Effect of tea catechin derivatives on stability of soybean oil/tea seed oil blend and oxidative stability of fried fish crackers during storage

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Abstract Effect of (–)-epigallocatechin gallate (EGCG) and (–)-epicatechin (EC) at different concentrations (100–300 ppm) on frying stability of soybean oil blended with tea seed oil was studied. Thermal stability of the blended oil increased with the addition of EGCG and EC, especially with increasing concentrations. Frying induced degradation of tocopherols and phenolics of oils, particularly tocopherols. Incorporation of catechin derivatives could retard tocopherol decomposition and formation of polar materials. The highest frying stability was found for the oil added with EC at 300 ppm. When the oil added with EC (300 ppm) was used to prepare fish crackers, lowered lipid oxidation of the resulting crackers than those prepared using the control oil was noted throughout 12 weeks of storage. EC could be effectively used as natural antioxidant in frying oil with carry through effect to enhance oxidation stability of the fried foods during a storage.

Keywords Soybean oil · Tea seed oil · (–)-Epigallocatechin gallate · (–)-Epicatechin · Deep frying · Fish crackers · Lipid oxidation

Introduction

Deep-frying using oils as heating media is a culinary process popularly implemented in both industrial and household levels. During the process, oil is exposed to elevated

temperature in the presence of moisture from food materials and the atmospheric oxygen. This extreme condition accelerates deterioration of oil through a series of chemical reactions, particularly oxidative reaction. Thermal stability of frying oils crucially affects characteristic, storage stability and safety of the fried foods. To enhance thermal stability of frying oils, antioxidants, particularly synthetic compounds have been widely used (Taghvaei and Jafari, 2015). However, the employment of these synthetic antioxidants becomes recently restricted due to consumer awareness on their safety.

Green tea is well known for its antioxidant properties, in which catechins and other flavonoids function as antioxidants (Taghvaei and Jafari, 2015). Catechins are accounted to 60–80% of total tea polyphenols, and the catechins predominantly observed in green tea are (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epigallocatechin gallate (EGCG) (Zhang et al., 2015). Among these compounds, EGCG is the most abundant component with the content more than 50% of total catechins of TSO, whereas EC contributed to ca. 3% of total catechins of TSO (Fazel et al., 2008). Being recovered from green tea with low cost, catechins are promising natural antioxidants that can be used in a wide range of products including foods, beverages, cosmetics and toiletries (Taghvaei and Jafari, 2015). By adding tea catechins, stability during frozen storage of bread dough was improved without negative effect on organoleptic properties of the products (Wang and Zhou, 2004). Moreover, tea catechins possess a wide range of bioactivities such as antioxidant, antiarteriosclerotic, anticarcinogenic, and antimicrobial activities, etc. (Wang and Zhou, 2004).

Heating adversely affects properties of antioxidants through several changes involving steam distillation, oxidation and polymerization with other food components

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(Fan et al., 2016). Antioxidants should have high thermal stability and also provide a protective effect toward the finished product, particularly during the storage. Higher heat tolerance of natural antioxidants compared to synthetic agents has been reported. Tea catechins could enhance stability of canola oil exposed to heating at 95 °C more effectively than BHT (Chen and Chan, 1996). Ability of catechins to improve frying stability of oils was expected due to their antioxidant capacity, particularly radical scavenging ability (Ananinsih et al., 2013). However, antioxidant efficiency of tea catechins depends on their structure, food system, and processing condition.

Soybean oil (SBO) is widely used as the frying oil. The blending with tea seed oil (TSO) rich in polyphenols could enhance the stability of SBO. Additionally, incorporation of catechin or derivatives was another means to improve the stability of oil and finished products. This work aimed to elucidate the effects of catechins on thermal stability of the SBO/TSO blended oil and on the oxidative stability of fried fish crackers during storage. EGCG and EC were selected as the representative of tea catechins.

Materials and methods

Materials

SBO (Thanakorn Vegetable Oil Products Co. Ltd., Samutprakarn, Thailand), TSO (Chaipattana Foundation, Chiangrai, Thailand), and raw fish crackers (Manora Food Industry Co. Ltd., Samutsakorn, Thailand) were purchased from a local market. SBO and TSO were used without further purification. EGCG, EC, butylated hydroxytoluene (BHT), thiobarbituric acid (TBA), and *p*-anisidine reagent were products of Sigma-Aldrich (St Louis, MO, USA). Iodine chloride and trichloroacetic acid were obtained from Carlo Erba Reagenti (Rodano, Italy). All chemicals were analytical grade, except for the reagents for HPLC analysis, which were of HPLC grade.

Sample preparation

Preparation of oil samples

Firstly, the blended oil was prepared by mixing SBO with TSO (60:40 *v/v*) in subdued light for 1 h. EC and EGCG were incorporated into the blended oils at different concentrations (100, 200 and 300 ppm). The catechins were firstly dissolved in a required amount of absolute ethanol, before adding to the oil and sonicating at 50 °C for 30 min. The oil added with the same amount of ethanol was used as a control. BHT was also added to the blended oil at the legally permitted level of 200 ppm (Chirinos et al., 2015).

