.320: Linoleic acid (C18:2) (10.55%)]

Table 1. Changes in peroxide (PV), *p*-anisidine (*p*-An) and TOTOX values during 6 days storage at 180 ℃

Characteristic	Day	Control	PO+BHT$_{200ppm}$	PO+Ca.S$_{200ppm}$	PO+Ca.S$_{600ppm}$	PO+Ca.S$_{1000ppm}$	PO+Ca.S$_{1400ppm}$	PO+Ca.S$_{1800ppm}$
PV (meq O_2/kg)	0	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$	$2.67\pm0.10^{a}{}_{A}$
	2	$5.74\pm0.43^{a}{}_{B}$	$3.88\pm0.35^{b}{}_{B}$	$5.87\pm0.44^{a}{}_{B}$	$5.75\pm0.68^{a}{}_{B}$	$5.63\pm0.53^{a}{}_{B}$	$5.21\pm0.31^{a}{}_{B}$	$4.11\pm0.00^{b}{}_{B}$
	4	$7.67\pm0.32^{a}{}_{C}$	$4.32\pm0.21^{b}{}_{B}$	$8.62\pm0.05^{a}{}_{C}$	$8.51\pm0.93^{a}{}_{C}$	$8.22\pm0.51^{a}{}_{C}$	$8.19\pm0.06^{a}{}_{C}$	$7.47\pm0.05^{a}{}_{C}$
	6	$6.08\pm0.11^{a}{}_{B}$	$6.55\pm0.03^{a}{}_{C}$	$11.12\pm0.21^{b}{}_{D}$	$10.29\pm0.27^{c}{}_{C}$	$10.58\pm0.03^{bc}{}_{D}$	$9.38\pm0.21^{d}{}_{D}$	$8.59\pm0.46^{e}{}_{D}$
p-An value	0	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$	$0.68\pm0.00^{a}{}_{A}$
	2	$93.48\pm0.20^{a}{}_{B}$	$92.51\pm0.95^{a}{}_{B}$	$49.53\pm1.28^{b}{}_{B}$	$39.26\pm0.00^{c}{}_{B}$	$29.13\pm0.00^{d}{}_{B}$	$24.90\pm0.29^{e}{}_{B}$	$33.02\pm0.28^{f}{}_{B}$
	4	$196.12\pm0.00^{a}{}_{C}$	$177.47\pm0.44^{b}{}_{C}$	$113.24\pm0.00^{c}{}_{C}$	$80.37\pm1.17^{d}{}_{C}$	$72.79\pm0.00^{e}{}_{C}$	$70.48\pm1.99^{e}{}_{C}$	$77.70\pm1.54^{d}{}_{C}$
	6	$206.68\pm0.00^{a}{}_{D}$	$200.48\pm0.48^{b}{}_{D}$	$151.38\pm3.31^{c}{}_{D}$	$143.18\pm3.13^{d}{}_{D}$	$124.05\pm2.58^{e}{}_{D}$	$105.32\pm2.86^{f}{}_{D}$	$102.99\pm1.10^{f}{}_{D}$
TOTOX value	0	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$	$6.02\pm0.21^{a}{}_{A}$
	2	$104.98\pm1.08^{a}{}_{B}$	$100.29\pm1.67^{a}{}_{B}$	$61.28\pm1.30^{b}{}_{B}$	$50.76\pm1.07^{c}{}_{B}$	$40.40\pm0.88^{d}{}_{B}$	$35.33\pm0.93^{e}{}_{B}$	$41.26\pm1.64^{d}{}_{B}$
	4	$211.47\pm0.64^{a}{}_{C}$	$186.11\pm0.87^{b}{}_{C}$	$130.49\pm1.03^{c}{}_{C}$	$97.40\pm1.28^{d}{}_{C}$	$89.23\pm0.12^{e}{}_{C}$	$86.87\pm2.10^{e}{}_{C}$	$92.65\pm3.40^{de}{}_{C}$
	6	$218.84\pm0.23^{a}{}_{D}$	$213.59\pm0.55^{a}{}_{D}$	$173.62\pm3.37^{b}{}_{D}$	$163.78\pm3.68^{b}{}_{D}$	$145.23\pm2.66^{c}{}_{D}$	$124.80\pm3.28^{d}{}_{D}$	$120.18\pm2.04^{d}{}_{D}$

