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Volatile profile of heated soybean oil treated with quercetin and chlorogenic acid

Perfil de compostos voláteis do óleo de soja aquecido e tratado com quercetina e ácido clorogênico

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Abstract

Changes in the profile of volatile compounds after the heating of refined soybean oil without adding antioxidants, and treated with quercetin and chlorogenic acid (5-CQA) were investigated by GC/FID, GC/MS, and GC/SNIFFING. The heating temperature of the oil sample was 20 °C for the first minute, and then it was increased up to 160 °C at the rate of 10 °C min^{-1}. The final temperature was kept for 10 minutes. 19 volatiles were identified in the heated samples without antioxidants. Medium-chain carbonyls predominated in the volatile fraction, mainly 2-heptenal, 2,4-heptadienal and 2,4-decadienal. Around 11 to 15 volatile compounds were detected in the heated samples treated with 5-CQA and quercetin, respectively. 5-CQA was not very efficient in delaying the formation of oxidative volatile compounds. The samples quercetin presented lower proportion of carbonyls with C_6-C_9. The *GC peak area* data were used as an approach to estimate the *relative* content of each *volatile compound and indicate* that the samples treated with quercetin ($p < 0.05$) had significantly lower values for, 1-pentanol, 2,4-heptadienal, and 2,4-decadienal compared with those without antioxidants and treated with 5-CQA. GC/SNIFFING analysis revealed a smaller odor perception in the samples treated with 5-CQA compared to those without antioxidants. No odor was perceived in the heated samples treated with quercetin. These results indicate greater effectiveness of quercetin in delaying the formation of oxidative volatile compounds in soybean oils subjected to mild heating conditions. Apparently, biopolyphenols used in the present work showed good oxidative stability since no new volatile compound was detected in the heated samples treated with them.
Keywords: *soybean oil; GC/MS; oxidative volatiles; quercetin; chlorogenic acid.*

Resumo

As alterações no perfil de compostos voláteis, após o aquecimento de óleo de soja refinado sem a adição de antioxidantes e tratado previamente com quercetina e ácido clorogênico (5-ACQ), foram investigadas através da CG/DIC, CG/EM e CG/SNIFFING. A temperatura de aquecimento do óleo foi de 20 °C no primeiro minuto e aumentada até 160 °C à taxa de 10 °C min^{-1}. A temperatura final foi mantida por 10 minutos. Um total de 19 compostos voláteis foi identificado nas amostras aquecidas sem a adição de antioxidantes. As carbonilas de cadeia média predominaram na fração volátil. Cerca de 15 e 11 compostos voláteis foram detectados no óleo aquecido com adição prévia de quercetina e 5-ACQ, respectivamente. As amostras tratadas com quercetina mostraram uma menor proporção de carbonilas com esqueletos de carbono C_6-C_9. A composição estimada de compostos voláteis mostrou que amostras tratadas com quercetina tiveram valores significativamente ($p < 0,05$) menores para 1-pentanol, 2,4-heptadienal e 2,4-decadienal em comparação com as amostras sem antioxidantes adicionados e com as tratadas com 5-ACQ. A análise por meio de CG/SNIFFING revelou um número menor de odores em amostras tratadas com 5-ACQ em comparação àquelas sem tratamento algum. Nenhum odor foi percebido nas amostras aquecidas com adição prévia de quercetina. Estes resultados indicam uma maior efetividade da quercetina no retardamento do processo oxidativo em amostras de óleo de soja sujeitas a aquecimento a 160 °C ± 10 °C min^{-1} por 10 minutos . Aparentemente, os compostos fenólicos naturais usados neste trabalho mostraram boa estabilidade oxidativa, já que nenhum composto volátil novo foi detectado nas amostras aquecidas e tratadas com eles.
Palavras-chave: *ácido clorogênico; CG/EM; compostos voláteis; óleo de soja; quercetina.*