The blended oil without antioxidant was referred to the control (Ct), and the blended oil incorporated with BHT was named as BHT200. The oils added with EC or EGCG at 100, 200 and 300 ppm were referred to EC100, EC200 and EC300 or EGCG100, EGCG200 and EGCG300, respectively.

Frying protocol

Frying was carried out using the wet cotton ball as a material. The oil (750 mL) was added to a frying pod (uncovered stainless steel pan, 16 cm i.d., 10 cm height) and pre-heated at 170 °C for 10 min. Five cotton balls (0.45 g cotton each containing 1.50 g distilled water) were fried in the oil for 3 min and then removed. The absorbed oil in the cotton balls was squeezed manually. Freshly prepared cotton balls were fried every 10 min for a total period of 2.5 h/day, referred to 1 frying cycle. After each cycle, the oil was allowed to stand overnight, and a required amount of oil was replenished to maintain the same level before starting a next cycle. The oil was heated for 3 consecutive days (3 repeated cycles) with a total heating time of 8 h. The oil was transferred into a screw capped bottle, flushed with N₂ and stored in the dark at – 20 °C, before being subjected to analyses.

Frying of fish crackers

Firstly, the selected oil was pre-heated at 170 °C for 10 min. Each batch (30 g) of crackers was fried for 1 min and drained in a frying basket for 1 min. A next batch of crackers was fried at 3 min interval, and 15 batches were processed in each day constituting 1 frying cycle. The frying was conducted for 3 repeated cycles, and the required amount of oil was replenished before beginning each cycle. The oil without antioxidants was also used for frying the crackers considered as the control. After frying, the crackers from each cycle were kept in polyethylene bag at room temperature (ca. 30 °C) for 12 weeks, before determining their characteristics at varying storage times.

Characterization of oil samples

Polyunsaturated fatty acid (PUFA) content

PUFA content of the oils was quantified using GC (Agilent technologies 7890A, Wilmington, DE, USA) equipped with a flame deionized detector and Varian's capillary column (VF-5 ms, length 30 m × width 0.25 mm with particle size of 0.25 µm; EZ-GRIPTM, Wilmington, CA, USA), following the procedure of Chowdhury et al. (2007).

Iodine value (IV) and acid value (AV)

IV and AV were measured using the standard methods No.cd 1–25 and ca 5a–40, respectively (AOCS, 1997).

Total oxidative degree (TOTOX)

TOTOX was calculated by the following equation (De Abreu et al, 2010).

$$TOTOX = 2PV + p - AV$$

where PV is peroxide value determined using the standard method No.cd 8–53 (AOCS, 1997), and *p*-AV is *para*-anisidine value quantified as per the method No. cd 18–90 (AOCS, 1994).

Total polar material (TPM) content

TPM was quantified using the standard method No.982.27 (AOAC, 2002).

Tocopherol content

Tocopherol was quantified using HPLC (Agilent Technologies 1200 series G1329A, Waldbronn, Germany) equipped with FLD detector and the analytical C18 column (Hypersil ODS, 5 µm spherical particles, 4.0 mm i.d. and 250 mm length; Agilent, Santa Clara, CA, USA) at the excitation and emission wavelengths of 295 and 325 nm, respectively. Total content of tocopherols was reported as mg γ-tocopherol Equiv/kg oil (Gliszczynska-Świątło and Sikorska, 2004).

Total phenolic content (TPC)

Firstly, phenolics were extracted from the oil using methanol:water (40:60, v/v) followed the method of Chen and Chan (1996). The extract was collected and the solvent was removed using a vacuum rotary evaporator (Eyela N-1000, Tokyo, Japan), before re-dispersing by methanol. The extract was filtered through a cellulose membrane (0.45 µm pored size) and introduced to the HPLC equipped with DAD detector and the analytical C18 column (Hypersil ODS, 5 mm spherical particles, 4.0 mm i.d. and 250 mm length; Agilent, Santa Clara, CA, USA). TPC was reported as mg gallic acid Equiv (GAE)/kg oil (Zhang et al., 2015).

Determination of storage stability of fried fish crackers

Oxidative stability of crackers

The residual oil absorbed in the crackers was extracted using hexane. Then, PV, *p*-AV, and TOTOX of the

extracted oils were measured as the aforementioned procedures, and content of thiobarbituric reactive substances (TBARs) was determined by the method of Aewsiri et al. (2009).

