

The Antioxidant Activity of Barley Malt Rootlet Extracts in Heated Corn Oil at Frying Temperature

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Abstract

In this study the antioxidant activity of barley malt rootlet (BMR) extracts was evaluated in heat treated corn oil up to 5 hours at 185°C frying temperature. The antioxidant activity of BMR extracts was measured at 25, 50, 100 and 150 ppm concentrations. The free and bound antioxidant phenolics were extracted from BMR using three different extraction methods. Conventional solvent extraction (CSE), microwave assisted extraction (MAE) and autoclave assisted pretreated solvent extraction (APSE). In the present experiment, the total phenolic content and antioxidant activity of the various extracts were measured. Thiobarbituric acid reactive substances (TBARS) assay was used to evaluate the ability of the BMR to protect lipid peroxidation in corn oil at 185°C frying temperature. The formation of TBARS at 5 hours of heat treated corn oil has shown similar antioxidant levels in 150 ppm butylated hydroxytoluene (BHT) or MAE free phenolic extract added to corn oil. TBARS value for BHT was 1.896 ± 0.013 µg/mL of corn oil and for MAE was 1.896 ± 0.034 µg/mL of corn oil. The highest level of antioxidant activity was found for the free phenolic extracts. The order of inhibition of oxidation was found to be for free phenolics as follows: BHT (100 ppm) > APSE (50 ppm) > MAE (100 ppm) > CSE (100 ppm).

Keywords

Antioxidant Activity, Autoclave Treatment, Barley Malt Rootlets, BHT, Bound and Free Phenolics, Corn Oil, Lipid Peroxidation, Microwave Assisted Extraction, Secondary Oxidation Products, Solvent Extraction

1. Introduction

Lipid oxidation is one of the major issues in food industry as it leads to the de-

velopment of undesirable flavors and changes in food matrices. Lipid peroxidation is a chain reaction so that the polyunsaturated fatty acids react sequentially with oxygen molecules by free radical mechanism making peroxides and secondary lipid peroxidation products such as aldehydes, ketones and reduced carbonyl compounds [1]. Oxidative deterioration of lipids reduces the shelf life of products and renders many foods unacceptable to consumers. Synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA) and tert-butyl hydroquinone (TBHQ) are currently in use to protect lipid oxidation in food systems. Some research suggests that, these synthetic antioxidants could cause the development of liver and other cancers [2] [3] [4]. Consumer interest for healthier products containing fewer synthetic additives and clean label products has created a demand for novel and cost-effective natural antioxidants.

Recent research has been intensified on extracting phenolic compounds from plant matrices and food industry by-products to utilize them as potential sources for natural antioxidants. Recent studies have shown that pretreating plant matrices can significantly increase the antioxidant activity of phenolic extracts [5]. In general conventional solvent extractions are the most common method for extracting phenolic compounds from plant matrices, however, these require increased volume of solvents and longer extraction times. The industries at present are looking for alternative green extraction methods such as microwave assisted extractions (MAE) which can considerably reduce the amount of solvents used, lower extraction time and produce higher extraction efficiencies. Using MAE, several classes of phenolic compounds have been efficiently extracted from a variety of matrices, such as apple pomace [6], red raspberries [7], green tea leaves [8] wheat bran [9], rice bran [10], peanut skins [11], distillers dried grains [12], and spent coffee [13].

Barley malt rootlets (BMR) are spent grain from the brewing industries, obtained after the cleaning process of malting. Studies conducted by investigators [14] and [15] have shown that BMR are potential source of antioxidant phenolic compounds. In the current study, free and bound phenolic compounds were extracted from BMR using three different extraction methods: conventional solvent extraction (CSE), autoclave pre-treated solvent extraction (APSE) and microwave assisted extraction (MAE). These extracts were added at various concentrations to corn oil and heat treated at frying temperature (185°C). The antioxidant activity of these extracts was measured by the Thiobarbituric acid reactive substances (TBARS) assay and the results were compared to a synthetic antioxidant, butylated hydroxytoluene (BHT).

2. Materials and Methods

2.1. Barley Malt Rootlets (BMR)

Barley malt rootlets (BMR), the byproduct of malting industry obtained after the kilning process were procured from Rahr Malting Corporation, Shakopee, Minnesota. The moisture content of BMR (Model: Smart TurboTM-5, CEM, Mat-

thews, NC) on arrival was 2.8%. It was ensured that the moisture content of the BMR was maintained below 5%. BMR was ground to a particle size < 0.5 mm using Udy mill (Cyclone Sample Mill, UDY Corporation, CO, USA) and was kept in a plastic bag stored at 4°C until used for the study.

2.2. Chemicals

Acetone, ethyl acetate, sodium carbonate were procured from Fisher Chemicals (Somerset, NJ, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin Ciocalteu reagent, trichloroacetic acid (TCA), thiobarbituric acid (TBA), malondialdehyde (MDA) was purchased from Sigma Aldrich (St. Louis, MO, USA). Gallic Acid (Chem-Impex, Illinois, USA), sodium hydroxide from Ricca Chemicals (Arlington, TX, USA), and concentrated hydrochloric acid from Alfa Aesar (Tewksbury, MA, USA). Commercial corn oil (Market Pantry, Distributed by Target Corporation, Minneapolis, MN, USA) was purchased from a local market. The oil sample was kept at 4°C until used for the study. All the chemicals were of analytical grade.

