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AKADÉMIAI KIADÓ

Efficiency of sea buckthorn extract in oxidative stability improvement of high oleic sunflower oil

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ABSTRACT

Sea buckthorn (*Hippophae rhamnoides*) with high antioxidant capacity is distributed all over the world, but has never been used as a natural antioxidant in oils to replace synthetic antioxidants. Therefore, this study was performed to investigate the effectiveness of sea buckthorn extract in comparison to a common natural antioxidant rosemary extract and a synthetic antioxidant on retarding lipid oxidation. First the extracts were characterised, and it was found that sea buckthorn extract had higher polyphenol contents, radical scavenging activity, and higher antioxidant capacity. Then the proper concentrations for the use of these antioxidants were determined. Additionally, the progress of lipid oxidation during cycles of frying was assessed in terms of free fatty acids content, peroxide value, *p*-anisidine value, TOTOX value, colour, total polar compounds, and Induction period. The general order of effectiveness for inhibition of high oleic sunflower oil degradation during frying was: sea buckthorn > BHT > rosemary > control (P <0.05).

KEYWORDS

sea buckthorn, rosemary, oxidative stability, high oleic sunflower oil

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1. INTRODUCTION

Traditionally, synthetic antioxidants are added to frying oils to reduce their oxidative deterioration (Shahidi and Ambigaipalan, 2015). However, the application of synthetic antioxidants has been restricted because of their potential carcinogenic effects and toxicity (Hou, 2003). Therefore, there is a trend in consumer preference for natural ingredients, such as phenolic compounds found in plants instead of synthetic compounds, which are still being used in the food industry (Yang et al., 2016; Wu et al., 2019). Bioactive components such as phenolics and flavonoids of plants tend to exhibit free radical scavenging activity, so they can be exploited in searching for novel antioxidants (Shahidi and Ambigaipalan, 2015).

Sea buckthorn (SBT) is a bush species with high radical scavenging activity (Negi et al., 2005; Papuc et al., 2008), but it has never been used as a natural antioxidant to improve the oxidative properties of frying oils. Therefore, the purpose of the current investigation was to evaluate the effectiveness of SBT extract during the frying process of high oleic sunflower oil in comparison with rosemary extract as a common natural extract and BHT as a common synthetic antioxidant. The most suitable concentration of these extracts added into the oil was also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Refined high oleic sunflower oil (HOSO) with no antioxidant content was gifted by Bunge Zrt., Hungary. The SBT plant (flesh) and rosemary plant (leaves) were obtained from Bio-Drog-Berta Kft., Hungary and dried at 80 °C. Butylated hydroxytoluene (BHT) was purchased from Sigma-Aldrich. All other chemicals used in this study were of analytical grade.

2.2. Preparation of extracts

Extraction was done with slight modification based on a method described by Da Porto et al. (2013). In summary, 10 g of powdered plant was mixed with 100 mL of methanol and sonicated at 35 kHz for 1 h at 30 °C. Then it was kept for 24 h at room temperature with intermittent shaking. The mixture was filtered, and the solvent was evaporated. The resulting residue was further dried in an atmospheric pressure oven to ensure the removal of any residual solvent and was stored at −18 °C till use. The final extract was a dark green powder.

2.3. Characterisation of the extracts

2.3.1. Polyphenols content. The total phenol content was determined using Folin–Ciocalteu reagent (FCR) according to Singleton et al. (1999). The concentration was measured with gallic acid, and values are reported in gallic acid equivalents per gram extract (GAE/g).

2.3.2. FRAP assay. The method is based on the reduction of the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ) at low pH. This reduction is monitored by measuring the absorption change at 593 nm. FRAP values were obtained by comparing the absorption change in the test mixture as described by Benzie and Strain (1996).

2.3.3. DPPH assay. The antioxidant activity of extracts was evaluated by determining the anti-radical activity of the sample against the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Xu and Chang (2007).

2.4. Frying tests

2.4.1. Determination of concentration. Antioxidants were added into the oil at different concentrations (100, 200, 400, 600, 800, and 1,000 ppm) selected based on a literature review (Wu et al., 2019). Oil samples containing the desired concentration of antioxidants were subjected to frying at 180 ± 2 °C for 15 cycles (each cycle 5 min), and samples were drawn at the end of this period. Samples were taken for free fatty acids content, peroxide value, *p*-anisidine value, and Totox value determinations.

