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Effect of carvacrol on the oxidative stability of palm oil during frying

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SUMMARY: Fats and oils deteriorate physically and chemically at frying temperatures due to several reasons. The objective of this study was to assess the effect of carvacrol on the oxidative stability of palm oil during a repeated frying process. Potatoes were serially fried in carvacrol-added palm oil, BHT-added palm oil and a control oil (without any antioxidants). After each tenth frying cycle, several chemical analyses were carried out on collected samples to evaluate deterioration in the oils. The free fatty acid, *para*-anisidine, iodine, and total polar component values of the fresh oil were 0.080, 2.85, 57.1 and 7.5, respectively. These values changed to 0.165, 11.80, 46.7, 11.0, respectively for the control oil; 0.151, 11.28, 49.2 and 10.5 for BHT-added oil; 0.140, 7.19, 51.7, 10.0 for carvacrol-added oil after 40 frying cycles. The results revealed that the use of carvacrol could significantly improve the oxidative stability of palm oil when compared to the control samples. This effect was also comparable to BHT. Using carvacrol in frying oil slowed down the rate of the formation of conjugated dienes and trienes compared to the oil with BHT and the control. The frying process significantly changed the viscosity of the oil samples.

KEYWORDS: *Carvacrol; Frying; Natural antioxidant; Oxidative stability; Palm oil*

RESUMEN: ***Efecto del carvacrol en la estabilidad oxidativa del aceite de palma durante la fritura***. Las grasas y aceites se deterioran física y químicamente a las temperaturas de fritura debido a diferentes razones. El objetivo de este estudio fue evaluar el efecto del carvacrol en la estabilidad oxidativa del aceite de palma durante el proceso de fritura repetida. Se sometió a fritura repetida patatas en el aceite de palma con carvacrol agregado, en aceite de palma con BHT agregado y en aceite control (sin antioxidante). Después de cada décimo ciclo de fritura, se realizaron diferentes análisis sobre las muestras recogidas para evaluar el deterioro de los aceites. Ácidos grasos libre, *para*-anisidina, índice de yodo y componentes polares totales del aceite fresco fueron: 0,080, 2,85, 57,1 y 7,5, respectivamente. Estos valores cambian a 0.165, 11.80, 46.7 y 11.0, respectivamente, para el aceite de control; 0.151, 11.28, 49.2 y 10.5 para el aceite con BHT añadido; 0.140, 7.19, 51.7 y 10.0 para el aceite con carvacrol añadido después de 40 frituras. Los resultados revelaron que el uso de carvacrol puede mejorar significativamente la estabilidad oxidativa del aceite de palma en comparación con las muestras control. Este efecto también era comparable a la del BHT. El uso de carvacrol en el aceite de fritura ralentizó la velocidad de formación de dienos y trienos conjugados en comparación con el aceite con BHT y el de control. Los procesos de fritura producen grandes cambios en la viscosidad de las muestras de aceite.

PALABRAS CLAVE: *Aceite de palma; Antioxidante natural; Carvacrol; Estabilidad a la oxidación; Fritura*

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1. INTRODUCTION

The great demand of food prepared in a cheap and quick way due to the rapid increase in population, excess urbanization and economical drawbacks has resulted in an increase in the production and consumption of the ready-foods, especially fried foods all around the world. Therefore, most of the fats and oils are used in the frying of food materials.

Frying is a fast, convenient and energy efficient method and therefore it is widely used in fast-food restaurants and domestic cooking. Despite the trend towards low-fat foods, fried foods have become more and more popular with their unique sensorial properties, since frying increases the palatability of foods due to fat absorption, crust formation and desired flavors and odors (Farhoosh and Moosavi, 2008).

Besides desirable changes, frying causes undesirable changes which finally result in the deterioration of oil leading to a shortening of the oil's frying life. These negative changes in the oil affect the quality of fried food at the same time. The most important reaction causing oil deterioration which affects both the sensory and nutritional quality of the food product is lipid oxidation. During the frying period, the oxidation process occurs rapidly during the heating of oil in the presence of air. The compounds formed as a result of oxidation cause off-tastes and off-flavors in the fried products, reducing the organoleptic characteristics and perhaps causing a health hazard (Moreira *et al.*, 1999, Innawong, 2001).