2.3. Barley Malt Extract Preparation

Approximately 5 g of BMR sample was extracted by autoclave pretreated solvent extraction (APSE), microwave assisted extraction (MAE) and conventional solvent extraction (CSE). Free and bound antioxidant phenolic compounds were extracted from each of the different methods. The extraction method for each of the process is detailed hereunder.

2.3.1. Conventional Solvent Extraction

Free phenolics were extracted under the optimum conditions of 80% acetone, 58.3°C temperature, 1:10.5 solid to solvent ratio, for 90 min based on parameters established in our previous studies [5]. Extractions were performed twice in a shaking incubator (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., NJ, USA).

2.3.2. Autoclave Pretreated Solvent Extraction

BMR samples were autoclave treated (3021-S, Gravity, AMSCO, Mentor, OH, USA) under the standard temperature (121°C) and pressure (15 psi) for 20 min, which is typically used for sterilization and enzyme inhibition. The samples were cooled to room temperature and used for extraction. Extractions were performed twice for APSE (80% aqueous acetone, 1:10 solid to solvent, 40°C, 60 min) in a shaking incubator (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., Edison, NJ, USA).

2.3.3. Microwave Assisted Extraction

The antioxidant phenolic compounds were extracted using a microwave assisted extraction system (Model: MARS 6, CEM Corporation, Matthews, NC). The optimum extraction conditions of 5.4 min extraction time, 42.1°C and 79.1% of solvent concentration as designed in previous research were used [5]. After

careful extraction in Xpress vessels, the samples were cooled down and transferred to centrifuge tubes for further extraction process of free and bound phenolic compounds.

2.3.4. Free Phenolic Extraction

After extraction, samples were centrifuged (Model: accuSpin™ 1R, Fisher Scientific, Pittsburg, PA, USA) at 3500 rpm for 15 mins. The supernatants were filtered using Whatman filter No. 1 and the extracts were pooled and dried at 35°C under nitrogen using an evaporator (34 Position N-EVAP Nitrogen Evaporator, Organomation, Berlin, MA, USA). The dried extracts were reconstituted with 80% methanol and kept at 4°C until used for analysis. All the extractions were conducted in duplicates. The residues from free phenolics were used to extract bound phenolics.

2.3.5. Bound Phenolic Extraction

Alkaline hydrolysis using sodium hydroxide (1N) was carried out for the extraction of bound phenolics from BMR. The crude free phenolics residues obtained after the free phenolic extraction was dried overnight and hydrolyzed using NaOH (10 mL/g) for 16 h at ambient temperature. The sample was then acidified with 1 N HCl to a pH of 2 and then extracted thrice with ethyl acetate (3 × 10 mL). The extracts were then pooled together and dried at 35°C under nitrogen gas. The extracts were reconstituted with 80% methanol and kept under 4°C until used for analysis.

2.4. Experimental Design

The functionality of each of the extracts in inhibiting lipid oxidation at 185°C for up to 5 hours was investigated at various concentrations and the results were compared to a synthetic antioxidant, BHT under the same concentrations.

The free and bound antioxidant BMR extracts from APSE, CSE, MAE and BHT were added to corn oil samples (5.0 ± 0.1 g) in a test tube in various concentrations (25 ppm, 50 ppm, 100 ppm, 150 ppm). Samples were carefully vortexed and placed in a sand bath (Fisher, Hi-Temp™ Bath, Model 160, Texas city, TX, USA) maintained at 185°C for 0, 1, 2, 3, 4, 5 hrs. The heated oil samples were collected, and the antioxidant activity of the added extracts were measured using the TBARS assay.

2.5. Analyses

2.5.1. Determination of Total Phenolic Content (TPC)

Method for determination of TPC was adopted from Devanto *et al.*, [15] with slight modifications. Gallic acid (0 - 300 µg/mL) was prepared for a standard curve. Extract of 0.125 mL from the reconstituted solution was added to 0.5 mL of distilled water and followed by the addition of 0.125 mL of Folin-Ciocalteu reagent (FCR). After 6 mins of reaction time, 1.25 mL of Na₂CO₃ (7% aqueous) was added. The total volume was adjusted to 3 ml by adding distilled water. Samples were incubated at room temperature in the dark for 90 mins and the

absorbance was measured at 760 nm using a spectrophotometer (Model: UV-1800, Shimadzu Corporation, Kyoto, Japan). All values were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight of the BMR. All experiments were done in quadruplicate and the mean values were used for the analysis.

2.5.2. DPPH Radical Assay (DPPH)

The total antioxidant activity of the BMR extracts was measured using DPPH assay with slight modifications [16] [17] [18] and was expressed as μg of trolox equivalents (TE) per gram of dry weight of the sample. Trolox stock solution of concentrations (0 to 500 μM) was prepared for producing a standard curve. All the reagents were prepared fresh every time before the analysis. About 100 μL of reconstituted extracts/standard were added to 3900 μL of DPPH reagent and were incubated for 1 hour in dark environment at room temperature. The absorbance was recorded at 515 nm using the above mentioned spectrophotometer. All the analyses were conducted in quadruplicate and the mean data were considered for statistical analysis.

