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ASSESSING THE ABILITY OF FREEZE-DRIED EXTRACTS FROM BLACKBERRY PROCESSING BY-PRODUCTS TO ENHANCE THE ANTIOXIDANT FUNCTION OF SUNFLOWER OIL DURING HIGH-TEMPERATURE HEATING

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Abstract

The aim of this study was to assess the ability of two freeze-dried extracts from blackberry processing by-products resulted after juice extraction, compared to butylhydroxytoluene (BHT) in preventing the lipid oxidation of sunflower oil (SFO) subjected to high-temperature heating at 180ºC up to 12 hours. The blackberries were collected from spontaneous flora of two regions of Romania, Zugau (Arad County) and Paltinis (Sibiu County) and the blackberry by-products extracts (BBE) were noted according to the place of origin as ZBBP, respectively PBBE. The evolution of lipid oxidation was tracked by means of specific indices as: peroxide value (PV), p-anisidine value (p-AV), total oxidation (TOTOX) value and the response of TBA-malondialdehyde (MDA) interactions evaluated by thiobarbituric acid (TBA) method. The results revealed that BBE displayed a high inhibitory effect on SFO thermo-oxidation. This response was dose-dependent, thus, the rate of lipid oxidation was inversely related to BBE concentration. The recorded data highlighted that BBE constitutes efficient natural antioxidants that can contribute to the development of sunflower oil with extended thermo-oxidative stability.

Key words*: blackberry processing by-products, freeze-dried extracts, inhibitory effect, lipid oxidation, thermo-oxidative stability.*

INTRODUCTION

Sunflower oil (SFO) is one of the top-quality and most widely used vegetable oils in industrial applications as well as in human nutrition, due to its composition of up to 85% unsaturated fatty acids, especially linoleic essential acid, and to its very high burning point (El-Hamidi & Zaher, 2018; Metzner Ungureanu et al., 2020c; Orsavova et al., 2015).

However, during food processing, due to the prolonged exposure of SFO to high temperatures, various chemical reactions such as thermal oxidation, polymerisation and hydrolysis take place, which produce changes in the physico-chemical properties and nutrient content (Abd Razak et al., 2021; Ramroudi et al., 2022).

The electric convection oven is an universal heat source, used in various thermal food applications. The main cause of the SFO degradation during convective heating, is lipid oxidation that involve a wide range of complex chemical reactions followed by the formation of primary oxidation compounds (Poiana et al., 2022). From primary oxidation compounds, secondary oxidation compounds will be developed, and so forth for tertiary compounds or further oxidation compounds. In recent years, emphasis has been placed on replacing the synthetic antioxidants previously used to improve the oxidative stability of SFO subjected to thermal processing, due to their damaging potential, with healthy, natural alternatives (Lorenzo et al., 2017). Therefore, the detection of new natural sources of bioactive compounds with antioxidant

potential, to replace synthetic additives such as butylhydroxytoluene (BHT), represent a priority in food industry (Fadda et al., 2022; Grebenteuch et al., 2021; Poiana et al., 2021; Raba et al., 2020).

Different studies have proved the potential of vegetable waste such as berry processing by-products, as sources of natural bioactive compounds with antioxidant activity due to their high phenolic content (Metzner Ungureanu et al., 2020b; Moraru Manea et al., 2022; Zeng et al., 2023). Berry processing by-products represent low-cost extraction material for natural antioxidants suitable in preventing food and oils lipid oxidation (Metzner & Poiana, 2018; Popa et al., 2011).

Antioxidant properties in terms of antioxidant activity, total phenolic content and individual polyphenolic compounds profile of the extracts obtained from different fruit processing by-products, have been previously exposed by methods specific to those type of investigations (Kuppusamy et al., 2020; Lyu et al., 2023; Metzner Ungureanu et al, 2020b).

For the most part, the impact of berry extracts on lipid oxidation, has been studied on meat products (Burri et al., 2020; Ganhão et al., 2013; Varzaru et al., 2020). Therefore, Ganhão et al. (2013), on their research on several Mediterranean berries, reported that the berries extracts were capable to inhibit the lipid oxidation in raw, cooked and cooked and chilled porcine burger patties and Varzaru et al. (2020) revealed that extracts resulted from bilberry, cranberry and raspberry leaves exposed an inhibitory response on lipid peroxidation on chickens meat. Also, Burri et al. (2020) demonstrated that bilberry and red currant extracts, are efficient against lipid oxidation developed in a processed meat model system.