Options for enhancing the stability of oils during frying process include the use of natural antioxidants rather than synthetic ones due to their adverse effects. Previous studies have generally focused on plant extracts including different plant organs such as seeds, fruits, leaves and others. These studies revealed that using plant extracts is more effective than directly adding the plants for stabilizing the oils. In addition to plant extracts, essential oils and active components have been used as natural antioxidants for stabilizing the polyunsaturated oils. However, essential oils generally have weak antioxidative effects at higher temperatures, whereas they are very effective at storage temperatures. This may also be due to decomposition and/or volatility of the essential oils taking place at very high temperatures (Tomaino *et al.*, 2005, Maestri *et al.*, 2006, Al-Jaber *et al.*, 2011, Inanc and Maskan, 2013).

Plants also have active components namely phenolics and polyphenolics that are known to act as antioxidants. Carvacrol, which is one of the strong active components, is predominantly present in oregano, but also naturally available in thyme, clove and cinnamon (Yanishlieva *et al.*, 2006). In only a few studies, the active components of plant extracts have been used to slow down the oxidation of oils at high temperatures instead of extracts and essential oils (Zunin *et al.*, 2010, Yeo *et al.*, 2011, Inanc, 2012). However, it is possible to utilize their advantageous properties in applications at high temperatures in the future.

The undesirable changes in oils during frying partially depend on the unsaturation degree of fatty acids, besides time and temperature of the frying process. Therefore, it is very important to choose the right frying oil to retard oil deterioration. High oxidative stability, high smoke point, low foaming, low melting point and bland flavor are important characteristics of good frying oils (Kochhar, 2000). Palm oil, which is the second largest source of oil in the world, next to soybean oil, is currently the oil most used for frying (FEDIOL, 2012). It has a good oxidative stability due to its high degree of saturation. This is the reason for the selection of palm oil as the frying medium in the current study.

In the present study, potato slices were serially deep-fried in palm oil enriched with a GRAS (Generally Recognized as Safe) active compound carvacrol at 150 °C. This temperature is in the range of adequate frying temperatures in the literature. The carvacrol concentration in the palm oil and the frying temperature were decided as a result of our previous study (Inanc, 2012). Carvacrol was added into palm oil at a concentration of 200 ppm, which is the legal usage limit of synthetic antioxidants in most countries according to the Food and Drug Administration (Shahidi, 2005). Thus, it could be comparable with a synthetic antioxidant, butylated hydroxytoluene (BHT). The intended results of the present study should facilitate the frying stability of oils by using natural antioxidants, especially active components instead of synthetic ones.

2. MATERIALS AND METHODS

2.1. Materials

Fresh potatoes were purchased from a local market. Palm oil was supplied from a manufacturer. Carvacrol and BHT were obtained from Sigma Aldrich Co. (St. Louis, MO).

2.2. Frying Experiments

Potato slices were fried in palm oil at 150 °C and 200 ppm antioxidant concentration. Three sets of frying experiments were performed; natural antioxidant (carvacrol) added oil, BHT added oil and the control oil (without any antioxidants).

The frying process was conducted in an electric deep fat fryer with a thermostate to regulate temperature (Arnica, Deep Fryer, ZG 27A, China). Before starting the frying process, 50 mL of the oil sample were collected into amber colored glass bottles in order to measure the initial properties of the oil. This oil represents the control oil. Frying was started 30 min after the temperature of the oil had reached the frying temperature. For each frying cycle, 100 g of sliced fresh potatoes ($6.0\pm1.23*7.8\pm1.25*49.5\pm4.09$ mm^3) were used. The slices were soaked in a 2.5% NaCl solution for 5 min prior to frying. This reduces the oil absorption capacity and prevents surface darkening of the potato slices due to oxidation. Following the draining of the water, the potato slices were dried by blotting with a paper towel before frying. The potatoes were fried in 2 liters of oil for 4 minutes. This period was decided from preliminary experimental studies to reach the 2% moisture content of potatoes. Then, the oil was heated for 5 min in order to attain the frying temperature again. For each set, a total of 40 frying cycles were done with the same oil. Fresh oil was not added between the cycles. After each tenth frying cycle, the oil was allowed to equilibrate for 30 min at the frying temperature and 50 mL of the oil sample were collected and stored at 4 °C for further analysis.