1 Introduction

Refined edible oils containing substantial amounts of polyunsaturated fatty acids undergo oxidative rancidity and produce off-flavors. Autoxidation is a major route of oil rancidity and its progression occurs via a free radical chain mechanism (SHERWIN, 1976). The addition of primary antioxidants (e.g. polyphenolic compounds) retards the autoxidation process by acting as chelators of transition metals and/or as hydrogen donors and produces relatively stable free radicals and non-radical products (HAMILTON et al., 1997). Synthetic phenolic compounds (e.g. TBHQ) are the most commonly antioxidants used in lipids. However, there is a tendency of consumer groups to decrease the amount of synthetic additives in foods. As a consequence, naturally occurring phenolic compounds (e.g. flavonoids and phenolic acids) have drawn attention because they have been ingested for centuries and are assumed to be relatively safe for human consumption. Indeed, the daily dietary intake of these natural components is approximately 1 g via the ingestion of vegetables (LIU, 2004). Quercetin (flavonol) and chlorogenic acid (5-CQA) (cinnamate) are typical phenolic compounds widely distributed in plants including many foods and beverages. In recent years, these biophenolic compounds have proved to provide protective

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effects against oxidative stress in animal and in vitro models (ZHANG et al., 2006; MORALES et al., 2006). Reactive oxygen species appear to be major contributors in the pathogenesis of degenerative diseases such as arteriosclerosis, cancer, and chronic inflammation.

In a previous work, some studies were performed aiming at elucidating the effectiveness of 5-CQA and quercetin in stabilizing refined soybean oil subjected to accelerated oxidation (MAGDA et al., 1997; MAGDA et al., 1998; DE MARIA et al., 2000). Chemical analysis of the oxidized oil samples included the determinations of the peroxide values (PV), the conjugated dienes at 233 nm (MAGDA et al., 1997; MAGDA et al., 1998), and the induction time obtained using an oxidative stability index instrument (DE MARIA et al., 2000). It was observed that primary antioxidative action of quercetin was greater than that of 5-CQA. In fact, results from another investigation have shown that quercetin is effective in delaying rancidity in canola oil (WANASUNDARA; SHAHIDI, 1994).

Nevertheless, the effect of natural phenolic antioxidants on the volatile profile of soybean oil submitted to forced oxidation has not been thoroughly studied yet. These biophenols could modify the volatile fraction not only by affecting the oxidative formation of volatile compounds, but also by providing volatiles through their thermal degradation. The present work aim is to study changes in the volatile profile of refined soybean oils treated with quercetin and 5-CQA and submitted to mild heating conditions.

2 Materials and methods

2.1 Materials

Reagents: Quercetin and 5-CQA were from Sigma (St. Louis, MO, USA). Tenax TA was from Supelco (Bellefonte, PA, USA). Absolute ethanol was from Merck (Darmstadt, Germany). All other chemicals were from Aldrich (Sheboygan, WI, USA).

Samples: Three fresh refined soybean oil samples were obtained from Cargill Agrícola S.A. (Brazil). The good quality of the samples was confirmed by the fatty acid composition and PV analysis (MAGDA et al., 1997).

2.2 Methods

Sample preparation: Due to the low solubility of the phenolic compounds in soybean oil, it was necessary to dissolve them in absolute ethanol. Quercetin or 5-CQA (40 mg) was dissolved in 1 mL of ethanol in a magnetic stirrer, added to the oil (120 mL), and mixed until dissolution. The sample without antioxidants contained the same amount of ethanol as the one that was used to dissolve the additives. Samples without adding antioxidants and with quercetin or 5-CQA were placed in a 10 cm diameter flat Teflon-coated pan (with deep of 4 cm) with flared sides and subjected to accelerated oxidation, as follows: the oil sample temperature was 20 °C for the first minute and then it was increased to up 160 °C at the rate of ~10 °C min^{-1}. The final temperature was kept for 10 minutes. The skillet was heated in a hot plate connected to an electronic thermometer used to regulate the temperature of the sample. Each heated sample was cooled at room temperature for 30 minutes and immediately submitted to the enrichment of the headspace fraction.