Although multiple studies recommend the exploitation of fruit processing by-products as reliable sources of bioactive compounds with antioxidant activities, so far, there are only a few data about the inhibitory response of berries processing by-products in limiting the lipid thermo-oxidation.

Taking into consideration the mentioned data, the purpose of this research was to assess the effect of freeze-dried extracts obtained from blackberry processing by-products resulted after juice extraction, to inhibit the lipid oxidation of SFO subjected to high-temperature convective heating in order to substitute BHT. Thus, the aim was to determine the most effective extract, the most efficient dose, and the impact of the origin area of the blackberries on the antioxidant properties of freeze-dried extracts, in order to obtain a superior inhibition of SFO thermo-oxidation. In this regard, the evolution of lipid oxidation initiated by the exposure of SFO to convective heating at 180ºC for different periods of time, was evaluated by means of specific indices as: peroxide value (PV), *p*-anisidine value (*p*-AV), total oxidation (TOTOX) value and the response of TBA-malondialdehyde (MDA) interactions was evaluated by thiobarbituric acid (TBA) method.

MATERIALS AND METHODS

Processing of blackberry by-products extracts

Blackberries (*Rubus fruticosus* L.) were collected at maturity stage in the year 2018, from two different Romanian areas: Zugau, Arad County and Paltinis, Sibiu County. Compared to the Paltinis area, Zugau area present a milder climate with moderate precipitation regime and higher temperatures. After juice extraction, resulted blackberry by-products were conditioned in an electric oven with convective heat (Esmach SpA-Ali Group/Italy, 1200 W, 50 Hz) at 60ºC for 12 h. Conditioned by-products were grinded using a laboratory mill (Grindomix Retsch GM200, Germany) and the extraction of bioactive compounds was completed by a maceration solvent extraction in a hydro-alcoholic ethanol/water mixture (1:1, v/v) where the solid:solvent extraction ratio was 1:10 (w/v), for 48 h at 20ºC. After percolation, the clear extracts resulted were evaporated until 100 mL, using a rotary evaporator (RV 10 auto pro V, IKA England Ltd., 100 W, 50 Hz). Further, the blackberry by-products extracts were subjected to freeze drying using a lyophilizer (Alpha 1-2 LD plus, 230 V/50 Hz, MARTIN CHRIST GmbH, Germany). According to the origin place of blackberries, the freeze-dried extracts obtained were noted as ZBBE for Zugau area, respectively PBBE for Paltinis area.

Application of freeze-dried blackberry by-products extracts to SFO

Freshly refined and free of synthetic antioxidants SFO was divided into eight portions. Six portion were supplemented with 200, 500 and 800 ppm freeze-dried blackberry by-products extracts (ZBBE and PBBE), the seventh portion was supplemented with 200 ppm BHT and the last portion of SFO, without any additive, was used as a control (C). For a better diffusion of supplements in SFO, before applying, the extracts and BHT were diluted in a minimum volume of absolute ethanol in an ultrasonic water bath and then were mixed with SFO for 10 min, followed by a vacuum evaporation. The control sample was prepared in identical conditions.

High-temperature heating of SFO

In order to evaluate the inhibitory effect of freeze-dried extracts and BHT on thermo-oxidative lipid degradation in simulated frying conditions, the SFO samples were exposed to a temperature of 180ºC for 3, 6, 9 and 12 h using a convection electric oven (Esmach SpA-Ali Group/Italy, 1200 W, 50 Hz). For each investigated sample 25.0 ± 0.5 g of SFO was weighted. Separate oil samples were used for different heating period.

Freeze-dried blackberry by-products extracts (BBE) analysis

Moisture content: The moisture content of the BBE was determined according to the method 925.09 of the AOAC (Horowitz & Latimer, 2006).

Total phenolic content (TPC): The TPC of BBE was spectrophotometrically assessed using the Folin-Ciocalteu method, as it was previously reported by Metzner Ungureanu et al. (2020c) and the results were expressed as mg gallic acid equivalent (GAE)/g dried substance (d.s).

Antioxidant activity (AA): The AA has been investigated with the DDPH assay by which the free radical scavenging ability of BBE was tested using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the previously described method. The results were expressed as mg GAE/100 g d.s. and the inhibition of DPPH was expressed as percentage (Metzner Ungureanu et al., 2020b).