2.3. Measurement of Oil Deterioration During the Frying Process

After each tenth frying cycle, several analyses were conducted such as free fatty acid content (AOCS, 1989), para-anisidine value (IUPAC, 1987), iodine value (AOAC, 1990), polar compounds (Hampikyan *et al*., 2011), conjugated dienes and trienes (PORIM, 1995). The rheological behavior of the used oil sample at the 40th frying was determined in the shear rate range of 1–200/s at 25 °C by using Haake Rheostress RS1 controlled stress rheometer as described by Maskan (2003). All analyses were made in triplicate. The data were analyzed for significant differences by one-way analysis of variance and compared by Duncan's multiple range test at 5% significance level using SPSS 16.0 software.

3. RESULTS AND DISCUSSIONS

3.1. Change in Free Fatty Acids (FFA)

During frying, oil is exposed to air and moisture at elevated temperatures resulting in the hydrolysis of triacylglycerols. This leads to a release of free fatty acids. The released fatty acids are more susceptible to thermal oxidation and cause off-flavors and odors in the frying medium and fried foods (Bensmira *et al*., 2007). In the current study, FFA values increased with the number of fryings in palm oil as shown in Figure 1. However, carvacrol slowed down the formation of FFA significantly ($p<0.05$). According to the statistical analysis, the effect of the number of fryings on the increase in FFA in all oil samples was significant ($p<0.05$) (i.e., at 10, 20, 30 or 40 frying cycles, the FFA contents in the BHT and carvacrol added and control oils increase). This is in good agreement with several previous works (Marco *et al*., 2007, Iqbal *et al*., 2008, Nor *et al*., 2009, Kalogianni *et al*., 2011).

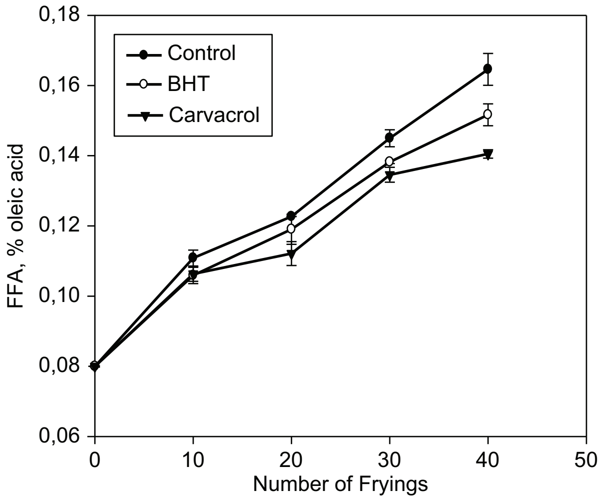

FIGURE 1. Changes in FFA against the number of frying sessions for palm oil samples (n = 3).

Treatment of oils with antioxidants significantly decreased the FFA of palm oil at each frying cycle as the results compared to the control oil ($p<0.05$). Carvacrol was found to be more effective than BHT. Multiple comparison tests showed that the positive effect of carvacrol was significantly different from BHT and control oils. Several researchers found similar results in their studies about the effectiveness of natural antioxidant in vegetable oils (Che Man and Jaswir, 2000, Bensmira *et al*., 2007, Iqbal *et al*., 2008, Nor *et al*., 2009).

3.2. Changes in *para*-Anisidine Value (*p*-AV)

The *para*-anisidine value is a measure of the contents of aldehydes which are secondary oxidation products formed when hydroperoxides decompose (Shahidi, 2005). Thus, it is reasonable to measure *p*-AV rather than peroxide value in order to determine the degree of deterioration in oil during frying. The changes in the *p*-AV of the palm oil samples during a series of frying are presented in Figure 2. The *p*-AV of all the oil samples increased as the frying number increased. These findings are in good agreement with several studies (Isabei and Mariano, 2001, Naz *et al*., 2008). A statistical analysis of the results showed the significant effect ($p<0.05$) of frying numbers on the increase in p-AV of the oils. From Figure 2, it could be determined that the *p*-AV of the oil samples treated with carvacrol were distinctly lower than that of the BHT and control samples. However, the final *p*-AV of BHT and carvacrol added oils were not very different from each other. The *para*-anisidine values finally increased from 2.85 to 7.19, 11.28, and 11.80 for samples with carvacrol, BHT, and the control, respectively.