2.4.2. Effect of frying cycles. Extracts were added to the oil at their optimum concentration (400 ppm as determined in the previous step) and were subjected to frying at 180 ± 2 °C for 15 cycles (each cycle 5 min). Sampling was done from every three batches (3^{rd}, 6^{th}, 9^{th}, 12^{th}, and 15^{th}) till the end of frying (five times and control). Oil samples containing BHT at the maximum legally permitted level of (200 ppm) and another with no added antioxidant were used as controls.

2.4.2.1. Total polar compounds (TPC). Testo 270 Deep-frying Oil Tester (Testo Inc., Germany) was used for the rapid measurement of the TPC level in oil samples during frying. The sensor calibration was done with an essential oil provided by the manufacturer.

2.4.2.2. Free fatty acids (FFA). FFA content as a percentage of oleic acid was determined by AOCS Official Method Ca 5a-40 (AOCS, 1998).

2.4.2.3. Peroxide value (PV). PV was determined by AOCS Official Method Cd 8-53 (AOCS, 1998).

2.4.2.4. p-Anisidine value (p-AnV). *p*-AnV was determined by AOCS Official Method CC 8-11 (AOCS, 1998).

2.4.2.5. Totox value. TV value was expressed as 2PV + *p*-AnV.

2.4.2.6. Colour analysis. The colours of the samples were analysed using CR-310 Chroma Meter (Konica Minolta, Japan). The instrument was calibrated using the standard white tile (L^* 98.03, a^* −0.23, and b^* 0.25).

2.4.2.7. Induction time. An automated rancimat instrument (Model 743, Metrohm Herisau, Switzerland) was used to test the oxidative stability of the oil samples. The accelerated oxidation conditions in the oil samples (5 g) were established using an airflow rate of 20 L h^{-1} and 120 °C (Upadhyay and Mishra, 2015).

2.5. Statistical analysis

Statistical analysis was carried out using two-way analysis of variance (ANOVA) and Tukey's as post hoc test when homogeneity of variances was accepted and Games-Howell when the

homogeneity of variances was rejected using SPSS software IMB SPSS-25. Significant levels were based on the confidence level of 95% ($P < 0.05$). Analyses and independent sampling were done in triplicates.

3. RESULT AND DISCUSSION

3.1. Characterisation of extracts

3.1.1. Polyphenols content. The phenolic content of the extracts was determined as 132 and 108 mg GAE/g extract for SBT and rosemary, respectively. For rosemary extract, this amount was less than that found by Erkan et al. (2008) and Chammem et al. (2015) for methanolic rosemary extract, which were about 162 mg GAE/g and 112 mg GAE/g extract, respectively. For sea buckthorn, this amount varied from 89 to even 165 in different studies (Papuc et al., 2008; Kant et al., 2012). This difference can be explained by the different polarity of methanol used in this study compared to mixes of water and ethanol used by Papuc et al. (2008). In another similar study reported by Negi et al. (2005) for SBT, polyphenols content was reported to be higher when extracted with methanol compared to non-polar solvents (chloroform, ethyl acetate, acetone). Other factors may also affect the polyphenols composition of the extracts, such as genetics, the season of collection, and the maturity of the plant (Liu et al., 2007).

3.1.2. FRAP assay. The highest antioxidant activity was observed for SBT (190.67 mg TE g^{-1} DW), followed by BHT (170.97 mg TE g^{-1} DW) and rosemary leaves (142.97 mg TE g^{-1} DW). This was comparable to the results found by Rodríguez-Rojo et al. (2012). Rosemary extract presented lower antioxidant activity than synthetic antioxidants.

3.1.3. DPPH assay. The scavenging activity values of the extracts at 0.1% concentration of BHT, rosemary, and SBT were 68.23, 71.88, and 86.45%, respectively. The antioxidant activity of SBT was significantly higher ($P < 0.05$) than that of rosemary and BHT. The result shows that the SBT extract acts as primary antioxidant. These findings are in agreement with other studies (Negi et al., 2005; Kant et al., 2012).

