# Stability of Fried Olive and Sunflower Oils Enriched with *Thymbra capitata* Essential Oil

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**Abstract**

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The stability of olive and sunflower oils for domestic uses after frying cow steak or only heating were evaluated in the presence or absence of the carvacrol-rich essential oil of *Thymbra capitata*. The treatments consisted of sunflower and olive oils either enriched with 200 mg/l of *T. capitata* oil or without it, heating at 180°C for 20 min, or frying 100 g cow steak at the same temperature and for the same period of time. In all assays, acid, peroxide, and *p*-anisidine values were followed over time. The fatty acid profile was estimated before heating or frying as well as at the end of the experiment. The results showed that the type of fat as well as the type of treatment (frying or heating) was determinant for the acid, peroxide, and *p*-anisidine values found. The presence of the essential oil also demonstrated to affect those values depending on the type of the oil as well as on the type of the treatment (frying or heating). In contrast, the fatty acid profile did not change greatly.

**Keywords**: vegetable oils; frying; heating; fatty acids

Frying is a common operation of cooking used for people because it is fast and generally gives rise to food with desirable sensory properties of colour, flavour, texture, and palatability (Dobarganes *et al*. 2000; Yaghmur *et al*. 2001). In frying, the food is completely surrounded by the frying fat or oil. The quality of the products cooked by using deep frying depends on several conditions: temperature of the heated oil, frying time, food weight and shape, frying oil volume, type of the oil, and the kind of food used (Varela 1994).

The positive changes occurring which are responsible for the desirable sensory properties, are generally accompanied by some undesirable modifications of the frying fat or oil. According to Pokorný (1998), five types of the main processes occur during deep fat frying of food products: hydrolytical processes due to the presence of water in the substrate that penetrates into oil; fat migration either from the pan into the substrate or from the substrate into the frying fat; oxidation reactions in both frying fat and in the substrate; pyrolytic and polymerisation reactions.

Zhang *et al*. (2012) reported in a review that the chemical reactions occurring during deep-fat frying roughly involved hydrolysis, oxidation, isomerisation, and polymerisation which resulted in the generation of free fatty acids, low-molecular alcohol, aldehyde, ketone, acid, lactone, and hydrocarbon, diglyceride and monoglyceride, cyclic and epoxy compounds, *trans* isomers, triacylglycerol monomer, dimer, and oligomer.

Oxidation by air oxygen is the most important deterioration reaction of frying oil, which is relatively rapid, particularly in the case of polyunsaturated oils (Pokorný 1998).

Aromatic plants and their essential oils contain components which have been reported as possessing antioxidant properties (Miguel 2010). *Thymbra capitata* essential oil is particularly rich in carvacrol which has been considered as possessing antioxidant properties (Miguel *et al*. 2003). Aromatic plants are traditionally included in many foods during processing.

The current study was conducted to evaluate the peroxidation of olive and sunflower oils and their decomposition into aldehydes in frying cow steak before and after the treatment with the essential oil of *Thymbra capitata*, an aromatic plant largely used in traditional Portuguese food.

## MATERIAL AND METHODS

***Material***. Sunflower and olive oils were acquired in a local supermarket. *Thymbra capitata* essential oil was isolated by hydrodistillation as previously reported (Coe 2007). Both olive and sunflower oils were heated without and with the addition of 200 mg/l of *T. capitata* essential oil. Cow rump steak (± 100 g) was used for frying.

***Conditions of the assay***. Four main assays were performed either for sunflower oil or olive oil: (1) Sunflower or olive oil (1 l) was heated alone, daily for 6 days at 180°C for 20 minutes. Between every new heating, the oil was kept at room temperature in the dark; (2) Sunflower or olive oil (1 l) with 200 mg carvacrol-rich *T. capitata* essential oil was heated in the same conditions as in (1); (3) Sunflower or olive oil (1 l) was heated up to 180°C, then 100 g steak was added for frying for 20 minutes. The oil was submitted to the frying of new similar steaks 6 times in the same conditions as previously; (4) Sunflower or olive oil (1 l) with 200 mg carvacrol-rich *T. capitata* essential oil and frying of steaks in the same conditions as in the previous treatment.