The polyphenolic compounds profile: The individual phenolic compounds of BBE were identified by chromatographic analysis using an UHPLC (ultra-high-performance liquid chromatograph) and the results were expressed as mg/100 g d.s (Metzner Ungureanu et al., 2020b).

Evaluation of lipid oxidation

The peroxide value (PV): The PV has been evaluated by the iodometric titration according to the standard methods for oils analysis and the results were expressed as milliequivalents of active oxygen per 1000 g oil sample (mEq O2/kg oil). PV represents a reliable indicator to assess the occurrence of primary lipid oxidation (AOCS, 1998).

The p-anisidine value (p-AV): The *p*-AV has been spectrophotometrically determined according to the standard methods for oils analysis and it provides information about the occurrence of secondary products of lipid oxidation (AOCS, 1998).

The total oxidation (TOTOX) value: TOTOX value reflects the contribution of both PV and *p*-AV to the total lipid oxidation and it was obtain by the following equation: TOTOX= 2PV+ *p*-AV (Esfarjani et al., 2019; Metzner Ungureanu et al., 2020c).

The thiobarbituric acid (TBA) assay: the TBA value was assessed spectrophotometically according to the slightly modified procedure of Singh et al. (2006) in agreement with the method reported by Metzner Ungureanu et al. (2020c) and the results were expressed as µg MDA/mL oil sample. The TBA assay depends by the reaction of MDA (malondialdehyde) with TBA and is used specifically in the evaluation of lipids secondary oxidation (Zeb & Ullah, 2016).

Statistical data analysis

All determinations were performed in triplicate and the results were reported as mean values ± standard deviation (SD). One-way ANOVA

analysis of variance was used for statistical data analysis. Computations Tukey post-hoc means comparisons and Levene's test for equal variance were included to estimate the statistical significance of variations among means. Data within the same row or column for tables, or bars, for charts, sharing different letters are significantly different ($p < 0.05$).

RESULTS AND DISCUSSIONS

Freeze-dried blackberry by-products extracts (BBE) analysis

Data exposed in Table 1 reveal the TPC of BBE and their antioxidant activity evaluated by the DPPH assay.

The residual moisture content of ZBBE and PBBE was determined in order to express the antioxidant characteristics relative to dried substance (d.s) content. The recorded moisture content was low in both ZBBE (3.18%) and PBBE (3.56%). Effective reduction of the humidity make the microbial and enzymatic degradation impossible, thus conferring a high shelf life.

Table 1. Antioxidant properties of freeze-dried extracts obtained from blackberry processing by-products

Sample	TPC (mg GAE/ g d.s)	DPPH	
		I (%)	(mg GAE/ 100g d.s)
ZBBE	105.34±0.54^{a}	92.84±0.51^{a}	1375.14±8.05^{a}
PBBE	76.13±0.38^{b}	90.82±0.44^{b}	1044.52±6.32^{b}

The results showed significant differences between the antioxidant characteristics of the freeze-dried extracts investigated. Thus, the TPC values recorded were 105.34 in ZBBE and 76.13 mg GAE/g d.s in PBBE. It is noted that the TPC value was 38% higher in ZBBE compared to the PBBE sample. Piasecka et al. (2022) reported a TPC value ranging between 48.28 and 50.16 mg GAE/g in extracts obtained from wild flora blackberry processing by-products dried in a desiccator under vacuum.

In terms of AA expressed as mg GAE/100 g d.s, a loss of 24% was noted in PBBE sample compared to ZBBE. The freeze-dried extract obtained from blackberry processing by-products from the Zugau area showed higher antioxidant attributes compared to those recorded for PBBE, therefore a milder climate results in a higher TPC and a superior AA.

Most polyphenolic compounds identified in BBE after UHPLC analysis, were consisted in phenolic acids as: p-coumaric acid, caffeic acid, rosmarinic acid, vanillic acid, gallic acid and syringic acid. Other polyphenolic compounds identified in freeze-dried extracts obtained from blackberry by-products were rutin and pyrocatechol. Of all polyphenolic compounds identified, pyrocatechol was recorded in the highest amount. The data obtained from the chromatographic analysis, correspond to the data reported by Jazić et al. (2019) for blackberry processing by-products.