3.2. Antioxidants concentration

As shown in Fig. 1, the antioxidant efficiency of extracts was generally enhanced by the increase in concentrations. Although high antioxidant activity was noticed in extracts up to concentrations of 1,000 ppm, the increase was mostly not significant above the concentration of 400 ppm. Therefore, 400 ppm of each natural antioxidant was chosen for further measurements during frying procedures defined as a minimum dosage of extract for achieving high oxidative stability.

3.3. Frying procedures

3.3.1. Total polar compounds (TPC). The level of TPC increased with the number of cycles ($P < 0.05$), which was in accordance with other studies (Urbančič et al., 2014). The order of increase in the TPC across the frying cycle was: control > BHT = rosemary extract > SBT extract. As shown in Fig. 2, oil without antioxidant should be discarded after 12 batches of

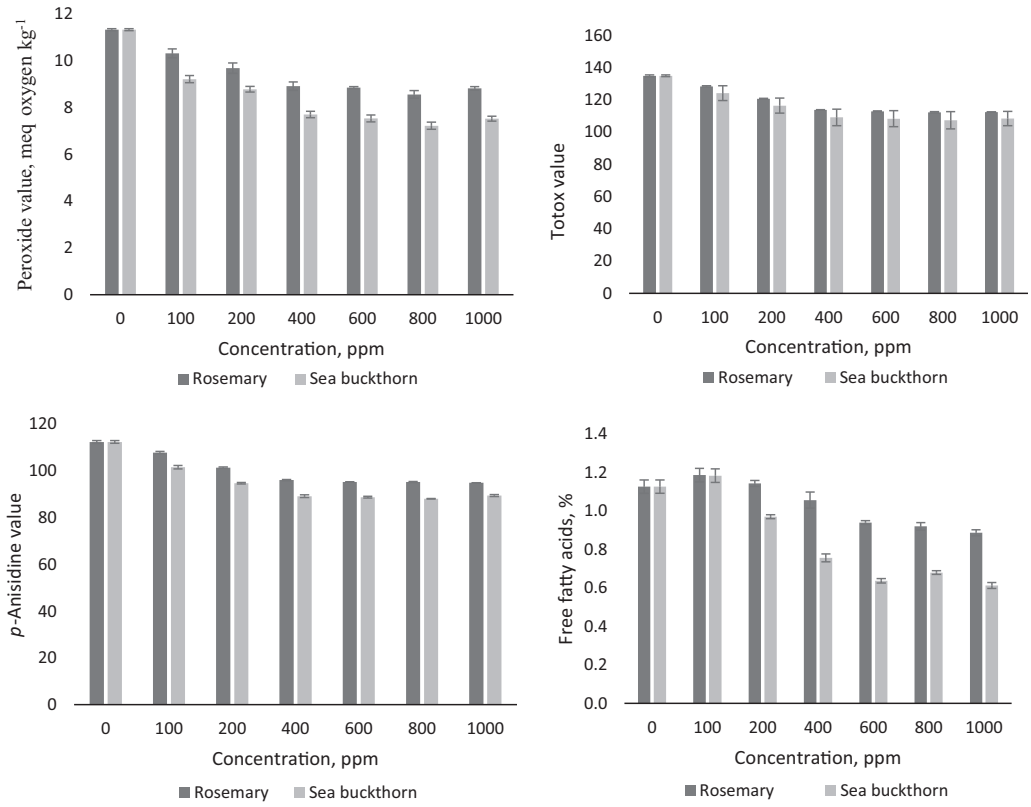

Fig. 1. Effects of antioxidant concentration on frying stability of high oleic sunflower oil samples with different antioxidants. Means for each antioxidant with different letters are significantly different ($P < 0.05$)

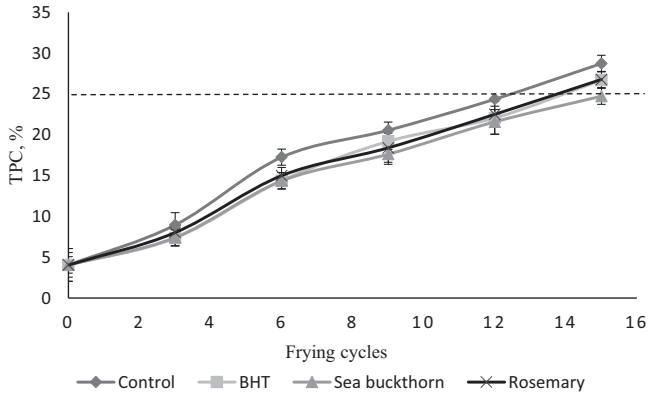

Fig. 2. Formation of TPC during frying of high oleic sunflower oil samples with different antioxidants

frying, and the oil with the highest stability containing SBT extract should be discarded after 15 cycles.