After oil cooling, one aliquot of the fat was taken for evaluating the acidity index, peroxide value, and *p*-anisidine value.

Fatty acid analysis was only performed at two distinct times: before the treatment and at the end of the experiment.

***Index of acidity***. Ten grams of each sample were weighed into an Erlenmeyer flask and 50 ml of ethanol/ethyl ether (1:1, v/v) mixture were added. The resulting mixture was titrated with 0.1M potassium hydroxide (KOH), using 1 ml of phenolphthalein as indicator, until obtaining a faint permanent pink colour persisting for 30 second. The index of acidity before any treatment was: 1.5 and 0.2 mg KOH/g olive or sunflower oil, respectively.

***Peroxide value***. A separate mixture of each sample and chloroform/acetic acid (3:2) (v/v) was left to react in the dark with saturated potassium iodide solution. The free iodine was titrated with a sodium thiosulfate solution. The peroxide value was determined in milliequivalents of active oxygen per kilogram of oil (mEq $O_2$/kg), according to the following formula: $Ip = 10(n_1 - n_2)/m$, where: $n_1$ – ml of sodium thiosulfate consumed in the sample titration, $n_2$ – ml of sodium thiosulphate consumed in blank titration, and m being the amount of the sample in g (AOCS 1989). The peroxide value before any treatment was 19.3 and 10.3 mlEq $O_2$/kg olive or sunflower oil, respectively.

***p-Anisidine value***. *p*-Anisidine value was determined according to the NP-1819 (1984) method. Briefly, it was carried out by the determination of the absorbance increase, measured at 350 nm, of the solution of 0.5 g of each sample (m) in *iso*-octane (25 ml), before ($A_1$) and after ($A_2$) the reaction with *p*-anisidine in glacial acetic acid in the dark. The *p*-anisidine value was determined using the following formula: $25\,(1.2A_2 - A_1)/m$. The *p*-anisidine value before the treatments was 16.0 and 5.3, respectively.

***Fatty acid analysis***. Fatty acid methyl esters for GC analyses were obtained using methanolic hydrogen chloride. Unsaturated fatty acids double-bond positions were determined by GC-MS mass spectra of their dimethyl disulphide adducts. The samples containing the fatty acid methyl esters were dissolved in 0.2 ml of dimethyl disulfide and 0.05 ml of the solution of iodine in diethyl ether (60 mg/ml) was added. After 24 h at room temperature, the mixture was extracted three times with 2 ml of *n*-pentane/ether (1:1). The *n*-pentane/ether fraction was washed with 5% sodium thiosulphate solution and evaporated to dryness. The product was dissolved in *n*-pentane.

*Gas chromatography*: GC analyses were performed using a twin FID Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, USA), a data handling system and a vaporising injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm *i.d.*, film thickness 0.25 mm) and a DB-17HT fused-silica column (30 m × 0.25 mm *i.d.*, film thickness 0.15 μm). The oven temperature was programmed for 170–270°C at 5°C/min and then held isothermally for 5 min; injector and detector temperatures were 300°C; carrier gas, $H_2$ at a flow of 30 cm/second. The samples were injected using the split-sampling technique with a ratio of 1:50. Percentage composition of

the samples was computed using the normalisation method from the GC peak areas without correction factors. The data shown are mean values of two injections of each sample.

*Gas Chromatography-Mass Spectrometry*: The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with a DB-1 fused-silica column (30 m × 0.25 mm *i.d.*, film thickness 0.25 mm) and interfaced with a Perkin-Elmer Turbomass mass spectrometer (Software version 4.1). The injector and oven temperature were as described above; transfer line temperature 280°C; ion source 220°C; carrier gas He at a flow of 30 cm/s; split ratio 1:40; ionisation energy 70 eV; ionisation current 60 μA; scan range 40–300 u; and scan time 1 second. The identity of the components was assigned by GC-MS data with the corresponding data of NIST and of Wiley mass spectral libraries and of laboratory-synthesised components and commercially available FAME standards from a lab-made library.