2.5.3. Thiobarbituric Acid Reactive Substances (TBARS) Assay

The method outlined by Duh *et al.*, [19] was used to measure the formation of secondary lipid peroxidation products in corn oil during heating at a frying temperature of 185°C. Malondialdehyde (MDA) of 10^{-5} concentration was used to prepare the calibration curve. Equal volumes of trichloroacetic acid (15% w/v) and 2-thiobarbituric acid (0.375% v/v) along with 0.25 N of hydrochloric acid were added to prepare the TBARS reagent. To 4 ml of TBARS reagent 0.2 ml of oil was added and the mixture was heated for 15 mins in a boiling water bath. After 15 mins of heating the samples were cooled, and the absorbance was measured at 535 nm by a spectrophotometer. All the measurements were performed in triplicates and the mean values were expressed as μmol MDA equivalents per gram of oil. The analysis was also conducted in triplicates and mean values of the data were used for statistical analysis.

2.6. Statistical Analysis

All the analytical parameters were measured in triplicates and the results obtained were statistically analyzed using two way-ANOVA and Tukey test at significance level of $p < 0.05$.

3. Results and Discussion

The total phenolic content and the antioxidant activities measured by % DPPH activity of APSE, CSE and MAE extracts are shown in **Table 1**. The antioxidant activities of various BMR extracts at different concentrations were analyzed and compared with the antioxidant activity of BHT. The significance of the extraction methods with respect to the concentration is shown in **Table 2**. All least mean square analysis data for various extracts used in the study are listed in **Table 3**. The detailed analyses are described in the following sections.

Table 1. Total phenolic content (TPC) and antioxidant activity (%DPPH) of Barley malt rootlets (BMR), extracted using various extraction methods.

Sample	Total phenolic content (TPC) (mg·g ⁻¹ dw. of BMR)		Antioxidant activity %DPPH	
	Free Phenolics	Bound Phenolics	Free Phenolics	Bound Phenolics
*APSE	3.76	1.49	71.6	24.8
**CSE	3.50	1.90	29.1	67.5
***MAE	4.82	2.80	53.8	52.3

*APSE: Autoclave pretreated solvent extraction; **CSE: Conventional solvent extraction; ***MAE: Microwave assisted extraction.

Table 2. Significance of MDA values for various extracts of BMR and comparison with just oil and BHT.

Sample extracts	Free Phenolics				Bound Phenolics			
	25 ppm	50 ppm	100 ppm	150 ppm	25 ppm	50 ppm	100 ppm	150 ppm
Corn oil	Corn oil ^a	Corn oil ^{ab}	Corn oil ^b					
Corn oil+ *APSE	APSE ^b	APSE ^b	APSE ^b	APSE ^c	APSE ^{ab}	APSE ^{ab}	APSE ^{ab}	APSE ^b
Corn oil+ **CSE	CSE ^a	CSE ^a	CSE ^b	CSE ^{ab}	CSE ^b	CSE ^{ab}	CSE ^a	CSE ^a
Corn oil+ ***MAE	MAE ^a	MAE ^b	MAE ^c	MAE ^c	MAE ^a	MAE ^{bc}	MAE ^b	MAE ^a
Corn oil+ ****BHT	BHT ^{ab}	BHT ^b	BHT ^c	BHT ^{bc}	BHT ^{ab}	BHT ^c	BHT ^c	BHT ^c

*APSE: Autoclave pretreated solvent extraction; **CSE: Conventional solvent extraction; ***MAE: Microwave assisted extraction; ****BHT: Butylated hydroxytoluene. Letters with same superscript in each column are not significantly different ($p < 0.05$).

Table 3. Analysis of Variance and summary of fit table for MDA equivalents ($\mu\text{g}\cdot\text{ml}^{-1}$ of corn oil) for free and bound phenolics of Barley malt rootlets (BMR) extracted using various methods for different source of variation at various concentrations (ppm).

Parameters analyzed	Concentration (ppm)	Source of variation	Degrees of freedom	Sum of squares	F-value	Prob (>F)
Free phenolics	25	Treatment	4	1.25	4.66	0.00
		Time (h)	5	37.87	112.82	<0.001
		Treatment*Time (h)	20	6.77	5.04	<0.001
	50	Treatment	4	4.41	24.62	<0.0001*
		Time (h)	5	44.80	199.89	<0.0001*
		Treatment*Time (h)	20	6.67	7.44	<0.0001*
	100	Treatment	4	4.21	16.57	<0.0001*
		Time (h)	5	28.46	89.60	<0.0001*
		Treatment*Time (h)	20	8.85	6.97	<0.0001*
	150	Treatment	4	3.65	12.54	<0.0001*
		Time (h)	5	56.99	156.39	<0.0001*
		Treatment*Time (h)	20	6.26	4.29	<0.0001*