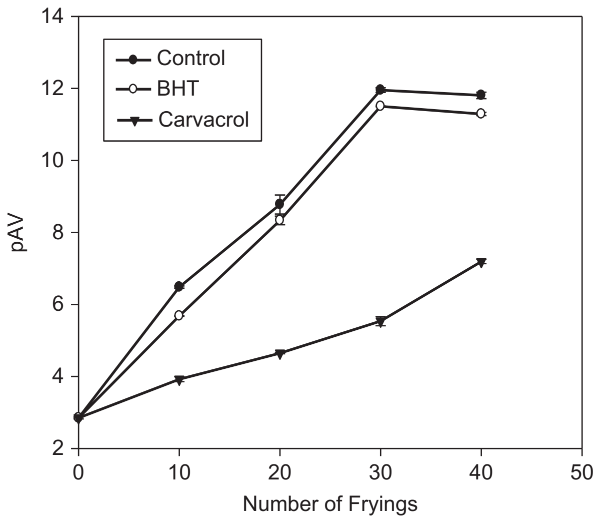

FIGURE 2. Changes in *p*-AV against the number of frying sessions for palm oil samples.

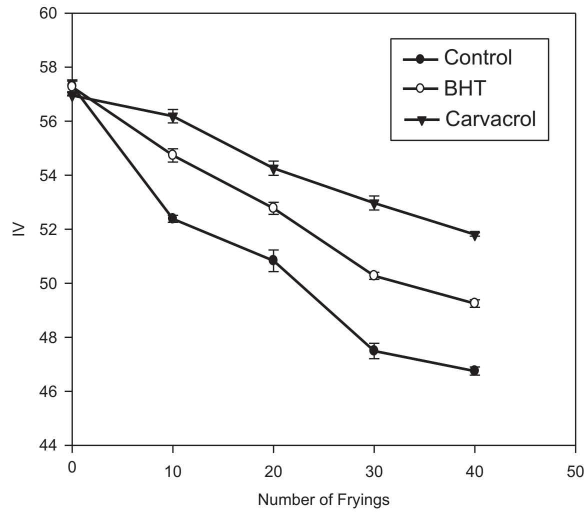

FIGURE 3. Changes in IV against the number of frying sessions for palm oil samples.

The ANOVA and multiple comparison test results showed that there were significant ($p<0.05$) differences among the antioxidant-treated oils and the control. From the graph it could be easily understood that carvacrol had a greater effect on the reduction in *p*-AV of the palm oil than the synthetic antioxidant, BHT (Figure 2). The results of several authors studying palm oil are in good agreement with our findings (Ismail, 2005, Nor *et al.*, 2009, Pambou-Tobi *et al.*, 2010).

3.3. Change in Iodine Value (IV)

Iodine value is a measure of the total number of unsaturated double bonds present in oil. The differences in iodine values of oils during frying are also good indicators of deterioration in the oils (Che Man and Jaswir, 2000). Oxidation, which consists of a complex series of chemical reactions, is characterized by a decrease in the total unsaturated content of the oil due to the abstraction of hydrogen adjacent to a double bond and the formation of free radicals (Naz *et al.*, 2004).

The change in the IV of the palm oil samples are presented in Figure 3. There was a decrease in IV from 57 to 51.7, 49.2, and 46.7 for samples with carvacrol, BHT, and the control, respectively. The decrease in IV can be attributed to the destruction of double bonds by oxidation and polymerization (Abdulkarim *et al.*, 2008).

The effect of frying numbers on the decrease in IV of all oil samples was generally found to be significant ($p<0.05$) according to the ANOVA results. This is in good agreement with the previous studies (Che Man and Jaswir, 2000, Naz *et al.*, 2004, Nor et al., 2009). However, the difference between the values of the 30th and 40th frying in all samples were not significant ($p>0.05$).