Although the same polyphenolic profile was identified in freeze-dried extracts, the amount recorded varied significantly depending on the region of origin. Thus, the highest amount of polyphenolic compounds was noted in ZBBE, except for pyrocatechol and syringic acid which had higher values in PBBE. Therefore, the region of origin, in the context of climatic conditions, may affect the concentration pattern of polyphenolic compounds. This finding is in agreement with similar previous studies, which have shown that area of origin, along with other factors such as variety, ripening stage, amount of hydration, storage and conditioning conditions, can influence the dynamics of bioactive substances (Dróżdż et al., 2017; Metzner Ungureanu et al., 2020a).

Evaluation of lipid oxidation

Peroxide value (PV)

Hydroperoxides are chemical compounds produced as a result of primary lipid oxidation. They are unstable and prone to enzymatic and non-enzymatic degradation, resulting in a wide range of secondary oxidation products. The peroxide value increases with increasing hydroperoxide formation coefficient when the rate of decomposition of the hydroperoxides into secondary oxidation products is exceeded by the rate of their formation (Poiana, 2012).

Table 2 shows the PV variations following the exposure of oil samples to a temperature of 180ºC for different periods of time.

Table 2. The impact of SFO supplementation with BHT and freeze-dried extracts obtained from blackberry processing by-products, on the peroxide value, during heat treatment

Time (h)	PV (meq O_2/kg Oil)	
	C	BHT **200** ppm
0	1.81±0.05[a]	1.81±0.05[a]
3	11.64±0.47[a]	8.12±0.26[d]
6	11.15±0.43[a]	7.87±0.23[b]
9	10.96±0.41[a]	7.53±0.25[b]
12	10.79±0.38[a]	7.38±0.31[b]

Time (h)	PV (meq O_2/kg Oil)		
	ZBBE (ppm)		
	200	**500**	**800**
0	1.81±0.05[a]	1.81±0.05[a]	1.81±0.05[a]
3	9.52±0.38[b]	8.56±0.29[d]	6.82±0.15[e]
6	8.76±0.34[b]	8.14±0.27[c]	6.68±0.14[d]
9	8.43±0.31[b]	7.98±0.28[b]	6.12±0.17[c]
12	7.92±0.25[b]	7.67±0.19[b]	5.92±0.18[c]

Time (h)	PV (meq O_2/kg Oil)		
	PBBE (ppm)		
	200	**500**	**800**
0	1.81±0.05[a]	1.81±0.05[a]	1.81±0.05[a]
3	9.65±0.39[b]	8.66±0.32[c]	6.97±0.16[e]
6	8.98±0.36[b]	8.52±0.33[b]	6.85±0.15[d]
9	8.61±0.30[b]	8.31±0.31[b]	6.69±0.19[c]
12	8.11±0.26[b]	7.84±0.27[b]	6.03±0.22[c]

One-way ANOVA test was used to compare the means differences registered for each heating period among the oil samples supplemented with BHT and different doses of BBE, relative to C; data within the same row sharing different letters are significantly different ($p < 0.05$).

A significant increase in PV value was observed in the first stage of thermal processing of oil samples, and in the interval 3-6 h of heat exposure, the peroxide value starts to decrease indicating the decomposition of primary oxidation products represented by hydroperoxides, into secondary oxidation products, aldehydes and ketones. By the end of the 12 h heating interval, PV follows a downward pattern.

Reduced primary oxidation was noted in response to supplementation with BHT and freeze-dried extracts obtained from blackberry processing by-products. Significant differences (p<0.05) in peroxide value were recorded during the heating process between the control sample and the samples supplemented with 200 ppm BHT and different doses of BBE. The inhibitory effect against primary oxidation is conditioned by the concentration of the applied extract. Therefore, the extracts in a dose of 800 ppm showed the highest inhibitory response in the first phase of thermo-oxidation. The results obtained are consistent with data recorded in similar studies in which the inhibitory response of BHT and natural extracts with antioxidant potential against primary oxidation promoted by exposure to high temperatures, was evaluated (Metzner Ungureanu et al., 2020c; Poiana, 2012).

Supplementation of SFO samples with BHT and freeze-dried extracts obtained from blackberry processing by-products resulted in a 30% reduction of PV in oil sample supplemented with 200 ppm BHT, respectively with 41% and 40%, in samples supplemented with 800 ppm ZBBE and PBBE, compared to the control sample, after 3 h of high temperature exposure, at which time the highest PV value was recorded.

