

BIOACTIVE PROPERTIES AND ANTIOXIDANT CAPACITY OF OILS EXTRACTED FROM CITRUS FRUIT SEEDS

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This study aimed at identifying the presence of bioactive compounds and evaluating the antioxidant capacity of oils extracted from citron, kinkan, and orange. Therefore, determination of fatty acid profile, tocopherol and phytosterol composition, phenolic compounds, and total carotenoids was performed in the oils extracted from seeds. To determine the antioxidant capacity, the tests DPPH[•], ABTS^{•+}, FRAP, β -carotene/linoleic acid, and oxidative stability were performed. The citrus fruit seeds presented high lipid level, with predominance of unsaturated fatty acids, especially linolenic essential fatty acid, with 7.9 and 8.5% in citron and kinkan seed oils, respectively. The presence of tocopherols and phytosterols induced a higher antioxidant capacity on the oils analysed. The collected information might help new alternative sources of vegetable oils obtained from agroindustrial waste to serve as raw material for food, chemical, and pharmaceutical industries.

Keywords: citrus fruit, extraction, vegetable oils, bioactive compounds, antioxidants, oxidative stability

Daily, a large volume of waste from fruit is generated by the agroindustry. Such waste has its use limited to animal feed industry or is disposed of in the environment, causing serious environmental damages. Faced with this inevitable generation of waste by food processing industries, several researches aim to find alternatives to better utilising such materials through the search for compounds of interest, such as antioxidants, vitamins, mineral nutrients, and phytosterols (BABBAR et al., 2011). These compounds can be isolated and directed to food industry for elaboration of functional foods, or even to the pharmaceutical industry for production of cheaper medicines. With the intention of obtaining alternative and cheaper sources of oils, many studies seek raw materials that provide oils of lipid profile that is similar to those of traditionally consumed oils and that are also alternative sources of phytosterols and phenolic compounds (DIAS et al., 2013). In this context, citrus fruit such as citron (*Citrus medica* L.), kinkan (*Fortunella margarita* Swingle), and orange (*Citrus sinensis* L. Osbeck) may be cited.

Recent researches have investigated citrus fruit seed oils, including orange and lemon (GÜNEŞER & YILMAZ, 2017a; 2017b). It demonstrates that this type of waste is source of high quality oils. The differences between these publications and the present work are in the method used to obtain the oil, the varieties used, the investigated compounds, and the methods applied.

The presence of bioactive compounds, such as essential fatty acids, phytosterols, tocopherols, phenolic compounds, and carotenoids, make the oils extracted from fruit seeds become alternative sources of oils with functional properties, that is, which reduce the risk of

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diseases if ingested frequently (ALU'DATT et al., 2013). In this context, the present study aimed at evaluating the oils extracted from citron, kinkan, and bitter orange disposed seeds, at the level of bioactive compounds, fatty acid profile, tocopherol and phytosterol composition, phenolic compounds, and total carotenoids. To determine the antioxidant capacity, the tests DPPH[•], ABTS^{•+}, FRAP, β -carotene/linoleic acid, and oxidative stability were performed.

1. Materials and methods

1.1. Materials

The fruit waste was obtained from Brazilian agroindustries located in the state of São Paulo, Southeastern Brazil. The collection of at least two lots of samples was performed between January 2011 and March 2012. The seeds of citron (*Citrus medica* L.), kinkan (*Fortunella margarita* Swingle), and orange (*Citrus sinensis* L. Osbeck) were separated from the residues, washed with running water, and dried in oven with air flow at 40 °C until the seeds reached humidity inferior to 10%. After dried, the seeds were packed in glass flasks protected from light and stored at room temperature. The oils were extracted by cold extraction with chloroform, methanol, and water in the proportion 2:1:0.8 (v/v/v), respectively, according to the method of BLIGH and DYER (1959). The obtained oils were packed in amber glass flasks, flushed with nitrogen gas, and stored until analyses.