Continued

Bound phenolics	25	Treatment	4	0.95	3.47	0.0129*
		Time (h)	5	60.22	175.85	<0.0001*
		Treatment*Time (h)	20	8.97	6.55	<0.0001*
	50	Treatment	4	2.55	8.10	<0.0001*
		Time (h)	5	40.29	102.53	<0.0001*
		Treatment*Time (h)	20	7.60	4.84	<0.0001*
	100	Treatment	4	5.82	17.46	<0.0001*
		Time (h)	5	28.61	68.69	<0.0001*
		Treatment*Time (h)	20	8.39	5.04	<0.0001*
	150	Treatment	4	8.33	28.75	<0.0001*
		Time (h)	5	77.11	212.99	<0.0001*
		Treatment*Time (h)	20	5.00	3.46	0.0001*

Columns with * on the superscript are significantly different.

3.1. Comparison of the Antioxidant Activities of Various BMR Extracts

3.1.1. Free Phenolics

For the various free BMR extracts ($p < 0.05$) using various concentrations for this study showed significant differences. The TBARS profile for all the free phenolic extracts used in the study and the formation of secondary lipid peroxidation products expressed as MDA equivalents ($\mu\text{g}/\text{mL}$ of corn oil) are shown in **Figures 1-4** for 25, 50, 100 and 150 ppm, respectively. Accordingly, with the increase in the heating time, the MDA values increased significantly from $-0.652 \pm 0.0 \mu\text{g}/\text{mL}$ to $2.748 \pm 0.0 \mu\text{g}/\text{mL}$ of corn oil. This indicates the formation of the secondary oxidation products in the oil due to the heat treatment for an extended period of 5 hours.

The added APSE extracts exhibited significant antioxidant activity at all concentrations (25, 50, 100, 150 ppm) over the 5 hour of heating period. With increase in the concentrations of the BMR extract from 25 to 50 ppm the inhibition of lipid oxidation increased by 12%. The addition of 100 ppm the MDA value was $1.948 \pm 0.57 \mu\text{g}/\text{mL}$ of corn oil compared to $1.694 \pm 0.30 \mu\text{g}/\text{mL}$ of corn oil at 150 ppm.

At lower concentrations, CSE extracts could not exhibit any antioxidant activity. The MDA values after 5 hours of heating at 185°C for 25 ppm and 50 ppm CSE extracts were found to be $2.389 \pm 0.77 \mu\text{g}/\text{mL}$ of corn oil, $2.72 \pm 0.146 \mu\text{g}/\text{mL}$ of corn oil compared to $2.748 \pm 0.282 \mu\text{g}/\text{mL}$ of corn oil (Control). With increase in concentration from 100 ppm to 150 ppm of CSE extracts, the MDA equivalents increased from $1.784 \pm 0.181 \mu\text{g}/\text{mL}$ of corn oil to $2.180 \pm 0.093 \mu\text{g}/\text{mL}$ of corn oil. APSE extracts at 50 ppm concentration performed equally as good as the CSE extracts at 100 ppm.

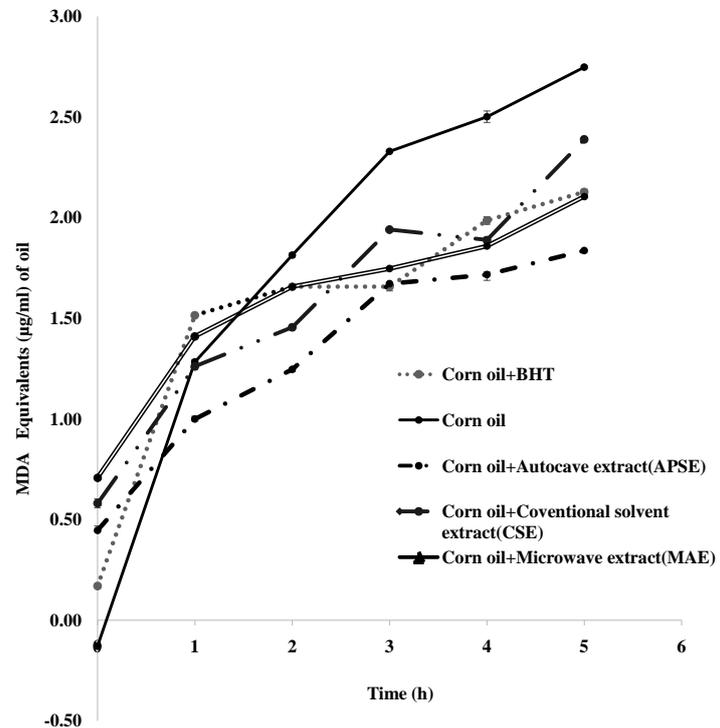


Figure 1. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C up to 5 hours and with 25 ppm of added free phenolic barley malt extracts and BHT.