In the study of Poiana (2012) regarding the improvement of oxidative stability of SFO subjected to convective and microwave heating up to 4 h by supplementation with grape seed extract, a steady increase in peroxide value was reported throughout the heating interval. At the end of the heating process, PV decreased by 32% in the oil sample supplemented with 200 ppm BHT and with 39% in the samples supplemented with 800 ppm grape seed extract. Also, in the study conducted by Khor et al. (2019) in which the effect of heating palm kernel oil at a temperature of 180ºC up to 24 h was investigated, the highest peroxide value was recorded after 4 h of heat treatment, and after this interval the PV was significantly reduced.

BBE obtained from blackberry collected from the region with a milder climate showed a higher inhibitory effect compared to BBE obtained from blackberry originating in the area with low temperatures and high rainfall. The obtained inhibitory response correlates with the antioxidant characteristics of the investigated extracts.

During thermal processing, freeze-dried extracts obtained from blackberry processing by-products at a concentration of 200 ppm showed a lower inhibitory response compared to BHT, at 500 ppm the response was similar to that recorded in samples added with BHT, and at a level of 800 ppm, the inhibitory effect of freeze-dried extracts was superior to that exposed by BHT (Metzner Ungureanu et al., 2020c).

p-Anisidine value (p-AV)

Under constant action of heat treatment, the primary products of lipid oxidation continue to decompose generating secondary oxidation products that affect sensory properties, being responsible for change of smell and flavour.

Table 3 presents variations of *p*-AV over the heating process, in additive SFO samples with 200 ppm BHT and various doses of BBE.

Table 3. The impact of SFO supplementation with BHT and freeze-dried extracts obtained from blackberry processing by-products, on the *p*-AV, during heat treatment

Time (h)	*p*-AV		
	C	BHT **200** ppm	
0	2.49±0.15[a]	2.49±0.15[a]	
3	47.86±2.07[a]	40.17±2.28[b]	
6	56.98±1.93[a]	50.39±2.43[a]	
9	62.27±2.21[a]	54.67±2.17[a]	
12	69.84±3.49[a]	58.85±2.52[b]	
Time (h)	*p*-AV		
	ZBBE (ppm)		
	200	**500**	**800**
0	2.49±0.15[a]	2.49±0.15[a]	2.49±0.15[a]
3	45.12±2.98[a]	41.89±2.53[a]	38.34±1.92[d]
6	54.05±3.04[a]	50.85±2.21[a]	49.21±2.33[b]
9	58.64±3.11[a]	56.14±3.20[a]	53.12±2.59[b]
12	64.28±3.15[a]	58.92±2.81[b]	55.79±2.64[c]
Time (h)	*p*-AV		
	PBBE (ppm)		
	200	**500**	**800**
0	2.49±0.15[a]	2.49±0.15[a]	2.49±0.15[a]
3	46.03±2.41[a]	42.35±3.03[a]	38.67±2.25[c]
6	54.51±2.56[a]	52.27±2.94[a]	50.02±2.97[b]
9	60.57±3.43[a]	56.73±3.42[a]	55.12±2.82[a]
12	65.72±3.95[a]	59.16±3.48[b]	58.51±3.34[b]

One-way ANOVA test was used to compare the means differences registered for each heating period among the oil samples supplemented with BHT and different doses of BBE, relative to C; data within the same row sharing different letters are significantly different ($p < 0.05$)

The *p*-AV has increased steadily throughout the heat treatment application. The high *p*-anisidine value implies the reduction of the smoke point which resides in quality decreasing of the investigated oil. In response to supplementation of sunflower oil with various doses of BBE and BHT, a significant ($p<0.05$) reduction in *p*-AV was noted compared to C.