Isolation of the headspace volatile fraction: 100 mL oil sample was placed in a 500 mL Pyrex heavy-walled filtering flask with a side hose-connection (Brand, Wertheim, Germany), o.d. 10 mm, and heated at 60 °C under stirring. Purified nitrogen (0.9-1.0 L min^{-1}) was passed through this system for 2 hours, and the entrained volatiles were adsorbed on a Tenax trap. This adsorvent was previously conditioned in a oven at 225 °C for 3 hours under a N_2 flow of 0.9-1.0 L min^{-1}. Desorption of the compounds was done with 200 mL of acetone. This volume was settled by monitoring 10 mL aliquots of acetone until no more volatiles could be detected using GC/FID. The eluate was then concentrated to 200 µL in a rotatory evaporator at 20 °C.

GC/FID: A Carlo Erba 4300 GC equipped with a 30 m x 0.25 mm i.d. x 0.25 µm Supelcowax™ 10 fused-silica polar capillary column (Supelco, Milford, USA) was used. The injector and FID temperatures were 230 and 240 °C, respectively. Helium was used as the carrier gas at 0.83 mL min^{-1} rate. The oven temperature ranged from 50 to 230 °C at 3 °C min^{-1}. The split ratio was 1:20. Linear retention indices (LRI) were estimated using the modified Kövatz method (VAN DEN DOOL; KRATZ, 1963). All oil samples were analyzed in two replicates. The *GC peak area* data were used as an approach to estimate the *relative* content of each *volatile compound*. The mean, standard deviation, and one-way analysis of variance ($p < 0.05$) were performed using a statistical graphics system (STSC, 1986).

GC/MS: A Shimadzu GC-17A/QP5050 quadrupole mass spectrometry with National Institute of Standards and Technology (NIST) 12.lib and 62.lib data system was used. The instrument was operated in the electron ionization mode at 70 eV, taking scans from 20 to 300 m/z in a 1 s cycle. The column and chromatographic conditions were the same as described for the GC/FID analysis. Tentative identification of the volatiles was based on the comparison of the mass spectra of unknown compounds against NIST library data. Wherever possible, the results were confirmed by comparison with authentic substances. Only the compounds identified using at least reference standards and mass spectra data were considered to be definitively identified.

CG/SNIFFING: The same above-mentioned chromatographic conditions were used, splitting the effluent of the capillary column (1:10) between FID, and the artisanal sniffing port. Odor port evaluation was carried out freely by two testers.

3 Results and discussion

3.1 PV analysis

The PV of the fresh oil was 0.7 ± 0.02 meq.L^{-1} which proved the good quality of the product (MAGDA et al., 1997). On the other hand, there were differences in PV of in the heated oil samples containing quercetin (12 ± 0.4 meq.L^{-1}) and 5-CQA (13 ± 0.5 meq.L^{-1}) to those without antioxidants (17 ± 0.7 meq.L^{-1}). Therefore, the addition of either quercetin or 5-CQA was capable of delaying oil peroxide formation.

3.2 Volatile profile

Changes in the volatile profile after the heating of refined soybean oil without antioxidants and treated with quercetin and 5-CQA were investigated. The estimated composition of volatiles in the headspace fraction is shown in Table 1. No *recovery method* or *GC standardization* were applied, and thus, the data presented are estimated values. A total of 19 volatile compounds were identified in the heated soybean oil without antioxidants. Among them, 63% were definitively identified by comparing the retention time and mass spectral data with those of authentic standards. The chief oxidative volatile compounds were aldehydes, especially 2-heptenal, 2,4-heptadienal, and 2,4-decadienal. The detection of 2-heptenal and 2,4-decadienal in the soybean oil samples is in accordance with reports from other authors (KAO et al., 1998; STEENSON et al., 2002). On the other hand, this is the first time, to our knowledge, that 2,4-heptadienal was detected in heated soybean oil. As previously reported, 2,4-heptadienal was detected during the autoxidation of methyl linolenate at room temperature (ULLRICH; GROSCH, 1987). In another study, tri cis,cis 9,15-linoleoylglycerol was submitted to accelerated oxidation forming allylic radicals that reacted with O_2 forming monohydroperoxides at the C_8-C_{11} positions (NEFF; SELKE, 1993). These monohydroperoxides could undergo additional reactions to produce oxidative volatiles. Thus, the occurrence of 2,4-heptadienal and 2,4-decadienal in heated soybean oil may be attributed to the thermal decomposition of 8, 9, 10 and 11 hydroperoxides of linoleic and linolenic esters.