The use of antioxidants caused a significant reduction in the IV of palm oils compared with the control values ($p<0.05$). It could be determined from Figure 3 and the multiple comparison tests that carvacrol was found to be more effective on the IV of the oils than BHT ($p<0.05$). Nor *et al.* (2008) reported similar results with a natural antioxidant used in their study. However, in another study, it was reported that there was no significant difference between thymbra spicata essential oil, whose active component is carvacrol, and BHT with respect to iodine value changes in the oils (Nacaroglu, 2006).

3.4. Changes in Total Polar Compounds (TPC)

The amounts of total polar compounds that are formed through oxidation, thermal and hydrolytic reactions during frying, indicate the degree of deterioration in frying oils (Hampikyan *et al.*, 2011, Osawa *et al.*, 2012).

In this study, TPC was measured by an instrument, Testo 265, which provides accurate results in a very short time compared to the traditional method. The principle of the instrument is based on the measurement of changes in the dielectric constant of frying oil and direct transformation of them into the percentage in the weight of polar compounds (Hampikyan *et al.*, 2011, Osawa *et al.*, 2012). When frying oil decomposes thermally or through oxidation processes, its dielectric constant increases (Hein *et al.*, 1998).

Figure 4 shows that in all oil samples the amount of TPC increased with increasing frying periods.

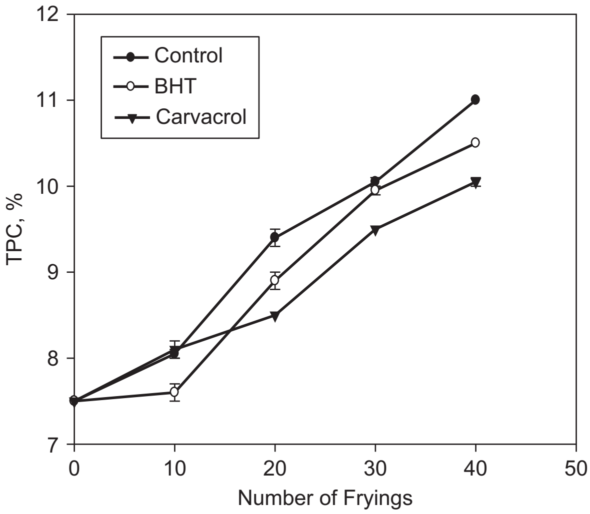

FIGURE 4. Changes in TPC against the number of frying sessions for palm oil samples.

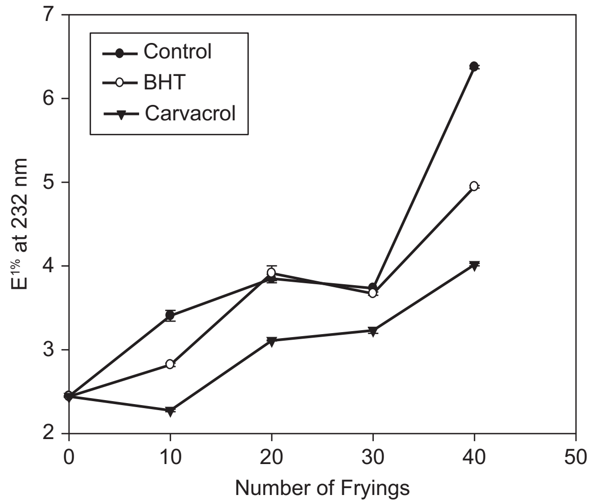

FIGURE 5. Changes in E1% at 232 nm against the number of frying sessions for palm oil samples.

The results of Pambou-Tobi *et al.* (2010) and Romano *et al.* (2012) agree well with our findings. However, it was determined that there was no significant change in the TPC of the oils with respect to successive frying numbers ($p>0.05$). At the end of the frying experiments, the level of polar compounds did not exceed 25%, which is the regulatory limit for frying oils in most European countries (Hampikyan *et al.*, 2011).