Volatile compound

Volatile compounds of the crackers were analyzed at the storage times of 6 and 12 weeks using a solid-phase microextraction (SPME) GC–MS, according to the method of Warner and Glynn (1997) with a slight modification. The crackers (ca. 3.5 g) were kept in a 6-mL glass headspace vial sealed with a Teflon-lined septum and aluminum cap. The sample was heated to 130 °C for 10 min to equilibrate. The fused silica fiber (75 µm CarboxenTM/PDMS Stable-flexTM; Supleco, Bellefonte, PA, USA) was pre-conditioned (270 °C for 10 min), before exposing to the headspace environment for 30 min to collect the volatile compounds. The volatile compounds were then introduced to the GC injector in a splitless mode at 270 °C for 10 min. The GC–MS analysis was conducted using the HP 5890 series II gas chromatography coupled with HP 5972 mass-selective detectors and equipped with a quadruple mass detector (Hewlett Packard, Atlanta, GA., USA). A HP-Innowax capillary column (30 m in length × 0.25 mm i.d., Hewlett Packard) was employed to separate the volatile compounds. The GC oven temperature was set at 35 °C for 3 min, followed by an increase to 70 °C (3 °C/min), then increase to 200 °C (10 °C/min), and finally increase to a final temperature of 250 °C (15 °C/min) and hold for 10 min. Helium was used as a carrier gas at a flow rate of 1 mL/min.

Identification of volatile compounds was performed based on the retention times by consulting ChemStation Library Search (Wiley 275L). Quantitative analysis was accomplished using an internal calibration curve with a single-to-noise ratio of 10. The volatile compounds related with lipid oxidation were identified as the normalized peak area of each compound using the software (G1701AA version A.03.00-HP 1986-1996, Chemstation, Agilent Technologies, Palo Alto, Calif., USA).

Statistical analysis

All measurements were run in triplicate, and mean values with standard deviations were shown. The results were statistically analyzed by analysis of variance (ANOVA) and the Duncan's multiple range test was used for mean comparison among the samples (SPSS 10.0 for windows, SPSS Inc., Chicago, IL, USA) at a 95% confidence level.

Results and discussion

Thermal stability of blended oils added with EC and EGCG

Table 1 shows the PUFA content and IV of the oils after frying for 8 h at 170 °C. As a result of frying, the decrease in PUFA was observed for all samples, which was in agreement with IV decreasing. Frying could destroy C=C of unsaturated fatty acids, mainly through oxidation and polymerization (Marmesat et al., 2012). When EC and EGCG were added, destruction of PUFA could be retarded in a dose dependent manner. After frying, lower decrease in PUFA was found for the oils added with EC (200 and 300 ppm) and EGCG (300 ppm), compared to the BHT (200 ppm) added oil. The EC300 showed the lowest decay of PUFA, which was coincidentally with its lowest decrease in IV as compared to other samples.

Frying can induce oil deterioration through a series of chemical reactions resulting in a formation of several compounds, so several indices should be determined to indicate quality changes of frying oils. Figure 1 shows AV, TOTOX, and TPM of the oils after frying. By adding EC and EGCG, thermal stability of the oils could be improved as indicated by the lowered AV and TOTOX of the EGCG and EC added oils than the Ct ($p \leq 0.05$). This might be due to antioxidant capacity of the catechins, particularly free radical scavenging ability, thereby retarding induction and propagation of lipid oxidation (Ananinsih et al., 2013).

However, difference in AV and TOTOX between the Ct and BHT200 after frying was not noticeable ($p > 0.05$).

TPM is considered as an appropriate index to evaluate quality of frying oil, because it has a good correlation with the oxidized fatty acids generated under frying condition (Sanibal and Mancini-Filho, 2004). After frying, the oils added with the catechin derivatives and BHT had lower TPM than the Ct ($p \leq 0.05$). The phenolic rich olive leaf extracts could successfully retard a formation of polar compounds in sunflower oil along frying at 180 °C for 5 days (Farag et al., 2007). At higher concentration (300 ppm), EGCG and EC could effectively lower TPM ($p \leq 0.05$), which was associated with the decreased oxidation degree of these oils. The lowest TPM was found for the EC300 ($p \leq 0.05$), implying the least polymerization degree. At 300 ppm, higher TPM was found for the oil added with EGCG than those with EC ($p \leq 0.05$), suggesting higher degree of heat induced polymerization of EGCG– than EC added oils. EGCG radicals could be easily oxidized by molecular oxygen to form O_2^- and EGCG quinone. The EGCG quinone was reactive to interact with another molecule of EGCG to form a dimer that could further transform to other compounds, particularly polymeric compounds (Hou et al., 2005).

The lowered AV, TOTOX and TPM were found for the oils incorporated with either EGCG or EC (300 ppm) than those added with BHT (200 ppm), indicating better thermal tolerance of the oils with added catechin derivatives. This might be owing to less thermal destruction of the catechins compared to BHT. EC and EGCG, with larger Mw and

Table 1 PUFA contents and IV of SBO/TSO blended oils before (0 h) and after (8 h) frying process

