



Effects of different processing methods on the polyphenolic compounds profile and the antioxidant and anti-glycaemic properties of horseradish roots (*Armoracia rusticana*)

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Abstract

Horseradish (*Armoracia rusticana*) provides human organisms with various effective treatments for improving human health, and it constitutes a salubrious root used for culinary purposes. This outcome derives from the chemical composition and polyphenols content of horseradish. This study's objective was to determine individual polyphenols, total phenolic (TPC) and total flavonoid (TFC) contents, colour parameters, inhibition of advanced glycation end product (AGEs) formation and Trolox Equivalent Antioxidant Capacity (TEAC) by DPPH and ABTS of horseradish products. Phenolic acids and flavonoids were analysed by HPLC-DAD-MS method. Horseradish products were obtained by thermal treatments such as boiling, baking and sous-vide. Fresh and sous-vide samples were characterized by the most abundant TPC values, whereas TFC horseradish indicated the highest value after boiling treatment. Moreover, the highest values of antioxidant capacity in fresh horseradish roots were detected. Flavonoids presented lower concentrations than phenolic acids, while syringic acid was the most abundant phenolic compound. Horseradish, after sous-vide treatment, appeared to have a higher inhibition of AGEs formation. Thermal treatment of horseradish root increased the saturation of yellow (b*) and red (a*) colours and decreased colour brightness. Our findings indicate that horseradish products are a valuable source of polyphenols, with their profile, content, and antioxidant and anti-AGEs formation activity modulated by applied processes.

Keywords Horseradish · Polyphenols · AGEs inhibition · Food treatment · Colour · Antioxidant capacity

Introduction

There is an increasing interest in the content of phenolic compounds and the antioxidant capacity of plant-based products. Recent studies focussed on the content of antioxidants in dietary sources of culinary roots used as spices or condiments, fruits and vegetables [1]. Within this context, plants of the Brassicaceae family have been highlighted as anti-inflammatory and antioxidant properties [2]. These characteristics are derived from their chemical composition and, more particularly, from their vitamins, minerals,

amino acids and bioactive constituents such as phenolics and tocopherols that have anti-inflammatory properties [3]. The Brassicaceae family contains cruciferous vegetables, including horseradish (*Armoracia rusticana*), a hardy edible perennial that prospers to south-eastern Europe and western Asia [4]. The plant's cylindrical, fleshy, yellowish-white root has an initial diameter of approximately 2.5 cm, although it may grow as long as 1 m [5]. The nutritional value and pungent savour of horseradish are the primary reasons for this plant's extensive use for medical and culinary purposes [6]. It is generally accepted that horseradish constitutes an antioxidant product with multiple health benefits because of its abundant bioactive compounds directly related to a positive effect on human health, including cancer [7]. Studies by Herz et al. [8] and Manuguerra et al. [9] have attested to the antibacterial, antiradical and anti-inflammatory properties of horseradish. Via spectrophotometric outcomes recorded that Brassicaceae family plants' total antioxidant capacities and significant amounts of phenolics provide the above properties [10].

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As far as it is known, there needs to be more scientific data about the process of horseradish as an independent vegetable because it constitutes an additional ingredient in various processed food products. More specifically, some products derived from processed horseradish are dry horseradish powder, paste, and prepared horseradish blended with other condiments [11]. This study documents several key contributions that could make to the field of industry, for instance, the development of new products rich in bioactive substances, which could be further suggested as a part of broader preventative strategies. This study's objective was to demonstrate the effects of the horseradish's cooking processes on the individual phenolic compounds, TPC and TFC, colour parameters, antioxidant activity, and inhibition of AGEs formation.

Material and methods

Chemicals and reagents

Methanol, acetonitrile, formic acid, ammonium formate, gallic acid, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminium chloride (AlCl_3), phosphate buffer (PBS), bovine serum albumin, 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and aminoguanidine solution were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of the samples

Fresh horseradish (*Armoracia rusticana*) roots were purchased from a local market in Olsztyn, Poland. Horseradish was divided into four parts, approximately 500 g each. The first one was the fresh sample, sliced into equal rings and stored in a polyethylene bag in a freezer at $-24\text{ }^\circ\text{C}$. The second was baked in a combi oven at $100\text{ }^\circ\text{C}$ with hot air for 1 h, the third was placed in 2 L of deionized water for 1 h in a stainless still pot with a lid, and the last followed the sous-vide method at $85\text{ }^\circ\text{C}$ for 2 h. Before sous-vide heat treatment, horseradish samples were vacuum packed in PA/PE bags (15 μm polyamide/60 μm polyethylene; temperature resistance $-20\text{ }^\circ\text{C}/+110\text{ }^\circ\text{C}$; Hendi, Austria) using a vacuum chamber sealer (Edesa VAC-20 DT, Barcelona, Spain). The last three samples were sliced in equal rings and stored in polyethylene bags in the freezer when they reached ambient temperature. At the concluding sample preparation phase, horseradish samples were freeze-dried, pulverized, and stored until analysed at $-24\text{ }^\circ\text{C}$.

Preparation of horseradish extracts

Powdered horseradish samples (200 mg) were extracted with 1 mL of methanol 80% (v/v), vortexed for 30 s, and placed in the ultrasonic bath for 30 s. The vortex and ultrasound application were repeated three times. After that, the samples were centrifugated at 14,000 rpm (VWR micro star 30R, Radnor, PA, USA) for 10 min at $4\text{ }^\circ\text{C}$. This procedure was repeated five times, and the obtained supernatant was collected in a volumetric flask with a capacity of 5 mL. Each horseradish sample was prepared in three replications.