1.2. Methods

1.2.1. Fatty acids. The lipid fractions of seeds (100 mg) were transesterified in methyl esters using a methanolic solution of potassium hydroxide and *n*-hexane (AOCS, 2009). The fatty acid methyl esters were analysed by gas chromatography GC 3900 (Varian Inc., Walnut Creek, CA, USA), equipped with flame ionization detector, split injector, automatic sampler, and fused silica capillary column CP-Sil 88 (60 m \times 0.25 mm i.d., 0.20 μ m film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA). The programming of the column temperature was initiated at 90 °C, for 4 min, heated at 10 °C min⁻¹ until it reached 195 °C, and kept isothermal for 16 min. The temperatures of the injector and the detector were 230 °C and 250 °C, respectively. The carrier gas was hydrogen, with speed of 30 ml min⁻¹. The fatty acids were identified by comparison between retention times of pure standards of methyl esters and separated sample compounds, and quantification was performed by normalization of the area. A mixture composed by 37 fatty acid esters (Supelco, Bellefonte, USA) from C4:0 to C24:1 was used as standard, with degree of purity between 99.1 and 99.0%. The amount of each fatty acid was expressed as percentage (%).

1.2.2. Tocopherols. The tocopherols were separated and quantified by high efficiency liquid chromatography through the method Ce 8-86 of AOCS (2009). Four hundred milligrams of oil were weighed in volumetric flask of 10 ml, and the volume was completed with *n*-hexane. An aliquot of 20 μ l of this solution was injected into the high efficiency liquid chromatograph (Varian Inc., Walnut Creek, CA, USA) equipped with fluorescence detector and stainless steel column packed with silica (LiChrosorb Si 100, 250 \times 4,6 mm i.d., particles of 5 μ m, Varian Inc., Walnut Creek, 121 CA, USA). Operation conditions: excitation wavelength of 290 nm and emission wavelength of 330 nm, chromatographic separation performed by isocratic elution of mobile phase constituted of *n*-hexane:isopropyl alcohol

(95.5:0.5) with flow of 1.2 ml min⁻¹. The tocopherols were identified by comparison with the retention time of pure standards, analysed under the same conditions as the samples. The quantification of each isomer was carried out by external standardization based on the peak areas, using standards of α -, β -, γ -, and δ -tocopherol (Supelco, Bellefonte, USA) with degrees of purity of 99.9, 98.0, 99.4, and 99.6%, respectively. The contents of tocopherols were expressed as mg kg⁻¹ of oil.

1.2.3. Phytosterols. The phytosterol composition was determined by gas chromatography with previous saponification of the sample (50–80 mg), which was performed according to DUCHATEAU and co-workers (2002). The analysis was carried out in gas chromatograph CG-2010 Plus (Shimadzu, Tokyo, Japan) with flame ionization detector, split injector, and automatic sampler. Analysis conditions: fused silica capillary column (30 m \times 0.25 mm i.d.), 138 and film thickness of 0.25 μ m (Restek RTX 5, Shimadzu, Tokyo, Japan). The programming of the column temperature was initiated at 100 °C for 2 min, heated at 15 °C min⁻¹ until 260 °C, and kept isothermal for 35 min. Injector and detector temperatures were 280 and 320 °C, respectively. The carrier gas was hydrogen with linear velocity of 40 ml min⁻¹. Samples of 1.0 μ l were injected with flow division of 1:50. The phytosterols were identified by comparison with the retention time of pure standards, analysed under the same conditions as the samples. The quantification of each isomer was performed by internal standardization (β -cholestanol = 5 α -cholestan-3 β -ol, degree of purity of 95%), based on the peak areas, using standards of cholesterol, campesterol, stigmasterol, β -sitosterol, and stigmastanol (Supelco, Bellefonte, USA) with degrees of purity of 99, 99, 95, 98, and 97.4%, respectively. The contents of individual phytosterols were expressed as mg by 100 g of oil (mg/100 g).

1.2.4. Phenolic compounds by HPLC. An aliquot of 1.0 g of oil was agitated in vortex with 3 ml of methyl alcohol. After that, the aliquot was centrifuged at 3000 r.p.m. for 10 min, and the supernatant was collected (PARRY et al., 2005). This procedure was repeated 3 times. The supernatants were combined, and the solution was injected into the high efficiency liquid chromatograph (model Prominence, Shimadzu, Nakagyo-ku, Kyoto, Japan) with diode array detector and manual injector (KIM et al., 2006). Analysis conditions: analytical column 5C18 (250 \times 4.6 mm i.d., particles of 5 μ m, Nakagyo-ku, Kyoto, Japan), mobile phase constituted of 2% acetic acid in water (v/v) (solvent A) and methanol (solvent B). Gradient programming: 100–70% A in 30 min, 70–0% A in 10 min, and 0–100% A in 10 min; 1.2 ml min⁻¹ flow and 20 μ l injection volume. The quantification of each isomer was performed by external standardization based on the areas of the peaks. The phenolic compounds were expressed in mg kg⁻¹.