Sample	PUFA content (% of total fatty acids)		Relative decrease of PUFA after frying (%)	IV		Relative decrease of IV after frying (%)
	0 h	8 h		0 h	8 h	
Ct	25.25 ± 0.23A	18.35 ± 0.17B	27.33	109.2 ± 2.4A	67.6 ± 2.4C	37.98
BHT200	25.19 ± 0.31A	19.66 ± 0.22AB	21.95	108.3 ± 1.2A	70.7 ± 1.9A	34.71
EGCG100	24.98 ± 0.32Aab	18.77 ± 0.13ABc	24.86	108.1 ± 1.3Aa	70.3 ± 2.5Ab	34.97
EGCG200	25.16 ± 0.11Aa	19.34 ± 0.09Aab	23.13	108.3 ± 2.2Aa	72.0 ± 2.4Aa	33.52
EGCG300	25.22 ± 0.11Aa	19.72 ± 0.10Aa	21.81	108.6 ± 2.1Aa	71.7 ± 1.4Aa	33.98
EC100	25.10 ± 0.20Aa	19.32 ± 0.21Ab	23.03	108.1 ± 1.5Aa	70.0 ± 3.0Ab	35.25
EC200	25.00 ± 0.31Aab	19.74 ± 0.28Aab	21.04	107.9 ± 3.5Aa	71.9 ± 2.7Aab	33.36
EC300	25.21 ± 0.18Aa	19.98 ± 0.17Aa	20.74	108.3 ± 1.2Aa	72.4 ± 3.1Aa	33.15

Ct the blended oil, BHT200 the blended oil added with BHT at 200 ppm, EC the blended oil added with EC, EGCG the blended oil added with EGCG

Numbers (100, 200, and 300) denoted the concentrations of EC or EGCG (ppm)

Means ± standard deviations ($n = 3$) were present

In each tested parameter, the data between before and after frying were significantly different ($p \leq 0.05$)

Different capital letters in a same column indicate significant differences between samples ($p \leq 0.05$). Different small letters in a same column within the same catechin derivatives indicate significant differences ($p \leq 0.05$)

Fig. 1 (A) AV, (B) TOTOX and (C) TPM of SBO/TSO blended oils after frying for 8 h at 170 °C. Ct: the blended oil, BHT200: the blended oil added with BHT at 200 ppm, EC: the blended oil added with EC; EGCG: the blended oil added with EGCG. Numbers (100, 200, and 300) denoted the concentrations of EC or EGCG (ppm). Bars represent the standard deviation ($n = 3$). The different capital letters indicate significant differences between the samples ($p \leq 0.05$). The different small letters within the same catechin derivatives indicate significant differences ($p \leq 0.05$)

higher boiling point than BHT, were less volatilized or decomposed during frying (Chen et al., 1996). Better thermal stability of natural antioxidants than the synthetic agents was previously reported. By exposing to frying,

TBHQ (0.02%) exhibited a rather low antioxidant activity in soybean and sunflower oils and showed a pro-oxidant effect in corn oil, whereas rosemary extracts (0.2%) provided the effective antioxidant ability in all tested oils (Iqbal and Bhanger, 2007).

Inferior quality of oils due to frying can be related with loss of bioactive compounds of the oils. Table 2 shows the contents of tocopherols and TPC of the oils. After frying, the greater loss was obviously evident for tocopherols than phenolics. Frying accelerated deterioration of tocopherols mainly through oxidation reaction. Loss of tocopherols in virgin olive oils (ca. 70–88% loss) markedly occurred after frying at 180 °C for 15 min (Messina et al., 2009), whereas the tocopherols of canola oil were degraded entirely after 6 days of frying at 215 °C (Aladedunye and Przybylski, 2009). Thermal stability of tocopherols was varied depending on their structures and chemical compositions of the oil, in which greater decomposition generally observed in the oil with lower thermal stability (Andrikopoulos et al., 2003). The present result suggested that BHT and the catechins could retard tocopherol degradation. EC and EGCG retained tocopherols of the oils more effectively than BHT, especially at the higher concentrations. As a result of frying, a significant decrease of phenolics was also observed for all oils, which was in accordance with other study (Srivastava and Semwal, 2015). Retention of phenolics of the oils after frying could be achieved by adding BHT or the catechins. The lowest tocopherol and phenolic degradations were found for the EC300, which was coincidental with its lowest TPM as compared to the other samples [Fig. 1(C)].

EC showed greater ability in improving frying stability of the oils than EGCG. This might be plausibly due to the higher antioxidant capacity in bulk oil model and less thermal induced structural change of EC than EGCG. According to the presence of gallate residue substituted at C-3 position, catechins are classified into gallated catechin (EGCG) and non-gallated catechin (EC). Moreover, catechins can be classified into pyrogallol type (EGCG) and non-pyrogallol type (EC) according to the additional hydroxyl group at B-5' position (Braicu et al., 2013). Chemical structures involving balance between hydrophilicity and hydrophobicity as well as the total number and location of hydroxyl groups on the aromatic rings crucially influence antioxidant ability of phenolic compounds (Chen and Chan, 1996). Due to the presence of gallate moiety, EGCG possesses higher hydrophilicity than EC and has lower solubility in lipophilic environment, resulting in less antioxidant ability of EGCG than EC in bulk oil system (Ananinsih et al., 2013).