3.3.2. Free fatty acids (FFA). The amount of FFA increased slowly during frying but did not exceed the limit of 2% established by European regulations (Firestone, 2009). At the end of the deep-frying process, the FFA contents of SBT and rosemary treatments were lower than those of the other treatments ($P < 0.05$), which is in accordance with other studies (Chammem et al., 2015; Guo et al., 2016).

3.3.3. Peroxide value (PV), p-Anisidine value (p-AnV), and Totox value. As shown in Table 1, all antioxidants significantly ($P < 0.05$) reduced peroxide formation during frying. Generally, with an increase in the frying cycle, frying oils showed an increase in PV, followed by a slight decrease in the last cycle. Previous studies have also reported an initial PV increase during frying, followed by a later decrease (Poiana, 2012). SBT extract was the most effective and was superior to the commercial antioxidant mixture in preventing peroxide formation in vegetable oil.

Table 1. Quality changes of high oleic sunflower oil samples with different antioxidants

Characteristic	Cycles	Control	BHT	Sea buckthorn	Rosemary
Free fatty acid (%)	0	0.09 ± 0.01 aA	0.09 ± 0.01 aA	0.09 ± 0.01 aA	0.09 ± 0.01 aA
	3	0.36 ± 0.05 dB	0.28 ± 0.02 bB	0.18 ± 0.03 aB	0.32 ± 0.03 cB
	6	0.71 ± 0.02 dC	0.60 ± 0.02 cC	0.38 ± 0.02 aC	0.50 ± 0.03 bC
	9	0.96 ± 0.03 dD	0.77 ± 0.03 cD	0.54 ± 0.03 aD	0.69 ± 0.03 bD
	12	1.12 ± 0.04 dE	0.88 ± 0.03 cE	0.59 ± 0.03 aDE	0.74 ± 0.04 bE
	15	1.13 ± 0.01 dE	0.92 ± 0.01 cF	0.60 ± 0.04 aE	0.89 ± 0.02 bF
Peroxide value (meq oxygen/kg)	0	2.27 ± 0.26 aA	2.27 ± 0.26 aA	2.27 ± 0.26 aA	2.27 ± 0.26 aA
	3	5.98 ± 0.14 cB	4.26 ± 0.04 bB	3.87 ± 0.46 aB	6.18 ± 0.06 dB
	6	7.92 ± 0.49 dC	5.94 ± 0.14 bC	5.03 ± 0.08 aC	7.33 ± 0.12 cC
	9	8.61 ± 0.13 dD	6.58 ± 0.08 aD	6.77 ± 0.11 bD	7.98 ± 0.14 cD
	12	12.32 ± 0.26 dE	8.00 ± 0.12 bE	7.90 ± 0.02 aF	9.17 ± 0.07 cF
	15	12.02 ± 0.40 dE	8.74 ± 0.20 cF	7.38 ± 0.45 aE	8.73 ± 0.34 bE
p-Anisidine value	0	8.89 ± 0.51 aA	8.89 ± 0.51 aA	8.89 ± 0.51 aA	8.89 ± 0.51 aA
	3	62.13 ± 0.81 dB	51.09 ± 0.95 bB	47.85 ± 0.38 aB	54.21 ± 0.24 cB
	6	73.87 ± 1.49 dC	60.19 ± 0.77 aC	61.60 ± 0.92 bC	62.00 ± 0.74 cC
	9	85.70 ± 1.16 dD	73.74 ± 0.34 aD	75.52 ± 1.12 bD	80.21 ± 0.84 cD
	12	95.80 ± 0.67 dE	79.58 ± 1.29 aE	80.34 ± 0.96 bE	83.63 ± 0.52 cE
	15	112.87 ± 1.48 dF	92.19 ± 0.33 bF	88.72 ± 0.40 aF	94.56 ± 1.18 cF
Totox (2PV+AnV)	0	13.43 ± 0.55 aA	13.43 ± 0.55 aA	13.43 ± 0.55 aA	13.43 ± 0.55 aA
	3	74.08 ± 0.63 dB	59.61 ± 0.94 bB	55.58 ± 0.87 aB	66.57 ± 0.24 cB
	6	89.70 ± 1.14 dC	72.07 ± 0.79 bC	71.65 ± 0.90 aC	76.65 ± 0.88 cC
	9	104.93 ± 5.00 dD	86.90 ± 0.50 aD	89.06 ± 0.90 bD	96.18 ± 0.92 cD
	12	119.84 ± 0.40 dE	95.59 ± 1.27 aE	96.13 ± 1.00 bE	101.96 ± 0.47 cE
	15	137.50 ± 0.99 dF	109.68 ± 0.72 bF	103.47 ± 1.26 aF	112.02 ± 0.50 cF