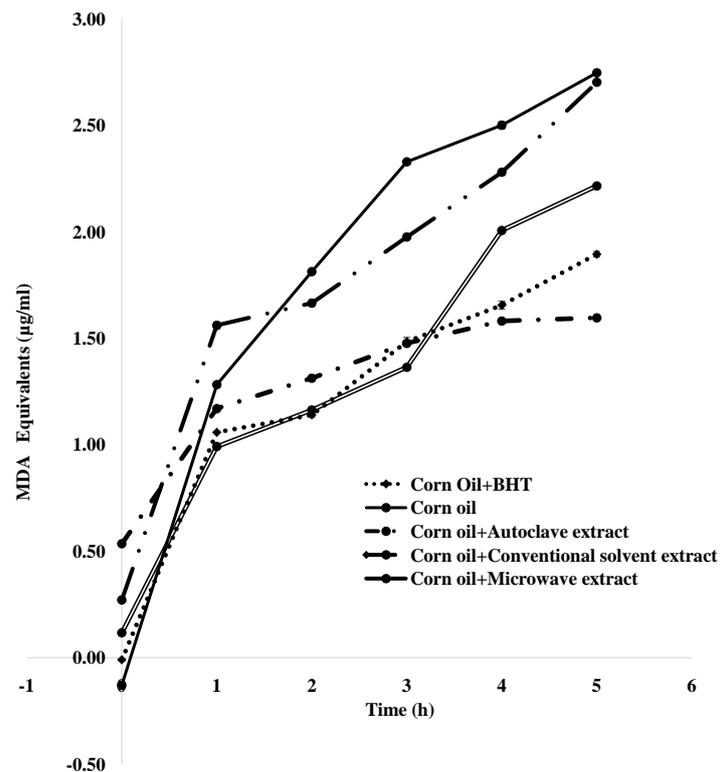


Figure 2. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in in corn oil heated at 185°C up to 5 hours and with 50 ppm of added free phenolic barley malt extracts and BHT.

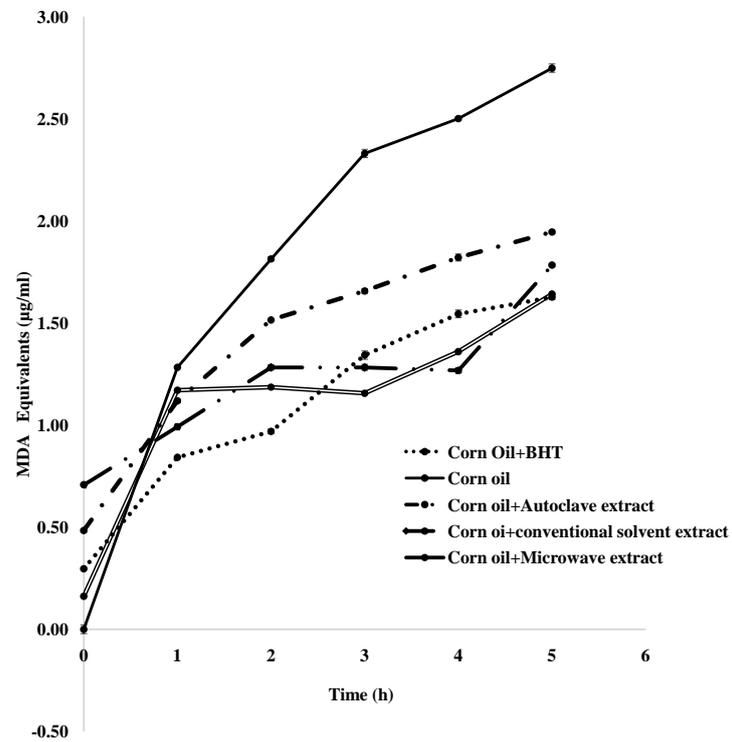


Figure 3. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) in corn oil expressed as MDA equivalents in heated at 185°C upto 5 hours and with 100 ppm of added free phenolic barley malt extracts and BHT.

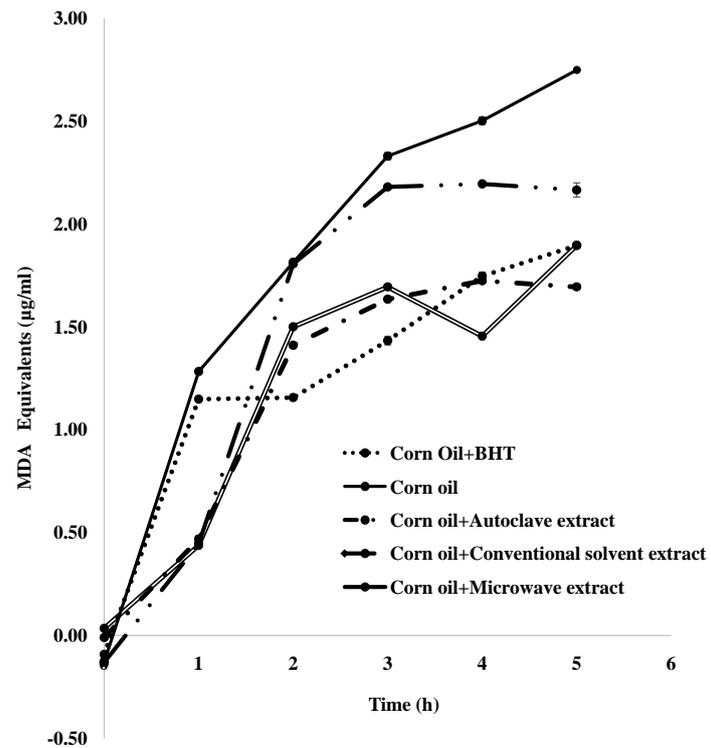


Figure 4. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C upto 5 hours and with 150 ppm of added free phenolic barley malt extracts and BHT.