Thermal process can induce structural changes of antioxidants through several chemical reactions involving hydrolysis, epimerization and oxidation/condensation,

Table 2 Tocopherol contents and TPC of the oil samples before (0 h) and after (8 h) frying process

Samples	Total tocopherols (mg γ -tocopherol Equiv/kg oil)		Relative decrease of tocopherols after frying (%)	TPC (mg GAE/kg oil)		Relative decrease of TPC after frying (%)
	0 h	8 h		0 h	8 h	
Ct	708.42 $\pm$ 13.33A	217.92 $\pm$ 11.25C	69.2	101.71 $\pm$ 3.22D	64.18 $\pm$ 5.03F	36.9
BHT200	717.36 $\pm$ 9.60A	289.27 $\pm$ 13.01B	59.7	310.28 $\pm$ 3.69B	209.74 $\pm$ 2.39D	32.4
EGCG100	748.03 $\pm$ 21.61Aab	319.98 $\pm$ 5.67Bbc	57.2	213.98 $\pm$ 7.01Cc	138.97 $\pm$ 1.89Ec	35.1
EGCG200	759.38 $\pm$ 9.50Aa	343.35 $\pm$ 14.63ABab	54.8	311.46 $\pm$ 3.98Bb	222.52 $\pm$ 9.11Db	28.6
EGCG300	749.77 $\pm$ 10.02Aab	372.60 $\pm$ 20.06Aa	50.3	458.00 $\pm$ 8.16Aa	351.25 $\pm$ 3.33Ba	23.3
EC100	674.74 $\pm$ 17.63ABb	299.07 $\pm$ 26.90Bc	55.7	212.81 $\pm$ 11.19Cc	144.16 $\pm$ 4.66Ec	32.3
EC200	716.23 $\pm$ 14.79Bab	327.90 $\pm$ 13.12ABab	54.2	336.83 $\pm$ 11.55Bb	255.03 $\pm$ 10.22Cb	24.3
EC300	678.02 $\pm$ 29.81ABb	348.80 $\pm$ 21.32ABa	48.6	471.95 $\pm$ 12.02Aa	378.11 $\pm$ 3.06Aa	19.9

Ct the blended oil, BHT200 the blended oil added with BHT at 200 ppm, EC the blended oil added with EC, EGCG the blended oil added with EGCG

Numbers (100, 200, and 300) denoted the concentrations of EC or EGCG (ppm)

Means $\pm$ standard deviations ($n = 3$) were present

In each tested parameter, the data between before and after frying were significantly different ($p \leq 0.05$)

Different capital letters in a same column indicate significant differences between samples ($p \leq 0.05$). Different small letters in a same column within the same catechin derivatives indicate significant differences ($p \leq 0.05$)

thereby diminishing their antioxidant capacity (Ananinsih et al., 2013; Fan et al., 2016). The hydrolysis was regarded as a typical heat induced structural change occurring to the pyrogallol catechins (Fan et al., 2016). After heating at 90 °C for 8 h, the percentage of hydrolysis conversion occurring for EGCG was 12.9%, whereas EC had a negligible hydrolysis conversion (Fan et al., 2016). Therefore, the larger phenolic degradation due to frying might be expected for the oils added with EGCG than those added with EC (Table 2).

The epimerization of catechins was initiated at the temperature above 60 °C, and markedly enhanced by heating (Fan et al., 2016). Catechins underwent structural change by converting epistucture to non-epistucture through the epimerization, in which EGCG and EC were transformed to gallocatechin gallate (GCG) and catechin (C), respectively (Chen and Chan, 1996). Greater heat induced structural change through the epimerization was reported for EGCG than EC (Fan et al., 2016). Moreover, GCG, which was converted from EGCG, could further transform into other products or radicals (Hou et al., 2005). Therefore, the lower thermal stability might be expected for the EGCG– than EC added oils.

During frying, oil is exposed to the atmospheric oxygen, thereby accelerating opportunity for oxidation/condensation of antioxidants by the active oxygen. EGCG and EC could tolerate thermal process at 90 °C within 0.5 h, whereas the prolonged heating from 0.5 to 4 h greatly induced oxidation/condensation of EGCG and EC (Fan et al., 2016). Due to a presence of gallic residue of EGCG,

thermal oxidation could be promoted, because gallic acid-related radicals were preferably formed as a common breakdown product generated by oxidation (Sharma and Zhou, 2011).

Since the EC300 showed the highest thermal stability, it was selected as the heating medium for frying fish crackers.

Storage stability of fish crackers prepared using the EC added oil

Figure 2 shows oxidative indices during storage of the crackers prepared using EC300 and Ct as frying media for several repeated frying cycles. With increasing numbers of frying cycle, lipid oxidation proceeded to a higher extent, suggesting the inferior quality of the crackers prepared using repeatedly used oils. Irrespective of frying cycles, higher oxidative stability was observed when the crackers were prepared using the EC300 compared to those fried with the Ct. PV of the crackers prepared by the Ct was increased initially, whereas PV of the crackers prepared using the EC300 showed 3 days of the induction period. Moreover, less formation of TBARs, *p*-AV, and TOTOX were noticeable for the crackers prepared using the EC300 than those fried with the Ct.