## RESULTS AND DISCUSSION

The presence of moisture during deep frying at relatively high temperatures due to the introduction of food (vegetables, fish, meat) induces hydrolysis of esters bonds in triacylglycerols with the consequent formation of free fatty acids, mono-, di-acylglycerols, and glycerols as well as the acceleration of the oxidation processes with the consequent rancid flavours and worsened organoleptic characteristics of the fried food (Pokorný 1998). This is not desirable and several approaches have been made trying to prevent these undesirable properties which may not only be unpleasant for the palate but also injurious to health because they destroy vitamins, inhibit enzymes, and can be potentially responsible for gastrointestinal irritations and mutations (Ng *et al*. 2007). One of these approaches for increasing the oil stability includes the addition of antioxidants such as tocopherols or tocotrienols, and synthetic antioxidants among other compounds (Tabee 2008).

A number of methods have been followed to test the quality of frying oils, including peroxide value, *p*-anisidine value, and free fatty acids, among others (Tabee 2008).

***Index of acidity***. The index of acidity is used as a chemical marker for monitoring the quality of frying operations and is often used for the assessment of frying oils suitability for human consumption. According to Matthaus (2006), the value of 2% is defined as the limit for oil rejection.

Olive and sunflower oils inidices of acidity before any treatment were 1.5 and 0.2 mg KOH/g, respectively. The acid index was superior in the olive oil to that in the sunflower oil, even at the beginning of the experiment (Figure 1). The addition of the essential oil to sunflower oil not used for meat processing was determinant on the lower acid index values detected during the first two days. Nevertheless, from this date on the value substantially increased, becoming higher than that in the absence of essential oil (Figure 1b). On the fifth and sixth days of heating, both samples with essential oil (with and without meat) showed significantly higher acidity index values when compared to the other treatments. A gradual increase of acidity was observed in sunflower oil without essential oil and submitted to heating as well as in the sample fried with cow steak plus essential oil (Figure 1b).

In olive oil (Figure 1a), differences were also registered between the treatments and along the assay, nevertheless not as evident as those observed for sunflower oil. Generally, heating the olive oil in the absence of essential oil induced the lowest values of acidity index while differences between the beginning and the end of the assay were not as evident as those observed for sunflower oil. Similarly, as reported for sunflower oil, at the end of the experiment olive oil with *T. capitata* essential oil alone and cow steak fried in just olive oil presented relatively higher indices of acidity than the other samples (Figure 1a).

Triacylglycerols' hydrolysis of frying oil is the most important reaction during the deep fat frying. It is affected by water present in the substrate, which is immediately heated to the boiling point, and the steam produced enters the hot oil. In such conditions, triacylglycerols are partially hydrolysed into free fatty acids and partial glycerol esters in a short period of time (Pokorný 1998). In our work, this was confirmed; nevertheless, the type of fat seems to be determinant in the results. Such data corroborate those already reported by some authors (Ndjouenkeu & Ngassouum 2002) obtained by submitting diverse types of vegetables to deep-frying. Nevertheless, the index values found in the work never exceeded the value in percentage (2%) of the main fatty acids of the oil, reported as the limit for oil rejection (Matthaus 2006).

***Peroxide and p-anisidine values***. Oxidation by air oxygen is the most important deterioration reaction of frying oil, which is relatively rapid, particularly in the case of polyunsaturated oils. The values of peroxide (primary step of lipid oxidation) and *p*-anisidine (secondary step of lipid oxidation) were also followed (Figures 2 and 3).