Data are presented as mean (± SD) (n = 3) [(a-f)] Means within each row for each parameter with different superscripts are significantly (p<0.05) different. (A-D) Means within each column for each parameter with different superscripts are significantly (p<0.05) different. (Control: Palm olein without antioxidant; PO+BHT 200ppm: palm olein containing BHT as antioxidant at concentration of 200 ppm; PO+Ca.S_{200}: palm olein supplemented with the extract at concentration of 200 ppm)

Table 2. Changes in thiobarbituric acid (TBA), iodine values (IV) and linoleic acid (LA) content of RBD palm olein during 6 days storage at 180 ℃

Characteristic	Day	Control	PO+BHT$_{200ppm}$	PO+Ca.S$_{200ppm}$	PO+Ca.S$_{600ppm}$	PO+Ca.S$_{1000ppm}$	PO+Ca.S$_{1400ppm}$	PO+Ca.S$_{1800ppm}$
TBA value (ppm)	0	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$	$0.81\pm0.01^{a}{}_{A}$
	2	$1.19\pm0.00^{a}{}_{B}$	$1.02\pm0.08^{ab}{}_{B}$	$0.94\pm0.05^{b}{}_{A}$	$0.93\pm0.13^{b}{}_{A}$	$0.84\pm0.17^{b}{}_{A}$	$0.88\pm0.04^{b}{}_{A}$	$0.90\pm0.15^{b}{}_{AB}$
	4	$1.50\pm0.11^{a}{}_{C}$	$1.43\pm0.00^{a}{}_{C}$	$0.97\pm0.00^{b}{}_{AB}$	$1.06\pm0.02^{b}{}_{AB}$	$1.00\pm0.10^{b}{}_{A}$	$0.90\pm0.05^{b}{}_{A}$	$0.92\pm0.13^{b}{}_{AB}$
	6	$2.47\pm0.03^{a}{}_{D}$	$2.00\pm0.03^{b}{}_{D}$	$1.77\pm0.12^{c}{}_{C}$	$1.53\pm0.04^{d}{}_{C}$	$1.40\pm0.07^{d}{}_{B}$	$1.28\pm0.06^{e}{}_{B}$	$1.04\pm0.07^{f}{}_{B}$
Iodine Value (g I_2/100 g)	0	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$	$58.06\pm0.01^{a}{}_{A}$
	2	$57.73\pm0.05^{a}{}_{A}$	$57.73\pm0.02^{a}{}_{A}$	$57.69\pm0.04^{a}{}_{B}$	$57.69\pm0.13^{ab}{}_{B}$	$57.92\pm0.09^{b}{}_{A}$	$58.00\pm 0.00^{b}{}_{A}$	$57.91\pm0.03^{b}{}_{AB}$
	4	$57.21\pm0.05^{a}{}_{B}$	$57.39\pm0.03^{b}{}_{B}$	$57.57\pm0.00^{c}{}_{B}$	$57.60\pm0.07^{c}{}_{B}$	$57.64\pm0.11^{c}{}_{B}$	$57.87\pm0.04^{d}{}_{A}$	$57.84\pm0.05^{d}{}_{B}$
	6	$56.67\pm0.06^{a}{}_{C}$	$56.97\pm0.06^{b}{}_{C}$	$57.04\pm0.04^{b}{}_{C}$	$57.08\pm0.03^{b}{}_{C}$	$57.25\pm0.09^{c}{}_{C}$	$57.56\pm0.07^{d}{}_{B}$	$57.22\pm0.11^{c}{}_{C}$
Linoleic acid profile (%)	0	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$	$10.55\pm0.01^{a}{}_{A}$
	2	$10.24\pm0.01^{a}{}_{B}$	$10.29\pm0.04^{a}{}_{B}$	$10.31\pm0.05^{a}{}_{B}$	$10.33\pm0.08^{a}{}_{B}$	$10.44\pm0.04^{b}{}_{B}$	$10.50\pm0.03^{b}{}_{A}$	$10.35\pm0.11^{ab}{}_{B}$
	4	$9.94\pm0.08^{a}{}_{C}$	$9.99\pm0.00^{a}{}_{C}$	$10.21\pm0.10^{b}{}_{B}$	$10.14\pm0.06^{b}{}_{C}$	$10.19\pm0.09^{b}{}_{C}$	$10.37\pm0.02^{c}{}_{B}$	$10.28\pm0.08^{bc}{}_{B}$
	6	$9.65\pm0.06^{a}{}_{D}$	$9.75\pm0.04^{ab}{}_{D}$	$9.84\pm0.06^{b}{}_{C}$	$9.91\pm0.00^{bc}{}_{D}$	$9.98\pm0.01^{c}{}_{D}$	$10.05\pm0.04^{c}{}_{C}$	$10.10\pm0.06^{c}{}_{C}$