Carvacrol was found to be significantly ($p<0.05$) effective on slowing down the formation of TPC in palm oil compared to the control samples. However, this effect was not statistically different from the BHT according to the multiple comparison test results ($p>0.05$). Nor *et al.* (2008) also reported the positive effect of a natural antioxidant on the prevention of polar compound formation.

3.5. Changes in Conjugated Dienes and Trienes in Oils

Lipids exhibit a shift in their double-bond position because of isomerization and conjugate formation during oxidation. The conjugated dienes (CD) formed show ultraviolet absorption at about 232 nm. Likewise, conjugated trienes (CT) exhibit absorption at about 270 nm. The increases in CD and CT might be proportional to the uptake of oxygen which is analogous to the oxidation of oils (Akoh and Min, 2002). Thus, the determination of CD and CT is known to be a reliable method for measuring the oxidative deterioration of oils. Several authors used CD and CT measurements to evaluate the effect of some antioxidants on vegetable oils. For instance, Houhoula *et al.* (2004), Arabshahi-Delouee *et al.* (2011), and Ramadan and Wahdan (2012) used absorption in the UV spectrum to evaluate the oxidative deterioration of oils in their studies.

The results in the current study revealed that there were increasing trends in both specific extinction, the $E^{1\%}$ values of 232 and 270 nm corresponding to the CD and CT contents for all of the oil samples with increasing frying numbers (Figures 5 and 6). However, the statistical analysis showed that the effect of frying numbers was not significant ($p>0.05$) on the increase in CD and CT formation in all oil samples. Using carvacrol in frying oil slowed down the rate of the formation of CD and CT compared to the samples with BHT and the control.

It was easily understood that the amounts of CT were much lower than those of CD in all the oil samples. The reason, as reported by Abdulkarim *et al.* (2008), may be due to higher linoleic acid contents of the samples than linolenic acid. Conjugated dienes formation is mainly caused by the presence of polyunsaturated fatty acid whereas trienes are mainly formed by the dehydration of conjugated diene hydroperoxides (Mohdaly *et al.*, 2010).

According to the statistical analysis, there were significant ($p<0.05$) differences among the extinction coefficients of the oils treated with antioxidants and the control samples. Serjouie *et al.* (2010) reported similar results to ours. Similarly, the study of Mohdaly *et al.* (2011) on treatment of natural antioxidant was found to be in good agreement with our results.

There was a decreasing trend in the specific extinction values of the oils between the 20^{th} and 30^{th} frying cycle and then an additional increase from the 30^{th} to the 40^{th} frying cycle. This might be explained by the probable equilibrium between the formation rate of conjugated dienes and the rate at which those

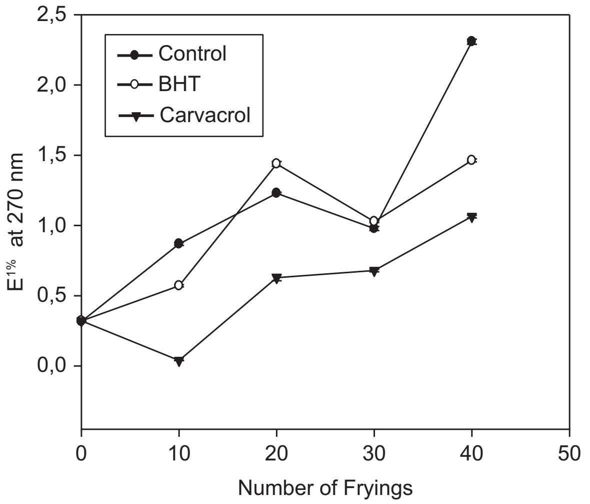

FIGURE 6. Changes in E1% at 270 nm against the number of frying sessions for palm oil samples.