1.2.5. Total carotenoids. The content of total carotenoids was performed by scanning spectrophotometer according to the methodology described by RODRIGUEZ-AMAYA (2001). The quantification was performed by absorption in the wavelength of maximum absorption and with absorptivity value of 2592, in petroleum ether. The values were expressed as μ g of β -carotene per gram of oil (μ g β -carotene/g).

1.2.6. Antioxidant capacity measurements. DPPH[•]: absorbance readings were performed at 517 nm. EC₅₀ value was expressed in mg ml⁻¹ (BRAND-WILLIAMS et al., 1995; KALANTZAKIS et al., 2006).

ABTS⁺ radical method: as reference, synthetic antioxidant Trolox was used in concentrations from 0.05 to 2.0 mmol l⁻¹ (RE et al., 1999; PELLEGRINI et al., 2001).

FRAP (Ferric Reducing Antioxidant Power) method: the absorbance reading was performed at 595 nm. The antioxidant capacity result was expressed as μmol ferrous sulphate/100 g of oil (SZYDLOWSKA-CZERNIAK et al., 2011).

β -Carotene/linoleic acid method: the absorbance at 470 nm was monitored every 15 min, for 2 hours, with the tubes kept in water bath at 50 °C during the readings (MARCO, 1968; MILLER, 1971).

1.2.7. Oxidative stability index The oxidative stability index was determined according to the method Cd 12b-92 proposed by AOCS (2009) by using the Rancimat instrument (Metrohm Ltd., Herisau, Switzerland). Oil samples (3.0 g) were transferred to reaction tubes and subjected to oxidation at a temperature of 100 °C, with 20 l h⁻¹ air flow. The induction period was determined in hours.

1.3. Statistical analysis

The analytical determinations were carried out in triplicate and the results presented correspond to the mean followed by standard deviation. The results obtained from the analytical determinations were submitted to variance analysis and to Tukey's test at 5% significance ($P > 0.05$) by using the Statistica program (Statsoft Inc., Tulsa, USA) version 7.0.

2. Results and discussion

2.1. Fatty acid profile

The lipid fractions of citron, kinkan, and bitter orange were, respectively, 25.5, 34.1, and 40.5%. The fatty acid profile of oils is given in Table 1. Among the saturated fatty acids, palmitic acid is predominant in vegetable oils, and its presence in high quantities is desirable from the technological point of view because of its high oxidative stability.

Table 1. Fatty acid composition of citrus seed oils

	Citron	Kinkan	Orange
Fatty acid (%)			
C16:0	19.8±0.1 ^c	20.6±0.0 ^b	26.2±0.1 ^a
C16:1	0.7±0.0 ^a	0.3±0.0 ^c	0.5±0.0 ^b
C18:0	4.3±0.0 ^c	6.0±0.0 ^a	5.8±0.0 ^b
C18:1 n-9	31.4±0.1 ^a	21.4±0.0 ^c	26.5±0.0 ^b
C18:2 n-6	35.2±0.0 ^c	42.5±0.0 ^a	37.4±0.0 ^b
C18:3 n-3	7.9±0.0 ^b	8.5±0.0 ^a	3.1±0.0 ^c
C20:0	0.2±0.0 ^b	0.4±0.0 ^a	0.4±0.0 ^a
SFA	24.3±0.1 ^c	27.0±0.0 ^b	32.4±0.1 ^a
PUFA	43.1±0.0 ^c	51.0±0.0 ^a	40.5±0.0 ^b

Means \pm standard deviation of the analyses performed in triplicate followed by the same letter do not differ by Tukey's test ($P > 0.05$). SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids

The oils of citrus fruit seeds showed to be predominantly unsaturated, with an average of 72% of unsaturated fatty acids, especially linoleic (n-6) and linolenic (n-3) essential fatty acids. The levels of essential fatty acid C18:3 were similar to what established by CODEX ALIMENTARIUS COMMISSION (2009) for soybean oil (4.5–11%). Essential fatty acids are important for the prevention of cardiovascular diseases because of their antithrombotic and anti-inflammatory properties.