A total of 15 and 11 oxidative volatile compounds were detected in heated soybean oil treated with 5-CQA and quercetin, respectively (Table 1). In general, 5-CQA was not very efficient in delaying oxidative volatile formation. The addition of quercetin, on the other hand, increased the oxidative stability of the heated oil samples. The estimated concentration of the volatiles by means of relative GC peak areas showed that soybean oil samples treated with quercetin had significantly lower values for 1-pentanol, 2,4-heptadienal, and 2,4-decadienal in comparison with those without antioxidants and treated with 5-CQA. Furthermore, samples treated with quercetin exhibited a lower proportion of aldehydes with C_6-C_9 skeletons. A previous study reported that 14, 15, 16, and 17 monohydroperoxides were expected precursors of medium-chain carbonyls (NEFF; SELKE, 1993). These monohydroperoxides are derived from the oxidation of allylic radicals at the $C_{15\text{-}16}$ position. Then, it is possible that quercetin reduces the formation of oxidative volatiles by acting as an inhibitor of the oxidation of allylic radicals or by delaying the thermal decomposition of the monohydroperoxides previously formed. These findings are in accordance with previous data from our laboratory (DE MARIA et al., 2000), which showed that the antioxidative potency of quercetin was significantly greater than that of 5-CQA. The structural characteristics imparting the highest antioxidant activity in the quercetin was found to be the following (DE BEER et al., 2002): a) the ortho 3',4'-dihydroxy moiety in the B'-ring; b) the 2,3-double bond in combination with the 4-keto group; and c) the 3- and 5-hydroxyl groups in the C- and A-ring. These characteristics favor the electron delocalisation in the C-ring promoting maximum scavenging potential. In contrast to quercetin, 5-CQA contains only a 3',4'-dihydroxy group in the aromatic ring (caffeic acid moiety) (Figure 1).

3.3 Odor perception

Aroma concentrates obtained from the headspace of heated soybean oil samples by adsorptive column chromatography,

Table 1. Volatile composition of heated soybean oil samples without antioxidants and treated 5-CQA and quercetin.

Compounds	Samples Percentual area (Mean ± SD)			Odor perception			LRI
	Oil	Oil + 5-CQA	Oil + QUE	Oil	Oil + 5-CQA	Oil + QUE	
Heptanal[a]	$0.30 \pm 0.06^{\alpha}$	$0.29 \pm 0.02^{\alpha}$	nd	slightly green	slightly green	nd	1164
2-hexenal[a]	$0.80 \pm 0.20^{\alpha}$	$0.77 \pm 0.15^{\alpha}$	nd	nd	nd	nd	1204
Octanal[a]	<0.01	nd	nd	nd	nd	nd	1238
1-pentanol[a]	$1.50 \pm 0.30^{\alpha}$	$1.13 \pm 0.15^{\alpha}$	$0.57 \pm 0.15^{\beta}$	nd	nd	nd	1248
2-heptenal[a]	$3.20 \pm 0.45^{\alpha}$	$4.03 \pm 0.15^{\beta}$	$3.10 \pm 0.30^{\alpha}$	nd	nd	nd	1295
2-penten-1-ol[b]	<0.01	nd	<0.01	nd	nd	nd	1323
Nonanal[a]	0.53 ± 0.25	nd	nd	nd	nd	nd	1335
1-hexanol[a]	<0.01	<0.01	<0.01	nd	nd	nd	1352
7-octen-4-ol[b]	$0.29 \pm 0.005^{\alpha}$	$0.32 \pm 0.07^{\alpha}$	$0.10 \pm 0^{\beta}$	nd	nd	nd	1438
2,4-heptadienal[a]	$2.77 \pm 0.45^{\alpha}$	$2.20 \pm 0.20^{\alpha}$	$1.17 \pm 0.30^{\beta}$	strongly oil	oily	nd	1446
2-nonenal[a]	<0.01	<0.01	<0.01	nd	nd	nd	1515
2,4-dimethyl-cyclohexanol[b]	<0.01	<0.01	nd	oily	oily	nd	1526
2-nonen-1-ol[b]	<0.01	nd	nd	nd	nd	nd	1607
2-decenal[b]	<0.01	<0.01	<0.01	slightly oil	nd	nd	1630
4-nonanol[b]	<0.01	<0.01	<0.01	nd	nd	nd	1642
Heptadecane[a]	<0.01	<0.01	nd	nd	nd	nd	1707
2-undecenal[b]	$0.53 \pm 0.15^{\alpha}$	$0.50 \pm 0.1^{\alpha}$	$0.34 \pm 0.04^{\alpha}$	nd	nd	nd	1743
2,4-decadienal[a]	$1.50 \pm 0.26^{\alpha}$	$1.33 \pm 0.15^{\alpha}$	$0.67 \pm 0.06^{\beta}$	heated oil	heated oil	nd	1806
hexanoic acid[a]	<0.01	<0.01	<0.01	unpleasant	nd	nd	1847