Data are presented as mean (± SD) (n = 3) [(a-d)] Means within each row for the same parameter with different superscripts are significantly (p<0.05) different. (A-D) Means within each column for the same parameter with different superscripts are significantly (p<0.05) different. (Control: Palm olein without antioxidant; PO+BHT 200ppm: palm olein containing BHT as antioxidant at concentration of 200 ppm; PO+Ca.S_{200}: palm olein supplemented with the extract at concentration of 200 ppm)

Table 3. Fatty acid profile of palm olein supplemented with tea leaves extract (*Camellia sinensis*) during the storage at frying temperature

Storage time (Days)	Fatty acid composition (wt%)						
	Palm olein sample	**C14 :0**	**C16 :0**	**C16:1**	**C18 :0**	**C18 :1**	**C18 :2**
0	PO (Control)	0.75±0.03	37.65±0.06	0.15±0.00	4.78± 0.03	46.09±0.02	10.55±0.01
	PO (Control)	0.73±0.00^{a}	37.81±0.00^{a}	0.14±0.00^{a}	4.71±0.07^{a}	46.34±0.08^{a}	10.24±0.01^{a}
	PO + BHT $_{200}$	0.76±0.04^{a}	37.78±0.05^{a}	0.16±0.02^{a}	4.77±0.03^{a}	46.21±0.08^{a}	10.29±0.04^{a}
	PO + *Ca.S* $_{200}$	0.72±0.02^{a}	37.89±0.00^{a}	0.14±0.00^{a}	4.79±0.02^{a}	46.12±0.05^{b}	10.31±0.05^{a}
2	PO + *Ca.S* $_{600}$	0.74±0.02^{a}	37.82±0.07^{a}	0.34±0.33^{a}	4.86±0.11^{a}	45.87±0.03^{c}	10.33±0.08^{a}
	PO + *Ca.S* $_{1000}$	0.72±0.00^{a}	37.70±0.01^{a}	0.14±0.00^{a}	4.81±0.09^{a}	46.14±0.02^{b}	10.44±0.04^{a}
	PO + *Ca.S* $_{1400}$	0.75±0.04^{a}	37.69±0.04^{a}	0.15±0.01^{a}	4.76±0.04^{a}	46.12±0.08^{b}	10.50±0.03^{b}
	PO + *Ca.S* $_{1800}$	0.72±0.01^{a}	37.66±0.23^{a}	0.56±0.35^{a}	4.90±0.21^{a}	45.78±0.20^{c}	10.35±0.11^{a}
	PO (Control)	0.75±0.02^{a}	37.97±0.09^{a}	0.24±0.01^{a}	4.84±0.09^{a}	46.23±0.10^{a}	9.94±0.08^{a}
	PO + BHT $_{200}$	0.74±0.02^{a}	37.88±0.03^{a}	0.23±0.04^{ab}	4.78±0.00^{a}	46.34±0.02^{a}	9.99±0.00^{a}
	PO + *Ca.S* $_{200}$	0.78±0.08^{a}	37.84±0.03^{a}	0.16±0.08^{b}	4.79±0.04^{a}	46.19±0.11^{a}	10.21±0.10^{b}
4	PO + *Ca.S* $_{600}$	0.72±0.02^{a}	37.81±0.00^{b}	0.16±0.02^{b}	4.77±0.03^{a}	46.37±0.07^{a}	10.14±0.06^{b}
	PO + *Ca.S* $_{1000}$	0.72±0.01^{a}	37.81±0.02^{b}	0.15±0.00^{b}	4.77±0.02^{a}	46.77±0.02^{b}	10.19±0.09^{b}
	PO + *Ca.S* $_{1400}$	0.73±0.00^{a}	37.68±0.03^{c}	0.13±0.01^{b}	4.81±0.09^{a}	46.25±0.07^{a}	10.37±0.02^{c}
	PO + *Ca.S* $_{1800}$	0.80±0.10^{a}	37.54±0.11^{c}	0.69±0.17^{c}	4.81±0.04^{a}	45.84±0.29^{c}	10.28±0.08^{d}
	PO (Control)	0.74±0.00^{a}	38.31±0.06^{a}	0.14±0.01^{a}	4.84±0.05^{a}	46.28±0.04^{a}	9.65±0.06^{a}
	PO + BHT $_{200}$	0.73±0.00^{ab}	38.05±0.05^{b}	0.17±0.03^{a}	4.86±0.09^{a}	46.40±0.03^{b}	9.75±0.04^{b}
	PO + *Ca.S* $_{200}$	0.75±0.01^{ac}	38.46±0.05^{a}	0.14±0.02^{a}	4.89±0.05^{a}	45.89±0.05^{c}	9.84±0.06^{c}
6	PO + *Ca.S* $_{600}$	0.72±0.01^{b}	38.10±0.03^{b}	0.22±0.10^{a}	4.87±0.08^{a}	46.15±0.06^{a}	9.91±0.00^{c}
	PO + *Ca.S* $_{1000}$	0.76±0.01^{c}	38.05±0.07^{b}	0.24±0.05^{b}	4.75±0.06^{a}	46.19±0.20^{a}	9.98±0.01^{d}
	PO + *Ca.S* $_{1400}$	0.74±0.00^{a}	37.82±0.02^{c}	0.41±0.06^{c}	4.73±0.10^{a}	46.22±0.11^{a}	10.05±0.04^{df}
	PO + *Ca.S* $_{1800}$	0.75±0.00^{ac}	38.06±0.04^{b}	0.14±0.00^{a}	4.90±0.12^{a}	46.02±0.00^{c}	10.10±0.06^{f}