Volatile compounds of the crackers were determined (Table 3). Thermal process induced oxidation and degradation of lipids, resulting in a formation of volatile compounds such as hydrocarbon, aldehydes, enals, dienals, ketones and organic acids (Matthäus, 2006). Carbonyl compounds were important products of oxidation strongly

Fig. 2 Storage time dependence on (A) PV, (B) p-AV, (C) TBARs and (D) TOTOX of the fish crackers prepared using the SBO/TSO blend (the open symbols with broken lines) and the blended oil added

relating with a rancid odor perception in food product (Messina et al., 2009). From Table 3, carbonyl compounds increased with increasing storage time and frying cycles, indicating higher degree of lipid oxidation. The total carbonyl contents of the crackers prepared using the EC300 were lower than those fried with the Ct. The total carbonyl compounds of the crackers prepared using the Ct with the 1st (3rd) frying cycle were 6.82 and 12.47 (9.84 and 20.08) after 6 and 12 weeks, respectively. The carbonyl compounds of the crackers fried with the EC300 with the 1st (3rd) frying cycle were 5.12 and 10.80 after (6.56 and 11.36) after 6 and 12 weeks, respectively. This tendency was in agreement with lower TBARs of the crackers prepared using the EC300 [Fig. 2(C)]. TBARs is the index determining secondary products of oxidative deterioration including aldehydes and ketones (Seppanen and Csallany, 2002).

Hexanal is a degradation product of linoleic acid and belongs to the major volatile constituents identified in frying oils containing linoleic acids such as SBO (Matthäus, 2006), and could be properly used to evaluate oxidative deterioration of fried food (Warner and Gupta, 2005). The increase in hexanal content of the crackers was

with EC at 300 ppm (the closed symbols with solid lines) with different repeated frying cycles (circle 1st cycle, triangle 2nd cycle and square 3rd cycle)

predominantly observed among aldehyde compounds, especially when storage time and number of frying cycles were increased. When EC300 was used as a frying medium, the lowered hexanal content of the crackers was observed, compared to those fried with the Ct. This result was correlated well with the lower p-AV of the crackers prepared using EC300 than those prepared with the Ct [Fig. 2(B)]. A good positive correlation between hexanal content and p-AV development was reported for the SBO exposed to frying process (Tompkins and Perkins, 1999). Hexanal had important role on rancid flavor perception in potato chips (Warner and Gupta, 2005). Therefore, less rancid flavor development during storage might be supposed to the crackers prepared using the EC added oil.

Linear aliphatic- (Ramírez and Cava, 2007) and short chain acids (Aladedunye and Przybylski, 2009) are considered as the secondary products of lipid oxidation generated through a degradation of lipid hydroperoxides. Development of volatile acids of the crackers took place with increasing storage times and numbers of frying cycles. Upon storage, increases in total acid content of the crackers prepared using the Ct was higher than the samples prepared by the EC300. With 3rd frying cycle, the total acid content

Table 3 Contents (% relative percentage) of volatile compounds present in the crackers fried using the SBO/TSO blend (Ct) and the blended oil added with EC at 300 ppm (EC300)