MAE extracts effectively reduced the lipid oxidation in heated corn oil at all the concentrations. The MDA equivalents from the TBARS assay at 25 ppm, 50 ppm, 100 ppm and 150 ppm are 2.101 ± 0.43 $\mu\text{g/mL}$ of corn oil, 2.217 ± 0.0 $\mu\text{g/mL}$ of corn oil, 1.642 ± 0.131 $\mu\text{g/mL}$ of corn oil, and 1.896 ± 0.770 $\mu\text{g/mL}$ of corn oil, respectively. There was no significant difference in the antioxidant activity between 25 and 50 ppm concentrations of MAE extracts. The highest inhibition of oxidation was observed at 100 ppm. There were no significant differences between the MDA value of BHT and MAE added samples at 100 ppm and 150 ppm. The order of inhibition of oxidation is as follows: BHT (100 ppm) > APSE (50 ppm) > MAE (100 ppm) > CSE (100 ppm).

It is evident that autoclaving as a pre-treatment (APSE) or microwave assisted extraction treatment (MAE) has significantly increased the total phenolic content and the antioxidant power of barley malt rootlet extracts. These results are in agreement to the fact that thermal treatments can enhance not only the yield of phenolic extracts but also the antioxidant activity, by breaking the lignocellulose of the cell components and creating disordered structures with or without the removal of inherent components [20] [21] [22] [23].

3.1.2. Bound Phenolics

The bound phenolic extracts followed a different pattern of antioxidant activity compared to the free phenolic extracts. The antioxidant activity of bound phenolics was comparatively low compared to the free phenolics. All values were significantly different between free and bound phenolics with all concentrations tested in the present study. The MDA values for all the bound phenolic extracts are presented in **Figures 5-8**, for 25, 50, 100 and 150 ppm concentrations of BMR extracts, respectively.

The MDA values for autoclave bound phenolic extracts at 25 ppm, 50 ppm, 100 ppm, 150 ppm concentrations are 2.285 ± 0.40 $\mu\text{g/mL}$ of corn oil, 2.105 ± 0.04 $\mu\text{g/mL}$ of corn oil, 1.941 ± 0.18 $\mu\text{g/mL}$ of corn oil, and 2.419 ± 0.66 $\mu\text{g/mL}$ of corn oil, respectively. With increase in the concentration from 25 ppm to 50 ppm there was an increase in inhibition of oxidation by 7%. However, there was a relative decline in antioxidant power after 100 ppm. The order of antioxidant activity of bound phenolic extracts with respect to the concentration is as follows 100 ppm > 50 ppm > 25 ppm > 150 ppm.

The bound phenolics of conventional solvent extracts (CSE) have shown poor antioxidant power compared to the autoclave treated extracts (APSE). The formation of MDA at 25 ppm of CSE was 2.913 ± 0.771 $\mu\text{g/mL}$ of corn oil compared to the MDA value of the control (2.748 ± 0.0 $\mu\text{g/mL}$ of corn oil).

For bound MAE extracts, at lower concentration the values were significant high for MDA (3.264 ± 0.207 $\mu\text{g/mL}$ of corn oil), however with the increase in concentration of MAE extracts, there was an increase in the inhibition of the oxidation. The highest level of inhibition of oxidation was observed at 100 ppm concentration of MAE, and it is similar to free phenolics from MAE. It is interesting to note that, among the bound phenolics, the MAE extracts at 100 ppm

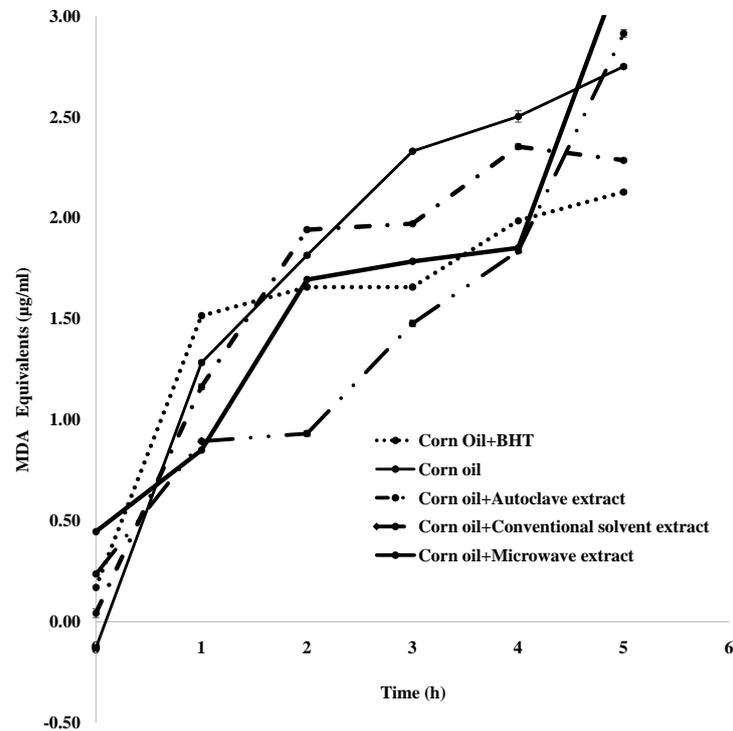


Figure 5. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C upto 5 hours and with 25 ppm of added bound phenolic barley malt extracts and BHT.