After 12 h of heating at 180ºC, a decrease in *p*-AV compared to C of 6%, 15% and 16% was observed for the oil samples supplemented with PBBE in concentration of 200 ppm, 500 ppm and 800 ppm, respectively 8%, 16% and 20% for the oil samples supplemented with ZBBE in concentration of 200 ppm, 500 ppm and 800 ppm, and 16% for the samples supplemented with 200 ppm BHT. Therefore, the effect of the investigated extracts in inhibiting secondary oxidation, is dose dependent. At a 200 ppm concentration, freeze-dried extracts showed a lower inhibitory response than BHT, at 500 ppm a similar response, and at 800 ppm, the inhibitory effect of PBBE was identical to that reported for BHT, while for ZBBE it was 4% higher. Recorded data indicate that at a dose of 800 ppm, ZBBE is a potential substitute for BHT. The results obtained are in agreement with data recorded in similar studies investigating the improvement of oxidative stability of sunflower oil subjected to heat by addition with extracts obtained from fruit processing by-products (El-Hadary & Taha, 2020; Metzner Ungureanu et al., 2020c). El-Hadary & Taha (2020) in their study focused on the enhancing of some vegetable oils oxidative stability by the addition of various doses of natural extracts obtained from pomegranate peel, found that increasing the dose of extract resulted in a significant improvement of the inhibitory response in the secondary oxidation stage.

Total oxidation (TOTOX) value

In order to estimate the quality of oils by determining total oxidation, it is necessary to establish in advance the specific chemical indices of primary and secondary oxidation. As a characteristic index for monitoring the oxidation process of vegetable oils, the TOTOX value corresponds to the level of total lipid oxidation. Therefore, increasing the total oxidation value proves an increased degree of lipid damage (Metzner Ungureanu et al., 2020c).

Figure 1 presents TOTOX variations noted in oil samples supplemented with various doses of freeze-dried extracts obtained from blackberry processing by-products, and 200 ppm BHT, during the entire heating process.

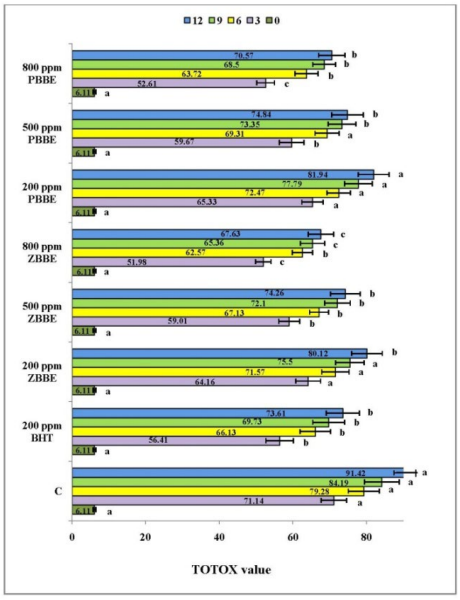

Figure 1. Effect of SFO supplementation with BHT and BBE on TOTOX value during high-temperature heating. One-way ANOVA test was used to compare the means differences registered for each heating period among the oil samples supplemented with BHT and different doses of BBE, relative to C; the values for bars sharing different letters are significantly different ($p < 0.05$)

As the heat treatment is prolonged, an increase in TOTOX value is observed. Compared to C, the total oxidation recorded in SFO samples supplemented with different concentrations of BBE, and BHT, was significantly reduced ($p<0.05$). Thus, at the end of the heating process, TOTOX value for oil samples supplemented with ZBBE and PBBE decreased in the range of 8-27%. The additive sample with 200 ppm BHT reported a 19% reduction of total oxidation after 12 h exposure to heat treatment. A higher efficiency in inhibiting total oxidation was recorded at BBE applied in an 800 ppm dose compared to BHT. The results obtained after TOTOX determination are in agreement with data recorded in similar studies in which various types of natural extracts were applied in order to limit the total oxidation of sunflower oil subjected to heating processes (Metzner Ungureanu et al., 2020c; Neves, 2020).

Thiobarbituric acid (TBA) assay

The TBA assay is based on the determination of the malondialdehyde (MDA) value in the investigated products. MDA is an essential factor in the production of non-specific odours and flavours. The TBA assay is the most suitable method in determination of oxidative rancidity because MDA is responsible for the specific reaction of thiobarbituric acid (Reitznerová, 2017).

Figure 2 shows the fluctuation of TBA value during high temperature exposure of oil samples supplemented with different concentrations of freeze-dried extracts obtained from blackberry processing by-products and 200 ppm BHT.

TBA indicates the stage of secondary oxidation and the appearance of related products. A high TBA value indicates a decreased oil stability and thermo-oxidation progression. Absorbance decreases with the amount of TBA-MDA complex which implies a higher protection of extracts against secondary oxidation.