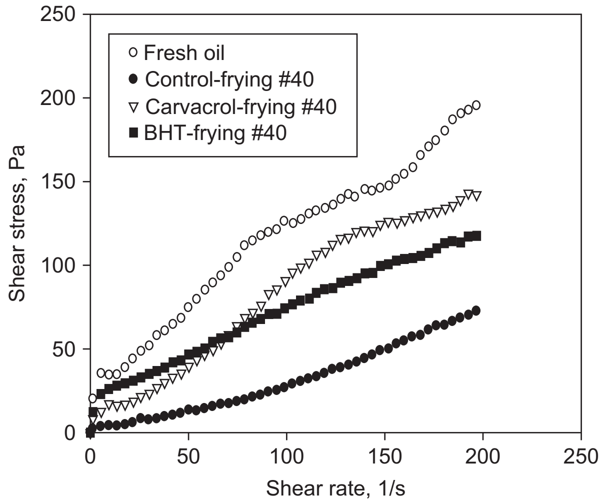

FIGURE 7. Changes in shear stress as a function of shear rate for palm oil samples.

compounds formed polymers. This might be due to both the formation and decomposition of peroxides at the same time during frying (Pambou-Tobi *et al.*, 2010; Serjouie *et al.*, 2010).

3.6. Changes in Rheological Behavior of Oils

Rheological changes in the oils can be also considered an indicator of oil deterioration. During frying at elevated temperatures, the carbon in the fatty acid molecules causes cross linking of the carbon to form cyclic compounds, dimers, trimers, epoxides and polymers, resulting in increased oil viscosity (Kusucharid *et al.*, 2009). It was reported in several studies that the viscosity of frying oils increased mainly as a result of oxidation and polymerization reactions (Bensmira *et al.*, 2007, Kalogianni *et al.*, 2011).

Shear stress measurements as a function of shear rate for palm oil samples collected after the 40th frying are shown in Figure 7. The graph demonstrated that palm oil exhibits non-Newtonian behavior because of the non-linear relationship between shear stress and shear rate. Figure 8 shows the change in apparent viscosity against the shear rate for palm oil samples. Increasing shear rate resulted in decrease in the apparent viscosity of palm oil. This behavior represents shear thinning behavior.

Both of the figures show that neither frying numbers nor antioxidant treatment importantly changed viscosity of the oil samples. However, according to the statistical analysis there was a significant difference ($p<0.05$) among the viscosities of palm oil samples. Multiple comparison tests also show that fried palm oils were significantly different ($p<0.05$) from the fresh control sample.

On the other hand, in several studies it was reported that long periods of frying caused a high increase in viscosity due to the highly formation of polar compounds (Che Man and Jaswir, 2000, Kusucharid *et al.*, 2009, Serjouie *et al.*, 2010). The differences between the results might be due to fewer frying applications in the present study. The other reason might be the lower temperature that was used in frying than the other studies reported in the literature. The slight increase in polar materials in our study also confirms these findings, since there is a strong correlation between formation of polar compounds and increase in viscosity (Abdulkarim *et al.*, 2008).

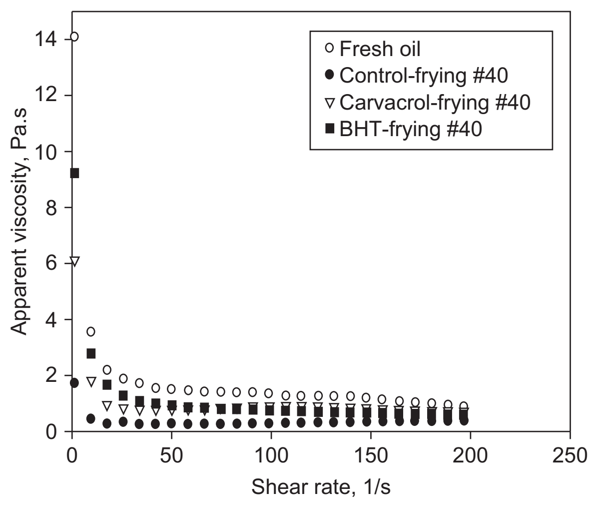

FIGURE 8. Changes in apparent viscosity as a function of shear rate for palm oil samples.

4. CONCLUSIONS

The results of the chemical analysis in oils correlated the good impact of carvacrol on reducing oil oxidation during the frying process. This effect was also comparable to the synthetic antioxidant BHT. Thus, it can be considered as an alternative to the BHT. However, the strong and characteristic flavor of natural antioxidants may adversely affect the sensorial properties of both the oil and the fried food. Therefore, this important aspect should be evaluated in further studies. Exploring natural antioxidants by means of toxicology is also recommended in order to conveniently use them in foods.

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