a-d, Means with different letters in each row are significantly different ($P<0.05$).
A-F, Means with different letters in each column are significantly different ($P<0.05$).

The *p*-AnV also increased consistently for every cycles of frying (Table 1), and there was a significant ($P < 0.05$) increase in *p*-AnV throughout the whole frying period. *p*-AnV reached a peak in the following order: control > rosemary > BHT > SBT ($P < 0.05$). This was consistent with findings of other studies (Che Man and Jaswir, 2000; Chammem et al., 2015).

Totox value was also obtained to compare the overall oxidative stability of the samples. As shown in Table 1, the Totox value for oil samples subjected to frying increased with the time of frying. Totox values of samples with sea buckthorn, BHT, and rosemary were lower than that of the values measured for the control samples ($P < 0.05$).

3.3.4. Colour analysis. The changes in the colour of the oils during the deep-frying process are shown in Table 2, with L^* as brightness/darkness, a^* greenness/redness, and b^* as an indicator of yellowness/blueness. All treatments decreased L^*, indicating darkening of the oil samples with the increase of frying cycles. From a starting value of L^* of 58.52 it decreased to 50.89, 53.11, 54.38, and 55.18 after 15 deep-frying cycles for control, BHT, rosemary, and SBT extracts, respectively.

At the same time, the initial b^* value of 11.65 increased to 15.99, 14.99, 14.83, and 14.56 for control, BHT, rosemary, and SBT extracts, respectively. This is probably due to the presence of natural carotenoids and xanthophylls in the oil (Guo et al., 2016).

Table 2. L^*, a^* and b^* colour values of high oleic sunflower oil samples with different antioxidants

Colour values	Cycles	Control	BHT	Sea buckthorn	Rosemary
L*	0	58.52 ± 0.54 aF	58.52 ± 0.54 aE	58.52 ± 0.54 aE	58.52 ± 0.54 aE
	3	57.11 ± 0.50 aE	57.82 ± 0.53 bDE	58.29 ± 0.56 dDE	58.25 ± 0.57 cDE
	6	55.41 ± 0.28 aD	56.97 ± 0.12 bCD	57.62 ± 0.29 dCD	57.55 ± 0.47 cCD
	9	54.68 ± 0.22 aC	55.78 ± 0.90 bC	57.12 ± 0.56 dBC	56.84 ± 0.02 cC
	12	53.32 ± 0.13 aB	54.22 ± 0.24 bB	55.62 ± 0.43 cAB	55.95 ± 0.60 dB
	15	50.89 ± 0.56 aA	53.11 ± 0.36 bA	55.18 ± 0.30 dA	54.38 ± 0.12 cA
	Change	−7.63	−5.41	−3.34	−4.14
a*	0	−3.55 ± 0.25 aA	−3.55 ± 0.25 aA	−3.55 ± 0.25 aA	−3.55 ± 0.25 aA
	3	−3.30 ± 0.10 aAB	−3.25 ± 0.00 cB	−3.25 ± 0.12 cB	−3.27 ± 0.20 bB
	6	−3.19 ± 0.25 dBC	−3.22 ± 0.11 aB	−3.22 ± 0.11 bB	−3.23 ± 0.11 bC
	9	−3.13 ± 0.68 bBC	−3.12 ± 0.06 cC	−3.14 ± 0.37 aC	−3.13 ± 0.20 bD
	12	−2.90 ± 0.32 dC	−3.03 ± 0.11 bD	−3.06 ± 0.05 aD	−3.00 ± 0.17 dE
	15	−2.55 ± 0.38 dD	−2.93 ± 0.21 dE	−3.01 ± 0.15 aE	−2.94 ± 0.21 bF
	Change	1.01	0.63	0.52	0.62
b*	0	11.65 ± 0.20 aA	11.65 ± 0.20 aA	11.65 ± 0.20 aA	11.65 ± 0.20 aA
	3	12.03 ± 0.16 dAB	11.77 ± 0.02 bA	11.73 ± 0.25 aAB	11.82 ± 0.07 cA
	6	12.38 ± 0.14 dB	12.26 ± 0.42 cB	11.96 ± 0.01 aB	12.24 ± 0.34 bB
	9	13.63 ± 0.96 dC	13.00 ± 0.65 cC	12.81 ± 0.25 bC	12.81 ± 0.66 aC
	12	15.15 ± 0.26 dD	13.71 ± 0.15 aD	13.72 ± 0.34 bD	13.77 ± 0.11 cD
	15	15.99 ± 0.49 dE	14.83 ± 0.03 bE	14.56 ± 0.04 aE	14.99 ± 0.10 cE
	Change	4.34	3.18	2.91	3.34