2.2. Bioactive compounds

The total quantity of these compounds in the lipid samples analysed ranged from 143.74 to 166.61 mg/100 g, of which around 80% is represented by β -sitosterol (Table 2). Regarding fruit seed oil, NYAM and co-workers (2009) found 477.25 mg/100 g of total phytosterols in bitter melon and 864.69 mg/100 g in pumpkin. Among the tocopherol homologs, only α -tocopherol was detected. ARANHA and JORGE (2013) studied the seed oils of four different orange varieties and also quantified only α -tocopherol with 135.50, 134.07, 137.43, and 135.63 mg kg⁻¹ in the varieties Hamlin, Natal, Pera, and Sweet, respectively.

Table 2. Bioactive compounds of citrus seed oils

	Citron	Kinkan	Orange
Phytosterol (mg/100 g)			
Cholesterol	0.91±0.01 ^b	nd	1.01±0.01 ^a
Campesterol	33.16±0.02 ^a	15.24±0.02 ^b	10.30±0.01 ^c
Stigmasterol	nd	10.89±0.02	nd
β -Sitosterol	132.53±0.02 ^b	117.62±0.02 ^c	146.90±0.02 ^a
Σ	166.61±0.02 ^a	143.74±0.02 ^c	158.21±0.02 ^b
Tocopherols (mg kg ⁻¹)			
α -Tocopherol	57.53±0.40 ^b	nd	153.67±0.06 ^a
Phenolic compounds (mg kg ⁻¹)			
<i>p</i> -Coumaric acid	35.04±0.64 ^a	7.13±0.76 ^b	tr
Salicylic acid	49.46±0.28 ^a	30.70±1.51 ^b	5.60±0.24 ^c
Quercetin	47.18±0.35 ^a	6.53±0.16 ^b	tr
Totals	131.68±0.48 ^a	44.36±0.94 ^b	5.60±0.24 ^c
Totals carotenoids (μ g β -carotene/g)	6.85±0.49 ^c	21.64±0.21 ^a	7.85±0.21 ^b

Means \pm standard deviation of the analyses performed in triplicate followed by the same letter do not differ by Tukey's test ($P>0.05$). nd: not detected. Detection limit of cholesterol ≤ 0.65 mg/100 g; stigmasterol ≤ 5.60 mg/100 g; α -tocopherol ≤ 3.15 mg kg⁻¹. tr: lower than 5 mg kg⁻¹ (*p*-coumaric acid, quercetin).

The main phenolic compounds found in the citrus fruit seed oils were *p*-coumaric acid, salicylic acid, and quercetin (Table 2). ALU¹DATT and co-workers (2013) quantified the level of total phenolic compounds extracted with methanol in soybean, linseed, and olive crude oils, and found 1.30, 1.90, and 3.95 mg g⁻¹, respectively. The authors used water, methanol, and acetone as solvents for the extraction of phenolic compounds and concluded that methanol is the most used one by researchers and the most efficient as well. The small

quantity of phenolic compounds found in the samples, when compared to citrus fruit seed oils, can be explained by the fact that soybean, linseed, and olive oils were obtained by hot extraction by using hexane, a non-polar solvent, which does not favour the extraction of phenolic compounds from the samples. Carotenoids, beside pro-vitamin A function, and pigments present antioxidant capacity. Total carotenoids ranged from 6.85 to 21.64 μg β -carotene/g. Such variation can be explained by the different colours of the oils obtained, which ranged from light yellow in orange seed oil to dark green in kinkan seed oil.

2.3. Antioxidant capacity

Antioxidant capacity of oil, measured by DPPH^{*} free radical assay, was described by the efficient concentration (EC_{50}) (Table 3). Orange seed oil had higher antioxidant activity when compared with citron and kinkan seed oils. The results can be expressed in several different forms: percentage of oxidation inhibition, antiradical efficiency, efficient concentration, Trolox equivalent, and tocopherol equivalent. Therefore, the lack of standardization for the tests causes differences in analytical protocols, which limit comparison possibilities among samples, due to the different expression of antioxidant capacity.