[a]Definitive identification via mass spectra + reference compound; LRI = linear retention index; [b]tentative identification: based on mass spectra data only; Oil = oil without added antioxidant; Oil + 5-CQA = oil treated with chlorogenic acid; Oil + QUE = oil treated with quercetin nd = not detected; D data are given as mean values of three independent experiments with two replicates each; and means with different Greek letters (α, β) in the same row are significantly different at $p < 0.05$.

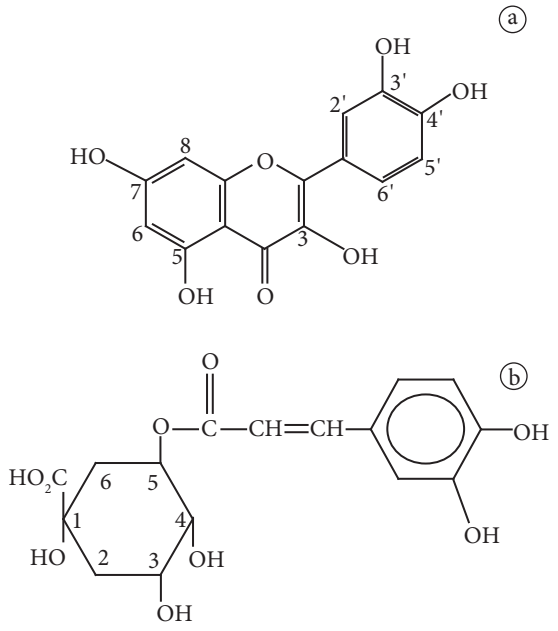

Figure 1. Chemical structures of biopolyphenols. a) quercetin; and b) chlorogenic acid (5-CQA).

followed by desorption with acetone, provided similar odors to those of the original samples. The perceived odor qualities obtained by means of the GC-SNIFFING technique are showed in Table 1. Different odors emanated from the heated soybean oil samples without antioxidants were related to the volatile compounds previously identified by GC/MS. Among these, heptanal had a slightly green odor, while 2,4-heptadienal, decenal, and 2,4-decadienal had a intense oily odor. These findings are in accordance with those from previous works which reported similar odor notes to heptanal (GASSER; GROSCH, 1988), 2,4-heptadienal, and 2,4-decadienal (ULLRICH; GROSCH, 1987) in different model systems.

Oil samples treated with 5-CQA provided less odoriferous notes in comparison with those without antioxidants while no odor was perceived when samples treated with quercetin were analyzed. Probably, the concentrations of the volatiles in the samples treated with quercetin were below the perception threshold.

4 Conclusion

The results mentioned above indicate the greater effectiveness of quercetin in delaying the formation of oxidative volatile compounds in soybean oil samples subjected to mild heating conditions. Apparently, the biopolyphenolic compounds used in the present work showed good oxidative stability since no new volatile compounds were detected in the heated samples treated with them.

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