Compounds	Cont rol ^a	Crackers prepared by Ct				Crackers prepared by EC300			
		1st frying cycle		3rd frying cycle		1st frying cycle		3rd frying cycle	
		6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks
<i>Hydrocarbons</i>									
Pentane	0.41	0.40	<i>na</i>	<i>na</i>	<i>na</i>	9.28	7.80	1.12	0.74
2,4 Dimethyl heptane	0.35	<i>na</i>	<i>na</i>	1.12	0.83	<i>na</i>	<i>na</i>	1.35	1.29
4-Methyl octane	<i>na</i>	<i>na</i>	<i>na</i>	1.61	1.17	0.23	<i>na</i>	1.74	1.47
Dodecane	<i>na</i>	<i>na</i>	<i>na</i>	2.10	1.92	<i>na</i>	<i>na</i>	0.58	1.22
4,5-Dimethyl nonane	<i>na</i>	<i>na</i>	<i>na</i>	0.75	0.85	0.22	<i>na</i>	1.37	0.46
Pentadecane	<i>na</i>	<i>na</i>	<i>na</i>	0.60	0.55	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
7-Methyl-3-methylene-1,6-octadiene	1.71	2.15	2.76	2.37	2.10	2.32	2.75	2.80	2.34
1-Methyl-4-(1-methylethenyl)-cyclohexene	<i>na</i>	18.53	19.33	16.31	<i>na</i>	<i>na</i>	22.61	16.74	15.11
2-Methyl-5-(1-methylethenyl)-1,3-cyclohexene	<i>na</i>	0.47	0.46	<i>na</i>	<i>na</i>	<i>na</i>	1.65	0.57	0.57
Benzene	0.49	0.58	0.17	<i>na</i>	<i>na</i>	0.24	0.29	<i>na</i>	<i>na</i>
Methyl-benzene	10.01	<i>na</i>	9.79	4.10	<i>na</i>	4.86	3.35	<i>na</i>	3.56
1,2,3,4-Tetramethyl-benzene	3.47	4.20	3.99	4.07	<i>na</i>	3.12	4.42	4.40	4.14
1-Methylethyl cyclopropane	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	0.27	<i>na</i>	<i>na</i>	14.62
1-Methyl-4-(1-methylethylidene)-cyclohexene	<i>na</i>	0.33	0.35	<i>na</i>	15.1	16.13	18.59	<i>na</i>	<i>na</i>
Dihydro-2(3 <i>H</i>)-furanone	0.90	0.79	0.65	1.91	<i>na</i>	0.97	0.89	1.89	1.76
Dihydro-2,5-furadione	<i>na</i>	<i>na</i>	0.52	<i>na</i>	1.83	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
3,5-Octadiene-2-one	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	0.59	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
<i>Aldehydes</i>									
3-Methyl-butanal	0.39	0.46	<i>na</i>	<i>na</i>	<i>na</i>	0.26	0.27	<i>na</i>	<i>na</i>
Hexanal	0.23	1.53	2.70	5.26	10.33	0.53	1.69	1.86	3.78
Heptenal	0.42	0.41	0.29	<i>na</i>	0.54	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
2,4-Heptadienal	0.32	0.33	0.23	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
Pentanal	<i>na</i>	0.78	0.88	0.96	1.78	0.56	0.66	0.73	0.69
Propanal	<i>na</i>	<i>na</i>	1.40	<i>na</i>	0.69	<i>na</i>	1.01	1.16	<i>na</i>
2-Furan-carboxaldehyde	<i>na</i>	<i>na</i>	<i>na</i>	0.76	0.72	0.24	0.24	0.84	0.77
<i>Alcohols</i>									
1-Acetoxy-2-propanol	0.44	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
1-Methoxy-2-propanol	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	0.53
3-Methyl-1-butanol	0.30	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
3,5-Dimethylpyeazole-1-methanol	0.31	0.31	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
1-Pentanol	<i>na</i>	0.46	0.42	0.59	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
1-Hexanol	<i>na</i>	<i>na</i>	0.28	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
3-Furanmethanol	<i>na</i>	<i>na</i>	<i>na</i>	0.83	0.84	0.24	0.23	0.87	0.83
<i>Acids</i>									
Propanoic acid 20.43	0.95	0.78	0.85	0.82	1.27	0.70	0.68	0.67	0.66
Butanoic acid 21.69	1.70	1.08	1.01	0.78	0.83	1.04	0.94	0.89	0.84
Pentanoic acid 23.06	0.29	<i>na</i>	0.18	<i>na</i>	0.45	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
Hexanoic acid 24.29	0.65	0.43	0.34	0.92	2.03	0.40	0.30	0.53	0.58
<i>Esters and ethers</i>									
Octanoic methyl ester	0.42	0.30	<i>na</i>	<i>na</i>	<i>na</i>	0.25	<i>na</i>	0.42	<i>na</i>
1-Methyl-4-(1-methylethyl)-cyclohexadiene	0.32	0.40	0.45	0.49	0.6	0.39	0.49	0.50	0.48