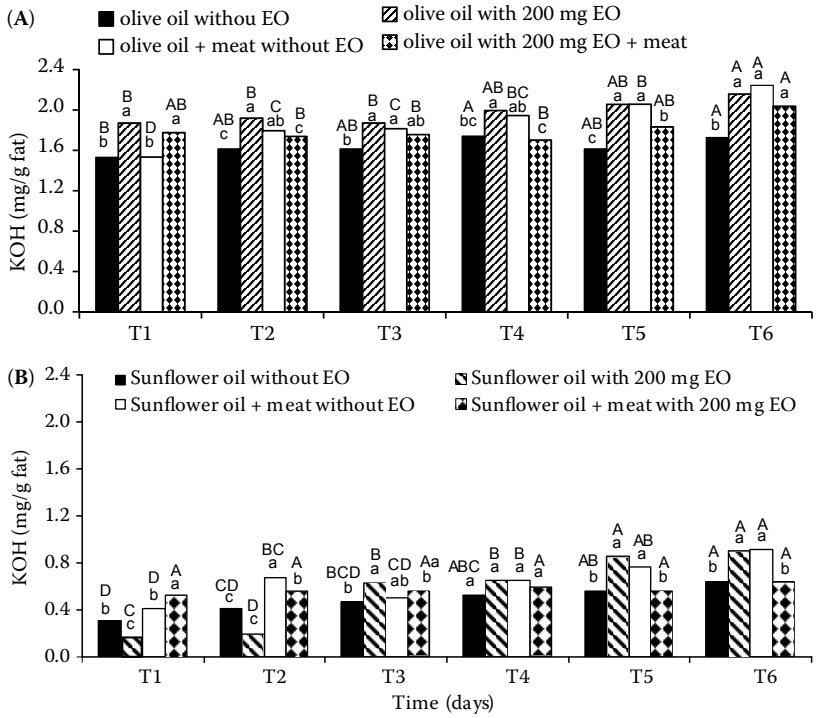


Figure 1. Acid index of olive (**A**) and sunflower (**B**) oils after heating or frying in the presence of ± 100 g cow steak for 20 min at 180°C, everyday, and for 6 days. In both treatments, one sample had essential oil (EO) (200 mg/l) and in the other had no EO

Values in the same day followed by the same lower case letter and in the same treatment followed by the same upper case letter are not significantly different by Tukey's test ($P < 0.05$)

Generally, the peroxide values decreased over time (Figure 2). Peroxide values on day 1 of the assay were substantially higher when compared to the remaining days (Figure 2) and practically around or slightly superior to the maximum allowed by EEC Regulation (1991). On the first day and for sunflower oil, frying cow meat triggered the formation of hydroperoxides more extensively than oil alone and more in oil with *T. capitata* essential oil addition. On the last sampling date, cow meat fried in sunflower oil plus essential oil had the lowest peroxide value, in contrast to that registered at the beginning (Figure 2b). Sunflower oil plus essential oil heating alone induced a higher accumulation of hydroperoxides than the remaining treatments, particularly in the last three days of the experiment. In olive oil samples, these differences were not so evident despite the decrease of the peroxides over time (Figure 2a). This diminution of the peroxides did not mean that heating or frying prevented the primary oxidation because, when *p*-anisidine was evaluated in our assay, it was possible to record the increase of these values over time, while that of peroxide diminished (Figure 3). In fact, the peroxide value is related to the hydroperoxides, the primary oxidation products, which are unstable under heating or deep-fat frying conditions and readily decompose into mixtures of mainly volatile aldehyde compounds (Vieira & Regitano D'arce 1999; Yaghmur *et al.* 2001; Farhoosh & Moosavi 2009).

The *p*-anisidine value, despite it being more empiric determination, is considered to be well correlated with the level of secondary oxidation products, for example aldehydes which are more stable than hydroperoxides (Casal *et al.* 2010).

Sunflower oil in the absence of essential oil and only submitted to the heating presented an increase of *p*-anisidine value over time particularly remarkably (Figure 3b), in contrast to that observed for olive oil samples in which a significant increase of that value was registered only in the samples in which cow steak had been fried, independently on the presence of the essential oil (Figure 3a).

It is noteworthy to refer that the lowest peroxide value observed in sunflower oil with meat and essential oil at the end of the experiment did not correspond to the highest value of *p*-anisidine (Figures 2b and 3b). Such results may be partly explained by the possible reaction of both peroxy radicals and hydroperoxides with the cow steak, particularly thiol, sulphide, disulphide, and primary amine groups of proteins, being deactivated and partially attached to the protein moiety (Pokorný 1998).