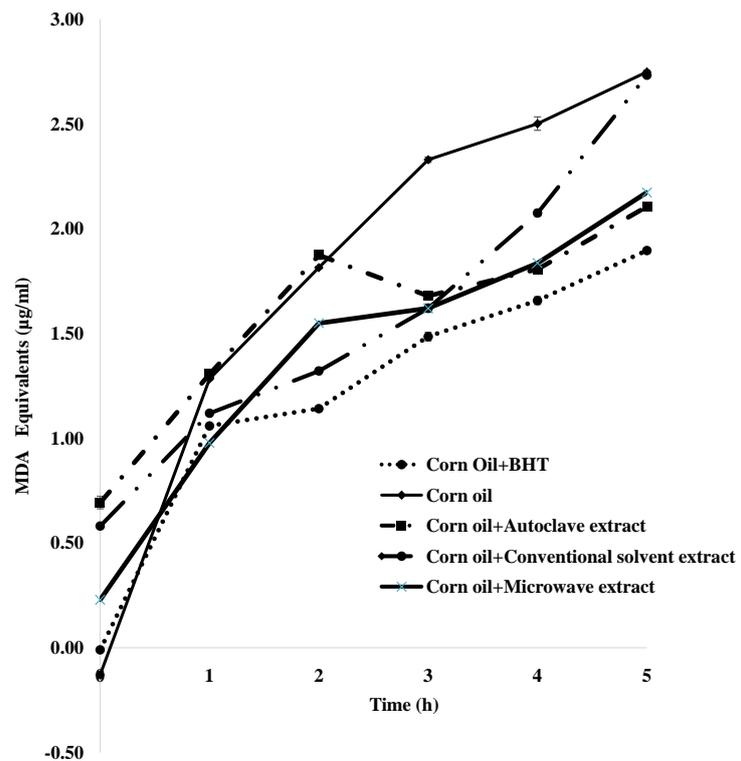


Figure 6. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in in corn oil heated at 185°C upto 5 hours and with 50 ppm of added bound phenolic barley malt rootlet extracts and BHT.

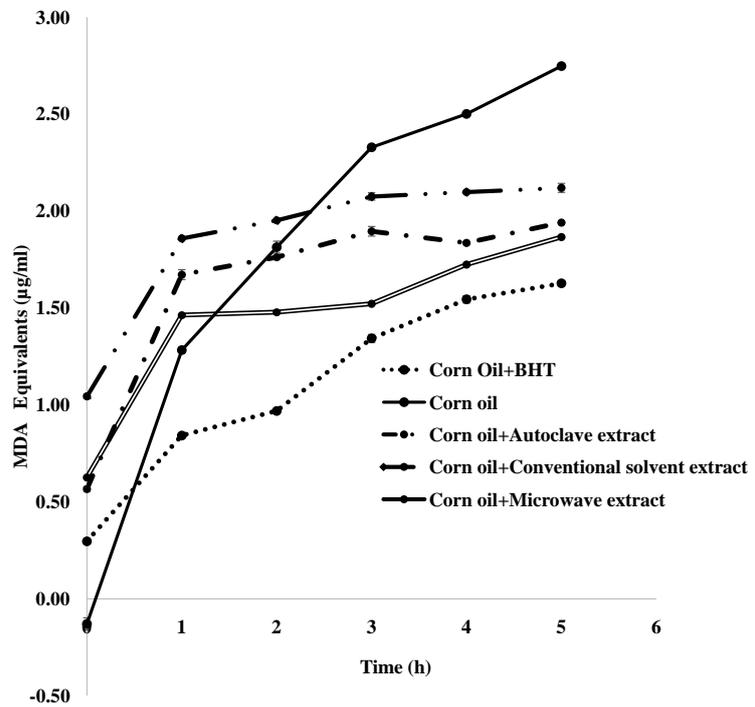


Figure 7. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C up to 5 hours and with 100 ppm of added bound phenolic barley malt extracts and BHT.