a–d, Means with different letters in each row are significantly different ($P < 0.05$).
A–F, Means with different letters in each column are significantly different ($P < 0.05$).

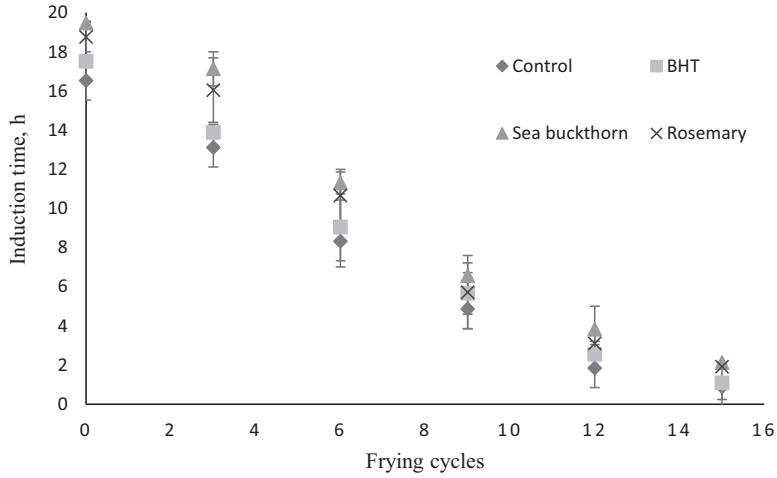

Fig. 3. Changes in the induction time of high oleic sunflower oil samples with different antioxidants during frying

Finally, there was also a significant ($P < 0.05$) increase in a* values. The results were similar to those obtained for the peroxide content, which reflected a continuous change in the frying oil. Therefore, the samples with no antioxidants changed more dramatically than those containing BHT, rosemary, and sea buckthorn.

3.3.5. Induction time. The result showed that SBT and rosemary significantly ($P < 0.05$) improved the resistance to oxidative rancidity of frying oils in the first 6 cycles of the frying, and the effect gradually decreased till the end of frying cycles (Fig. 3). After the 6th cycle, the oxidative rancidity of frying oils with or without antioxidants seemed to get closer to each other during continuous heating with further deterioration of antioxidants. This observation is in accordance with the study of Yang et al. (2016).

4. CONCLUSION

The present study has revealed that SBT extract exhibited the strongest scavenging activity and effectively slows oil deterioration during frying. In terms of TPCs, FFAs, *p*-AnV, Totox value, TPC, rancidity, and colour (L*, a*, b*), the data showed that the oil treated with SBT extract had higher oxidative stability compared to the control oil and the oil with other natural and synthetic antioxidants. Natural extract at the concentration of 400 ppm showed considerable antioxidative effects with minimal consumption of antioxidants. Overall, this is a preliminary report on the extraction and antioxidant capacity from SBT, and further studies are needed for the identification of individual phenolic components to explain the mechanisms underlying its antioxidant properties and the existence of possible synergism, if any, among different phenolic compounds.

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SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1556/066.2021.00080.

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