Table 3 continued

Compounds	Cont rol ^a	Crackers prepared by Ct				Crackers prepared by EC300			
		1st frying cycle		3rd frying cycle		1st frying cycle		3rd frying cycle	
		6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks
2-Pinene	2.08	2.12	1.60	0.93	0.84	1.56	1.47	1.10	1.00
2(10)-Pinene	3.02	2.85	1.80	0.00	1.17	2.17	1.88	1.44	1.33
3-Thujene	0.29	0.35	0.69	1.27	<i>na</i>	0.36	0.44	<i>na</i>	<i>na</i>
4(10)-Thujene	0.56	0.52	1.68	<i>na</i>	<i>na</i>	0.45	0.70	0.78	0.55
3-Carene	30.81	32.61	23.45	14.62	15.0	24.80	<i>na</i>	16.40	<i>na</i>
Phellandrene	3.14	4.37	4.93	4.33	3.9	4.16	5.68	5.31	4.35
δ -Elemene	1.27	1.68	1.56	1.70	1.75	1.53	1.72	1.52	1.54
α -Cubebene	0.40	0.39	0.35	0.54	0.51	0.62	0.37	<i>na</i>	<i>na</i>
Caryophyllene	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	5.83
4,11,11-Trimethyl-8-methylene bicycle undec-4-ene	0.40	0.37	0.29	0.51	0.50	0.33	0.31	0.50	0.42
4,11,11-Trimethyl-8-methylene bicyclo undec-4-ene	8.73	8.83	7.22	6.81	6.50	7.05	7.15	5.98	5.83
1,5,9,9-Tetramethyl-Z,Z,Z-1,4,7-cycloundecatriene	0.40	0.41	0.38	<i>na</i>	<i>na</i>	0.36	0.39	<i>na</i>	<i>na</i>
<i>Ketones</i>									
2-Propanone	1.50	1.42	1.99	0.71	0.62	0.80	<i>na</i>	0.97	0.74
3-Hydroxy-2-butanone	0.33	<i>na</i>	0.22	<i>na</i>	<i>na</i>	0.25	<i>na</i>	<i>na</i>	<i>na</i>
1-Hydroxy-2-propanone	1.03	0.78	0.96	1.91	2.20	1.19	1.21	2.59	2.44
3A,4,5,6-Tetrahydro-2(3H)-pentalenone	0.32	0.32	<i>na</i>	0.96	0.78	0.32	0.59	0.76	1.18
Formic pentyl ester	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	1.12	0.24	0.26	<i>na</i>	0.43
Dodecyl octyl ether	<i>na</i>	<i>na</i>	<i>na</i>	0.54	0.48	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
Nitrogen and sulfur containing compounds									
<i>N,N</i> -Dimethyl methanamine	5.05	<i>na</i>	<i>na</i>	0.61	0.52	<i>na</i>	<i>na</i>	<i>na</i>	0.69
<i>N</i> -Methyl formamide	<i>na</i>	<i>na</i>	0.90	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	0.67	0.89
<i>N,N</i> -Dimethyl-formamide	<i>na</i>	<i>na</i>	0.24	<i>na</i>	0.76	<i>na</i>	0.32	<i>na</i>	0.55
<i>N,N</i> -Dimethyl acetate	<i>na</i>	<i>na</i>	0.66	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
Acetonitrile	0.40	0.31	0.29	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
2-Pyridinamine	3.58	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
2,5-Dimethyl pyrazine	0.78	0.78	0.41	1.88	1.58	0.91	0.84	1.83	1.64
Ethyl-pyrazine	0.42	0.36	0.24	1.25	1.08	0.40	0.34	1.26	1.08
2,3-Dimethyl pyrazine	0.32	0.28	0.28	1.03	0.85	0.26	0.25	0.86	0.77
Pyrazine	<i>na</i>	0.32	<i>na</i>	0.63	0.52	<i>na</i>	0.22	0.83	0.69
Methyl pyrazine	<i>na</i>	<i>na</i>	2.47	10.00	8.10	3.87	<i>na</i>	11.27	9.99
2-Ethyl-3,5-dimethyl pyrazine	<i>na</i>	<i>na</i>	<i>na</i>	0.45	0.46	<i>na</i>	<i>na</i>	0.41	0.40
3,5-Dimethylpyrazole-1-methanol	<i>na</i>	0.31	0.26	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
1-Ethenyl-1H-imidazole	<i>na</i>	2.97	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	3.11	<i>na</i>	<i>na</i>
2-Propen-1-ol	<i>na</i>	0.40	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>
2-Oxo-propionamide	<i>na</i>	<i>na</i>	0.24	<i>na</i>	<i>na</i>	<i>na</i>	0.21	<i>na</i>	<i>na</i>
4,5-Dimethyl pyrimidine	<i>na</i>	<i>na</i>	<i>na</i>	0.82	0.71	0.30	0.26	0.72	0.82
Allyl methyl sulfide	0.72	<i>na</i>	0.33	<i>na</i>	<i>na</i>	0.25	0.26	0.50	0.49
Diallyl disulfide	0.82	0.87	0.72	0.61	0.56	0.69	0.67	0.71	0.66

The oils were used for 1 and 3 repeated frying cycles, and the crackers were kept for 6 weeks and 12 weeks

na not available

^aControl: The crackers fried in the Ct without any storage time

of the crackers fried using the Ct was 2.52 and 4.58 after 6 and 12 weeks, whereas the acid content of the crackers prepared using the EC300 was almost constant throughout 12 weeks.

By using the EC300 as frying medium, oxidative stability of the crackers could be successfully enhanced. This might be due to better thermal tolerance of the EC added oil with higher retained tocopherols and phenolics. These antioxidative compounds might be transferred to the crackers via oil adsorption during frying, thereby improving oxidative stability of the crackers. Loss of antioxidants during heating is one of important factors contributing to loss in antioxidant activity of the compounds (Chen and Chan, 1996).

Incorporation of EGCG and EC could improve thermal stability of SBO/TSO blended oil as indicated by lower TPM and TOTOX. Degradation of bioactive compounds occurred as a result of frying, and the higher decomposition was found for tocopherols than phenolics. Addition of EC or EGCG could retard thermal degradation of the bioactive compounds. The highest thermal stability was found for the oil added with EC (300 ppm). When the oil with EC (300 ppm) was used for preparation of fish crackers, higher oxidative stability of the crackers was attained throughout 12 weeks of storage, compared to those prepared using the blended oil without EC. Therefore, EC recovered from green tea could be used as a potential natural antioxidant in frying oil.

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Conflict of interest The authors declare no conflict of interest.

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