Table 3. Antioxidant capacity of oils extracted from citrus fruit seeds

	Citron	Kinkan	Orange
EC_{50} (mg ml ⁻¹)	93.25±1.13 ^b	115.59±0.63 ^a	40.58±0.13 ^c
ABTS ⁺⁺ (μmol Trolox/100 g)	62.20±6.14 ^b	70.45±0.75 ^b	94.10±3.85 ^a
FRAP (μmol FeSO ₄ /100 g)	143.95±5.61 ^a	128.99±5.85 ^{ab}	120.89±5.35 ^b
β -Carotene/linoleic acid (%)	48.08±1.61 ^b	13.35±0.71 ^c	58.49±6.11 ^a
Oxidative stability (h)	4.88±0.34 ^c	9.37±0.50 ^b	13.60±0.19 ^a

Means \pm standard deviation of the analyses performed in triplicate followed by the same letter do not differ by Tukey's test ($P>0.05$).

Regarding antioxidant capacity determined by ABTS⁺⁺ radical assay (Table 3), the oils presented values from 62.20 μmol Trolox/100 g in citron oil to 94.10 in orange oil. PELLEGRINI and co-workers (2003), in a study performed with oils of soybean, corn, sunflower, and peanut and extra virgin olive oil found 220, 129, 117, 61, and 179 μmol Trolox/100 g of oil, respectively. The comparison between these values and those found in the present study must be made with reservations, since PELLEGRINI and co-workers used *n*-hexane for the dilution of commercial oils, and the solvent employed in the methodology may change solubility of the antioxidant compounds, such as tocopherols, phenolic compounds, and carotenoids.

FRAP system is commonly used to study the antioxidant capacity of more polar fractions of vegetable oils, rich in phenolic compounds, since this method is incompatible with organic solvents. The antioxidant capacity values ranged from 121 μmol FeSO₄/100 g for orange seed oils to 144 μmol FeSO₄/100 g for citron seed oils. Citron, kinkan, and orange seed oils presented oxidative inhibition with 40.08, 13.35, and 58.49% of β -carotene remaining after 120 min of reaction, respectively (Table 3). The test of β -carotene/linoleic acid is conducted in an oil–water emulsion matrix. Thus, differences in solubility of antioxidant compounds interfere with their activities. In this context, hydrophobic antioxidants, such as tocopherols,

tend to be more efficient than hydrophilic antioxidants, such as phenolics (MIRALIAKBARI & SHAHIDI, 2008).

Oxidative stability of the samples was 4.88, 9.37, and 13.60 hours in citron, kinkan, and orange seed oils, respectively (Table 3). According to SILVA and JORGE (2011), soybean oil has oxidative stability of 14.75 hours under the same analysis conditions.

3. Conclusions

The analyses of oils obtained from citron, kinkan, and orange seeds indicated the presence of bioactive compounds such as phytosterols, phenolic compounds, carotenoids, and tocopherols, presenting antioxidant capacity and helping to protect the oil against damage caused by lipid oxidation. The oils were predominantly unsaturated, with considerable quantity of essential fatty acids, mainly in the kinkan seed oil. β -Sitosterol was the major sterol quantified. The citron seed oil showed higher level of phenolic compounds. Beside food industry, pharmaceutical and cosmetic industries may also benefit from the bioactive compounds obtained from these oils. The results obtained in this study are also expected to attract the attention of fruit processing industries, to better manage their waste, thus avoiding a growing environmental problem. Further studies on the applications of these oils in different products are suggested.