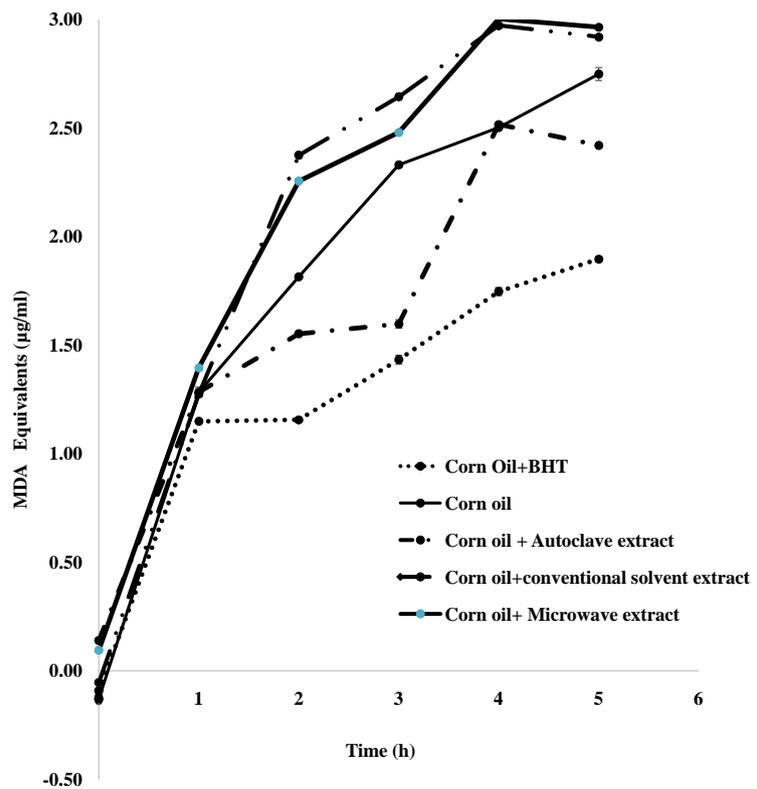


Figure 8. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C up to 5 hours and with 150 ppm of added bound phenolic barley malt extracts and BHT.

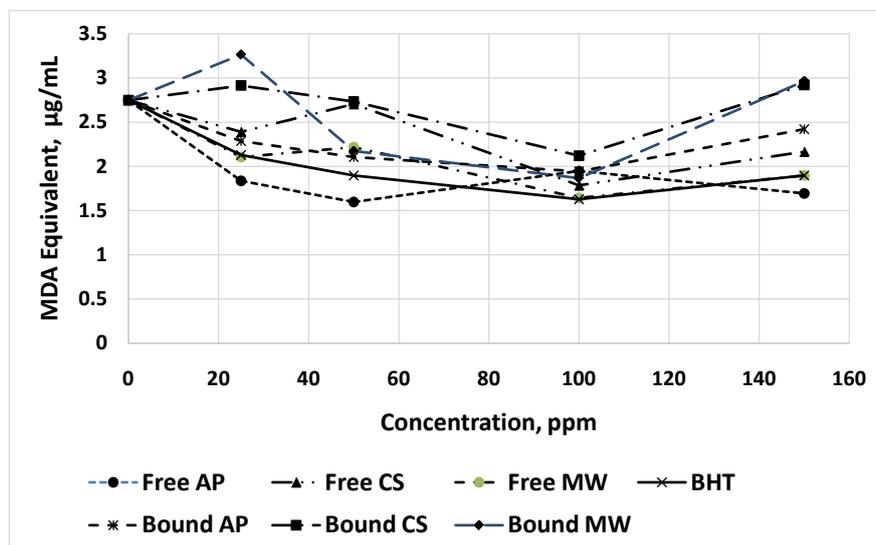


Figure 9. Comparison of formation of Thiobarbituric acid reaction substances (TBARS) expressed as MDA equivalents in corn oil heated at 185°C for 5 hours and with varying concentration of free and bound phenolic barley malt extracts and BHT.

exhibited similar antioxidant activity to BHT. The order of inhibition by the various bound extracts is as follows: MAE (100 ppm) > BHT (50 ppm) > autoclave (100 ppm) > CSE (100 ppm). Overall it was found that the bound phenolic extracts did not exhibit as good antioxidant activity as the free phenolics. In the present study, the antioxidant activity declined in around 100 ppm concentrations for all the BMR extracts.

4. Conclusions

In the current study, BMR extracts exhibited high antioxidant activity in inhibiting lipid peroxidation of heat treated corn oil at frying temperature of 185°C. The current study shows that thermal autoclave pre-treatment or microwave assisted extraction can significantly increase the phenolic content of BMR and therefore its antioxidant activity. The formation of MDA at 150 ppm concentration in heated corn oil has shown similar inhibition values for BHT and those of MAE free phenolic extracts, after 5 hours. The maximum inhibition happened for APSE (50 ppm) and for MAE (100 ppm) and BHT (100 ppm) (Figure 9). The order of inhibition of lipid oxidation in the formation of MDA for BHT and for free phenolics is: BHT (100 ppm) > APSE (50 ppm) > MAE (100 ppm) > CSE (100 ppm). There was a great variability in the data obtained from bound phenolic extracts of APSE, MAE, CSE. This could be due to the possible pro-oxidant activity of bound phenolic extracts at various concentrations.

A detailed investigation of the bound phenolic extracts from BMR is essential to understand the behavior of these extracts under frying temperatures. In the current research, the antioxidant effect of free and bound phenolics was investigated separately, a synergistic effect of the extracts on the antioxidant and antimicrobial properties yet to be investigated. In addition, evaluating these natural

antioxidants for their cost-effectiveness is vital to reduce the financial burden on food industries.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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