$^{(a-f)}$ *Means within each column, for each day with different superscripts are significantly ($p < 0.05$) different [PO (Control): Palm olein without antioxidant; PO+BHT 200ppm: palm olein containing BHT as antioxidant at concentration of 200 ppm; PO+Ca.S$_{200}$: palm olein supplemented with the extract at concentration of 200ppm…]*

4. CONCLUSION

It could be concluded from the results of the present study that the methanolic extract of *Camellia sinensis* at concentrations 200, 600, 1000, 1400 and 1800ppm is a powerful natural antioxidant for improving the oxidative stability of palm olein when subjected at frying temperature. This extract was more efficient and more stable at high temperature than BHT. The order of stability of palm olein samples during the storage was PO+Ca.$S_{1800ppm}$ > PO+Ca.$S_{1400ppm}$ > PO+Ca.$S_{1000ppm}$ > PO+Ca.S_{600ppm} > PO+Ca.S_{200ppm}> PO+BHT_{200ppm} = Control.This suggests that tea leaves might be explored as a viable source of potent antioxidants for the protection of palm olein from oxidation at high processing temperature.

ACKNOWLEDGEMENTS

The authors would like to thank the TWAS-CSIR, and the Department of Science and Technology, Government of India, for the financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aladedunye FA, Przybylski R. Degradation and nutritional quality changes of oil during frying. J. Am. Oil Chem. Soc. 2009;86: 149-156.
DOI: 10.1007/s11746-008-1328-5
2. Andrikopoulos NK, Kalogeropoulos N, Falorea A, Barbagianni IMN. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. Int J Food Sci Tech. 2002;37 (2):177-190.
DOI: 10.1046/j.1365-2621.2002.00555.x
3. Cuvelier ME, Maillard MN. Stabilité des huiles alimentaires au cours de leur stockage. OCL. 2012;19(2):125-132.
DOI: https://doi.org/10.1051/ocl.2012.0440
4. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2010;89(3):1-17.
5. Fan HY, Sharifudin MS, Hasmadi M, Chew HM. Frying stability of rice bran oil and palm olein. Int Food Res J. 2013;20(1): 403-407.
6. Hou DX. Potential mechanism of cancer chemoprevention by anthocyanin. Curr. Mol. Med. 2003;3(2):149-159.
7. Chang SS, Ostric-Matijasevic B, Hsieh OAL, Cheng LH. Natural antioxidants from rosemary and sage. J. Food Sci. 1977;42 (4):1102-1106.
DOI: 10.1111/j.1365-2621.1977.tb12676.x
8. Hamama AA, Nawar WW. Thermal decomposition of some phenolic antioxidants. J. Agric. Food Chem. 1991; 39(6):1063-1069.
DOI: 10.1021/jf00006a012
9. Ardabili AS, Farhoosh R, Khodaparast MHK. Frying stability of Canola oil in presence of pumpkin seed and olive oils. Eur J of Lipid Sci Tech. 2010;112(8):871-877.
DOI: 10.1002/ejlt.200900257
10. Navas JA, Tres A, Bou R, Codony A, Guardiola F. Optimization of analytical methods for the assessment of the quality of fats and oils used in continuous deep fat frying. Grasas Aceites. 2007;58(2):154-162.
DOI:http://dx.doi.org/10.3989/gya.2007.v58.i2.80
11. Chen ZY, Chan PT, Ma HM, Fung KP, Wang J. Antioxidative effect of ethanol tea extracts on oxidation of canola oil. J. Am. Oil Chem. Soc. 1996;73(9):375-380.
DOI: 10.1007/s11746-998-0126-4
12. Womeni HM, Tonfack DF, Anjaneyulu B, Karuna MSL, Prasad RBN, Linder M. Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract. NFS Journal. 2016;3():33-40.
DOI:https://doi.org/10.1016/j.nfs.2016.03.002
13. Osawa CC, Gonçalves LAG. Deep-fat frying of meat products in palm olein. Ciênc. Tecnol. Aliment. 2012;32(4):804-811.
DOI:http://dx.doi.org/10.1590/S0101-20612012005000109
14. Iqbal S, Haleem S, Akhtar M, Zia-ul-Haq M, Akbar J. Efficiency of pomegranate peel extracts in stabilization if sunflower oil under accelerated conditions. Food Res Int. 2008;41:194-200.
DOI: doi:10.1016/j.foodres.2007.11.005
15. Duh PD, Yen GC. Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. J. Am. Oil Chem. Soc. 1997;74(6):745-748.