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References

- ALU'DATT, M.H., RABABAH, T., EREIFEJ, K. & ALLI, I. (2013): Distribution, antioxidant and characterisation of phenolic compounds in soybeans and olives. *Food Chem.*, 139, 93–99.
- AOCS (2009): *Official and tentative methods of the American Oil Chemists' Society, including additions and revisions*. 6th ed, AOCS Press, Champaign, 457 pages.
- ARANHA, C.P. & JORGE, N. (2013): Physico-chemical characterization of seed oils extracted from oranges (*Citrus sinensis*). *Food Sci. Technol. Res.*, 19, 76–82.
- BABBAR, N., OBEROI, H.S., UPPAL, D.S. & PATIL, R.T. (2011): Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Res. Int.*, 44, 391–396.
- BLIGH, E.G. & DYER, W.J. (1959): A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.*, 37, 911–917.
- BRAND-WILLIAMS, W., CUVELIER, M.E. & BERSET, C. (1995): Use of a free radical method to evaluate antioxidant activity. *LWT – Food Sci. Technol.*, 28, 25–30.
- CODEX ALIMENTARIUS COMMISSION (2009): Codex-Stan 210: *Codex standard for named vegetable oils*. Rome.
- DIAS, L.S., LUZIA, D.M.M. & JORGE, N. (2013): Physicochemical and bioactive properties of *Hymenaea courbaril* L. pulp and seed lipid fraction. *Ind. Crop. Prod.*, 49, 610–618.
- DUCHATEAU, G.S.M.J.E., BAUER-PLANK, C.G., LOUTER, A.J.H., VAN DER HAM, M., BOERMA, J.A., VAN ROOIJEN, J.J.M. & ZANDBELT, P.A. (2002): Fast and accurate method for total 4-desmethyl sterol(s) content in spreads, fat-blends, and raw materials. *J. Am. Oil Chem. Soc.*, 79, 273–278.
- GÜNEŞER, B.A. & YILMAZ, E. (2017a): Effects of microwave roasting on the yield and composition of cold pressed orange seed oils. *Grasas Aceit.*, 68, e175.
- GÜNEŞER, B.A. & YILMAZ, E. (2017b): Bioactives, aromatics and sensory properties of cold-pressed and hexane-extracted lemon (*Citrus limon* L.) seed oils. *J. Am. Chem. Soc.*, 94, 723–732.

- KALANTZAKIS, G., BLEKAS, G., PEGKLIDOU, K. & BOSKOU, D. (2006): Stability and radical scavenging activity of heated olive oil and other vegetable oils. *Eur. J. Lipid Sci. Tech.*, *108*, 329–335.
- KIM, K.H., TSAO, R., YANG, R. & CUI, S.W. (2006): Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effects of hydrolysis conditions. *Food Chem.*, *95*, 466–473.
- MARCO, G.J. (1968): A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc.*, *45*, 594–598.
- MILLER, H.E. (1971): A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc.*, *48*, 91.
- MIRALIKBARI, H. & SHAHIDI, F. (2008): Antioxidant activity of minor components of tree nut oils. *Food Chem.*, *111*, 421–427.
- NYAM, K.L., TAN, C.P., LAI, O.M., LONG, K. & CHE MAN, Y.B. (2009): Physicochemical properties and bioactive compounds of selected seed oils. *LWT – Food Sci. Technol.*, *42*, 1396–1403.
- PARRY, J., SU, L., LUTHER, M., ZHOU, K., YURAWECZ, M.P., WHITTAKER, P. & YU, L. (2005): Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry and blueberry seed oils. *J. Agr. Food Chem.*, *53*, 566–573.
- PELLEGRINI, N., SERAFINI, M., COLOMBI, B., DEL RIO, D., SALVATORE, S., BIANCHI, M. & BRIGHENTI, F. (2003): Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.*, *133*, 2812–2819.
- PELLEGRINI, N., VISIOLI, F., BURATTI, S. & BRIGHENTI, F. (2001): Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agr. Food Chem.*, *49*, 2532–2538.
- RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.*, *26*, 1231–1237.
- RODRIGUEZ-AMAYA, D.B. (2001): *A guide to carotenoid analysis in food*. ILSI Press, Washington. 64 pages.
- SILVA, A.C. & JORGE, N. (2011): Antioxidant properties of *Lentinus edodes* and *Agaricus blazei* extracts. *J. Food Quality*, *34*, 386–394.
- SINGLETON, V.L. & ROSSI, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Viticult.*, *16*, 144–158.
- SZYDŁOWSKA-CZERNIAK, A., TROKOESKI, K., KARLOVITS, G. & SZLYK, E. (2011): Effect of refining process on antioxidant capacity, total content of phenolics and carotenoids in palm oils. *Food Chem.*, *129*, 1187–1192.