Bensmira *et al.* (2007) reported on a decrease of peroxide value in sunflower oil treated with the aerial parts of thyme when submitted to heating. Such results were attributed to the aromatic chemical composition of the plant, in which thymol dominated. In our case, the essential oil predominantly constituted by carvacrol, an isomer of thymol, also

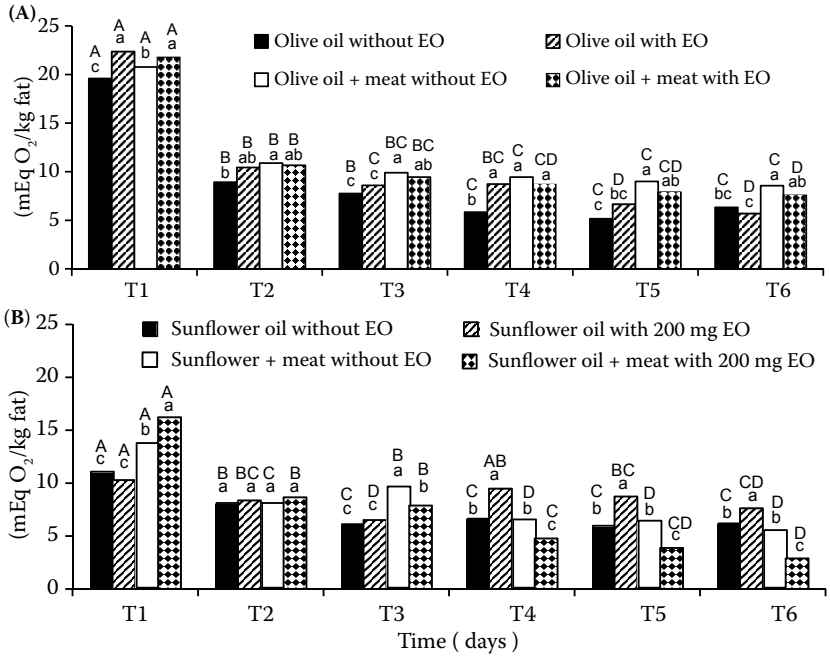


Figure 2. Peroxide values (mEq $O_2$/kg fat) of olive (**A**) and sunflower (**B**) oils after heating or frying in the presence of ± 100 g cow steak for 20 min at 180°C, everyday, and for 6 days. In both treatments, one sample had essential oil (EO) (200 mg/l) and in the other had no EO

Values in the same day followed by the same lower case letter and in the same treatment followed by the same upper case letter are not significantly different by Tukey's test ($P < 0.05$)

possessed antioxidant activity, but on the level of preventing the formation of products resulting from the degradation of peroxides.

In contrast to the sunflower oil, frying the cow steak in olive oil had negative effects on its quality, because a significantly higher accumulation of aldehydes could be detected. Almost the same tendency could be observed for the peroxide value (Figure 2a), at least during the two last days of frying. Nevertheless, the presence of the essential oil prevented the formation of the secondary products of oxidation when compared to the samples without the essential oil also submitted to the meat frying (Figure 3a). The highest peroxide and *p*-anisidine values found for the olive oils in which meat was fried, may indicate that the formation of secondary stable oxidation products occurs at similar rates in these samples as also reported by Casal *et al.* (2010) when they compared this fat with other ones.