16. Mundkinajeddu D, Agarwal A. Residual Methanol in Botanical Dietary Ingredients - Perspectives of a Manufacturer. Herbal E Gram. 2014;11.
17. European Parliament and the Council of the European Union. Directive 2009/32/EC; 2009.
18. Japan External Trade Organization. Specifications and standards for foods, food additives, etc. under the food sanitation act; 2010.
19. Sultana B, Farooq A, Muhammad RA, Shahzad ASC. Antioxydant potential of extracts from different agrowastes: Stabilization of corn oil. Grasas Aceites. 2008;59(3):205-217.
20. International Dairy Federation (IDF). International IDF Standard 74A, Brussels, Belgium; 1991.
21. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990; 186:421-431.
22. American Oil Chemist's Society (AOCS). Official methods and recommended practices of the American Oil Chemist's Society. 5th ed. Methods Cd 8–53, Cd 18–90, Cd 19–90. American Oil Chemist's Society, S Champaign, IL, USA; 2003.
23. Shahidi F, Wanasundara UN. Methods for measuring oxidative stability in edible oils. In Akoh, C.C. and Min D.B. (Eds). Food Lipids: Chemistry, Nutrition and Biotechnology. New York: CRC Press. 2008;387-388.
24. Christie WW. Preparation of ester derivatives of fatty acids for chromatographic analysis in "Advances in Lipid Methodology - Two". (Christie, W.W. ed.) Oily Press: Dundee, Scotland. 1993; 69-111.
25. Mc ginely L. Analysis and quality control for processing and processed fats. In: Analysis of Oilseeds, Fats and Fatty Foods, Rossell JB and Pritchard JLR (Eds.), New York, Elsevier Applied Science. 1991;460–470.
26. Choe E, Min DB. Chemistry of Deep-fat frying oils. J. Food Sci. 2007;(5):R1-R10. DOI: 10.1111/j.1750-3841.2007.00352.x
27. Che Man YB, Tan CP. Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized palm olein during deep-fat frying of potato chips. J. Am. Oil Chem. Soc. 1999;76(3):331-339.
28. Roman O. Mesure et prédiction de la réactivité des lipides au cours du chauffage d'huiles végétales à haute température, Thèse de Doctorat Agro Paris Tech, Institut des Sciences et Industries du Vivant et de l'Environnement, Paris, France. 2012;168.
29. Abd-El Ghany, Ammar MS, Hegazy AE. Use of olive waste cake extract as a natural antioxidant for improving the stability of heated sunflower oil. World Appl Sci J. 2010;11(1):106-113.
30. Raza SA, Rashid A, William J, Razzaq A. Evaluation of oxidative stability of sunflower oil at frying temperature in presence of butylated hydroxytoluene and methanolic extracts of medicinally important plants of Pakistan. Int Food Res J. 2014;21(1):331-334.
31. Izzreen NQMN, Fadzelly MAB. Phytochemicals and Antioxidant properties of different part of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia. Int Food Res J. 2013;20(1): 307-312.
32. Iqbal S, Bhanger MI. Stabilization of sunflower oil by garlic extract during accelerated storage. Food. Chem. 2007; 100:246-254.
33. Tynek M, Hazuka Z, Pawlowicz R, Dudek M. Changes in the frying medium during deep-frying of food rich in proteins and carbohydrates. J. Food Lipids. 2001;8 (4): 251-261. DOI: 10.1111/j.1745-4522.2001.tb00200.x