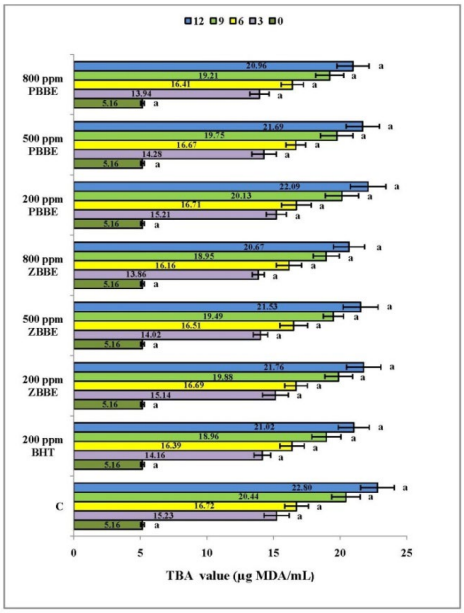

Figure 2. The effect of SFO supplementation with BHT and BBE, on TBA value, during high-temperature heating. One-way ANOVA test was used to compare the means differences registered for each heating period among the oil samples supplemented with BHT and different doses of BBE, relative to C; the values for bars sharing different letters are significantly different ($p<0.05$)

After 3 h of heating at 180ºC, the TBA value was decreased in all investigated samples, as a result of the fact that the rate of destruction of hydroperoxides exceeded their rate of formation. After 6 h of thermal exposure, when the rapport was reversed and the rate of formation of hydroperoxides was exceeded by their rate of decomposition into secondary oxidation products, the TBA value started to increase following this pattern throughout the rest of the heating program. The highest PV value was reported after 3 h of heating when the lowest TBA value was reported, and the lowest PV value was recorded after 12 h of thermal processing when the highest TBA value was noted, which establishes a direct relationship between the increase in hydroperoxide destruction rate and the TBA value. Previous studies conducted on this topic reported the same result regarding the inverse relationship between PV and MDA content (Okhli, 2020). Following the study conducted by Okhli et al. (2020) on the improvement of oxidative stability of sunflower oil by addition of extracts obtained from citron (*Citrus medica* L.) peels, it was found that the decomposition of hydroperoxides and the formation of aldehydes and ketones, contributed to the increase of TBA value simultaneous with the decrease of PV.

At the end of the heating program, ZBBE at the maximum concentration of 800 ppm was the most effective extract against secondary lipid oxidation, leading in a 9% decrease of TBA value compared to C.

CONCLUSIONS

Freeze-dried extracts obtained from blackberry processing by-products showed a strong inhibitory effect against primary and secondary oxidation initiated in sunflower oil following the exposure to 180ºC up to 12 hours. The antioxidant action of freeze-dried extracts obtained from blackberry processing by-products against thermo-oxidation of sunflower oil subjected to intense heat treatment was determined by the presence of polyphenolic compounds.

Supplementation with 200 ppm BHT and various concentrations of freeze-dried extracts obtained from blackberry processing by-products resulted in a significant increase ($p < 0.05$) of the thermo-oxidative stability of sunflower oil subjected to a heating program that simulated the conditions specific to alimentary processing. The effect of freeze-dried extracts ZBBE and PBBE in inhibiting lipid oxidation of sunflower oil, both in early and late stages, was dependent on the dose applied. At a concentration of 200 ppm, BBE showed a lower inhibitory effect compared to the inhibitory action of BHT. At a concentration of 500 ppm, BBE exhibit an inhibitory action similar to that of BHT and at a concentration of 800 ppm, both freeze-dried extracts proved a higher inhibitory effect than 200 ppm BHT.

Following the thiobarbituric acid test, a direct relationship was established between increased hydroperoxide consumption and increased TBA value.

The determination of specific indices for the assessment of oxidative stability revealed a more pronounced inhibitory response in the oil samples supplemented with freeze-dried extract obtained from blackberry processing by-product from an area with a milder climate characterized by higher temperatures and a moderate rainfall regime (ZBBE>PBBE).

The results obtained in this research are of applied importance in the edible oil industry by providing useful information on the evaluation of the ability of freeze-dried extracts obtained from blackberry processing by-products in limiting thermo-oxidative degradation of heat-processed sunflower oil and the evaluation of the opportunity offered by these extracts as potential BHT substitutes.

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