***Fatty acids***. The highest *p*-anisidine value found for olive oil, independent on the treatment, was inferior to the highest value found for sunflower oil, revealing a greater stability of that oil when compared to the sunflower oil. This may be due to the triacyglycerols that constitute the fat. In fact, the fatty acid profiles of both fats were different as expected (Table 1). In the sunflower oil, the ratios of polyunsaturated

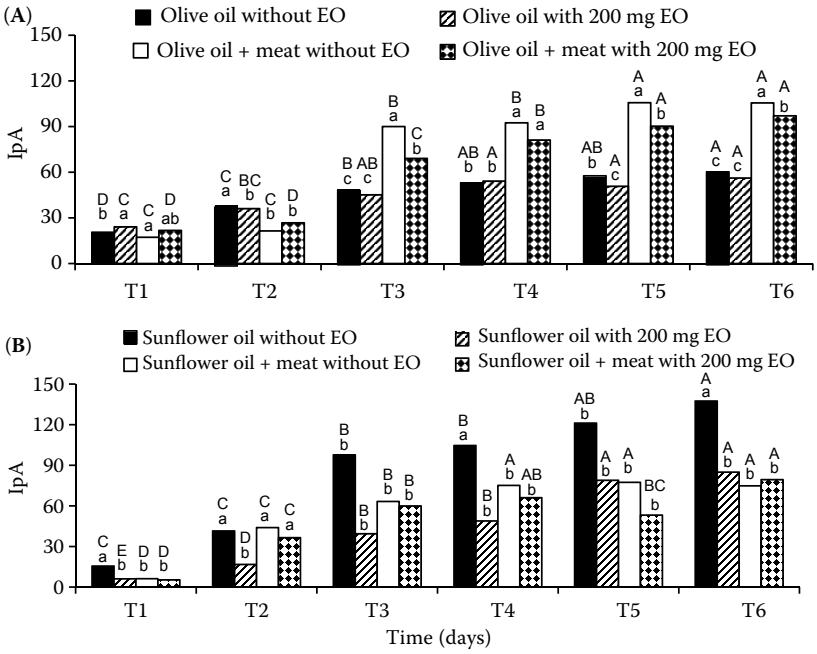


Figure 3. *p*-Anisidine values of olive (**A**) and sunflower (**B**) oils after heating or frying in the presence of ± 100 g cow steak for 20 min at 180°C, everyday, and for 6 days. In both treatments, one sample had essential oil (EO) (200 mg/l) and in the other had no EO

Values in the same day followed by the same lower case letter and in the same treatment followed by the same upper case letter are not significantly different by Tukey's test ($P < 0.05$).

Table 1. Fatty acid composition and their ratios in olive and sunflower oils, before (Day 1) and after heating and after deep-frying cow steaks at the end of the experiment (Day 6), in the presence or the absence of *Thymbra capitata* essential oil (EO)

| Fatty acid (%) | Sunflower oil | | Sunflower oil + EO | | Olive oil | | Olive oil + EO | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 6 | Day 1 | Day 6 | Day 1 | Day 6 | Day 1 | Day 6 |
| **After heating** | | | | | | | | |
| Palmitoleic | 0.15 | 0.12 | 0.14 | 0.16 | 0.69 | 0.69 | 0.73 | 0.73 |
| Palmitic | 7.20 | 7.67 | 6.92 | 7.03 | 11.48 | 11.08 | 10.94 | 11.35 |
| Linoleic | 54.47 | 52.53 | 52.50 | 52.78 | 5.13 | 4.73 | 5.38 | 5.09 |
| Oleic | 28.39 | 29.51 | 32.75 | 31.79 | 77.12 | 76.36 | 76.75 | 75.08 |
| Linolenic | 1.30 | 1.46 | 1.34 | 1.47 | 2.05 | 1.97 | 1.86 | 2.11 |
| Stearic | 3.86 | 4.12 | 3.36 | 3.53 | 2.66 | 2.89 | 2.79 | 3.15 |
| Ratio | | | | | | | | |
| Sat/mono[a] | 0.39 | 0.40 | 0.31 | 0.33 | 0.18 | 0.18 | 0.18 | 0.19 |
| Poly/mono | 1.95 | 1.82 | 1.64 | 1.70 | 0.09 | 0.09 | 0.09 | 0.09 |
| Poly/sat | 5.04 | 4.58 | 5.24 | 5.14 | 0.51 | 0.48 | 0.53 | 0.50 |
| Oleic/linolenic | 0.52 | 0.56 | 0.62 | 0.60 | 15.03 | 16.14 | 14.27 | 14.75 |
| **After deep-frying cow steaks** | | | | | | | | |
| Palmitoleic | 0.14 | 0.14 | – | 0.18 | 0.77 | 0.78 | 0.74 | 0.67 |
| Palmitic | 6.43 | 7.14 | 6.60 | 6.84 | 11.42 | 11.33 | 11.48 | 10.35 |
| Linoleic | 53.19 | 50.30 | 52.69 | 49.41 | 5.62 | 5.11 | 5.53 | 4.82 |
| Oleic | 32.08 | 32.75 | 33.01 | 34.14 | 73.34 | 76.99 | 75.92 | 75.77 |
| Linolenic | 1.38 | 1.37 | 1.33 | 1.50 | 1.86 | 1.75 | 1.99 | 1.59 |
| Stearic | 3.46 | 3.89 | 3.31 | 3.75 | 3.70 | 3.45 | 2.19 | 3.03 |
| Ratio | | | | | | | | |
| Sat/mono[a] | 0.31 | 0.34 | 0.30 | 0.31 | 0.20 | 0.19 | 0.18 | 0.18 |
| Poly/mono | 1.69 | 1.57 | 1.64 | 1.48 | 0.10 | 0.09 | 0.10 | 0.08 |
| Poly/sat | 5.52 | 4.68 | 5.45 | 4.81 | 0.49 | 0.46 | 0.55 | 0.48 |
| Oleic/linolenic | 0.60 | 0.65 | 0.63 | 0.69 | 13.05 | 15.07 | 13.73 | 15.72 |

[a]ratio saturated/mono-unsaturated fatty acids; [b]ratio poly-unsaturated/mono-unsaturated fatty acids; [c]ratio poly-unsaturated/saturated fatty acids; [d]ration oleic acid/linoleic acid

acids/monounsaturated acids and polyunsaturated acids/saturated acids were about twenty and ten fold higher than the ratios found for olive oil, respectively (Table 1). The predominance of linoleic acid in sunflower oil in contrast to the olive oil in which oleic acid dominated is responsible for the oleic acid/linoleic acid ratio found in these two samples. Therefore, the predominance of polyunsaturated acids in sunflower oil might have been responsible for the highest values of *p*-anisidine found in its samples submitted only to heating, mainly at the end of the experiment. In these samples, the presence of the essential oil revealed to be effective in preventing the formation of aldehydes. Its presence in olive oil samples did not produce any effective results in view of antioxidant activity. The minor resistance of sunflower oil to oxidation partially agrees with that reported by Casal *et al.* (2010). Nevertheless, the presence of the essential oil of *T. capitata* in sunflower oil may prevent the secondary oxidation.

## CONCLUSION

In conclusion, the type of fat used for frying cow meat or simply heated resulted in diverse acidity and oxidation parameters in heating. The presence of the essential oil also induced some variation in the final quality of the vegetable oils studied:

The acid index was higher in the olive oil than in the sunflower oil. In both oils, when they were submitted to heating, the presence of the essential oil increased this parameter, whereas the opposite could be detected when the oils were submitted to deep-frying in the presence of cow steak.

Peroxide value generally decreased over time. At the end of the experiment, the peroxide value was

superior in olive oil after frying cow steak, either in the presence or absence of the essential oil, than in the heated oil, whereas in sunflower oil under the same conditions as those reported for the olive oil, the peroxide value decreased. In this case, the presence of the essential oil of *T. capitata* was determinant for the peroxide value found.

Similarly as for the peroxide value, *p*-anisidine value was also superior in the fried olive oil than in those only submitted to heating. The presence of the essential oil in the fried fat was responsible for the lowest *p*-anisidine value in the fried samples. This was not evident in sunflower oil. The essential oil only prevented the formation of the secondary oxidation products when submitted solely to heating.

The fatty acid profiles of both vegetable oils were different since in olive oil oleic acid predominated, whereas in sunflower oil, linoleic acid dominated and the operations made with both fats did not change the fatty acid profile. Nevertheless, the chemical difference might be partly responsible for the results obtained for the acid index and peroxide and *p*-anisidine values.

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