Colour measurement

Colour measurement was performed according to Danowska-Oziewicz et al. [12]. A Konica Minolta CR-400 colourimeter (Konica Minolta, Sensing Inc., Osaka, Japan), equipped with a standard 2° observer, was used to determine the colour parameters. The equipment was calibrated using a white reference tile before taking measurements. Colour parameters were measured using a D65 light source. The CIE $L^*a^*b^*$ colour space was used to describe the colours. Based on the obtained data, the total colour difference (ΔE^* ; formula 1) and browning index (BI; formula 2) were calculated:

$$\Delta E_{ab} = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)} \quad (1)$$

$$BI = 100 * (X - 0.31)/0.17 \quad (2)$$

where $X = (a^* + 1.75L^*)a^* / (5.645L^* + a^* - 3.012b^*)$.

Determination of total phenolic (TPC) and total flavonoid (TFC) contents

The total phenolic (TPC) content was carried out using the Folin's phenol reagent according to the procedure described by Sawicki et al. [13]. A mixture containing 15 μL of appropriately diluted extract and 240 μL of Folin's phenol reagent were placed into wells of microplates and incubated for 10 min at room temperature (RT). Next, 15 μL of 20% sodium carbonate was added and shaken. Absorbance was measured at 765 nm using a microplate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany). Obtained results were expressed as mg gallic acid equivalents (GAE) per gram of dry matter (dm).

The assay of total flavonoid (TFC) content was carried out according to the procedure described by Sawicki et al. [13]. A mixture containing 25 μL of extract and 75 μL of ethanol (v/v) was mixed with 5 μL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ as well as 5 μL of 1 mol/L potassium acetate. Next, 140 μL of deionized water was added to each well, mixed and left for 30 min at RT. After this time, the absorbance was measured

at 415 nm using a microplate reader. The TFC of each sample was calculated by interpolating with the calibration curve built with quercetin as a standard. Results are expressed as mg quercetin equivalents (QE) per gram of dry matter. All measurements were performed in triplicates.

Determination of polyphenols profile

The analysis of polyphenols was performed according to the methodology described by Zakrzewski et al. [14]. Polyphenols qualitative and quantitative were carried out using a UHPLC system (Nexera XR, Shimadzu, Kyoto, Japan) coupled with a diode area detector (DAD) and mass spectrometer (LCMS-2020, Shimadzu, Japan). Measurement parameters were as follows: eluent 0.01% formic acid in water with 1 mM ammonium formate (A) and 0.01% formic acid in 95% acetonitrile solution with 1 mM ammonium formate (B); flow rate 0.37 mL/min; scanning in negative ionization; column Kinetex (2.6 μ m particle size; 100 \times 4.6 mm) (Phenomenex, Torrance, CA, USA); oven temperature was 40 $^{\circ}$ C; sample injection volume 10 μ L. An analysis was conducted in the selected ion monitoring mode (SIM). Analysed compounds were identified according to their qualitative ions, retention times and λ_{\max} summarized in Table 1. The quantity of polyphenols was calculated from the UHPLC-DAD-MS peak area against commercially available standards.

The method of least squares was used to obtain the equations of calibration curves ($y = ax + b$). The coefficient of determination (R^2) gave goodness of fit, which is the evidence of linearity for all analysed phenolic compounds in the concentration range from 0.01 to 150 μ g/mL (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) values were calculated based on the signal-to-noise (S/N) ratio. The level of noise was measured from the chromatograms obtained for the standard solutions at the lowest concentration level. The limit of detection was calculated

as three times higher than the noise level, and the limit of quantification was equal to ten times the noise level.

TEAC (Trolox equivalent antioxidant capacity) by ABTS and DPPH

The TEAC was performed as described by Sawicki et al. [13] to evaluate the antioxidant capacity of the obtained extracts. The TEAC by ABTS was conducted by placing 10 μ L samples, blanks, or standard into microplate wells. Subsequently, the reaction and time measurement were started by adding 290 μ L of ABTS $^{+\cdot}$ solution. The reaction was performed at ambient temperature in the dark for 6 min. In the case of the DPPH assay, antioxidant capacity was conducted by placed of 20 μ L samples, blanks or standard into microplate wells and then adding 300 μ L of DPPH $^{\cdot}$ solution. The reaction was performed at ambient temperature for 30 min in the dark. The absorbance was measured at 734 nm (ABTS assay) and 517 nm (DPPH assay) using a microplate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany). Results were presented as μ mol Trolox/g dm. All measurements were performed in triplicates.

Anti-glycation assay

To determine the anti-glycation properties of horseradish samples, the BSA-glucose model was used, describing the final stage of AGEs formation. In the first step, the obtained extracts were dried under nitrogen. After drying, samples were dissolved in this same amount of phosphate buffer (0.1 M, pH 7.4) and used directly for the anti-glycation tests as described by Przygodzka and Zieliński [15]. Fluorescence intensity (excitation wave 330 nm and emission wave 410 nm) was measured using a microplate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany). Triplicate samples were run for each sample, and the percent inhibition

Table 1 Analytical parameters of the detected polyphenols in horseradish products

No	Compounds	R_t [min]	$[M]^-$ (m/z)	λ_{\max} [nm]	a	R^2	LOD [μ g/g]	LOQ [μ g/g]
Phenolic acids								
1	Syringic acid	2.26	197	278	2E-7	0.999	0.020	0.061
2	Caffeic acid	4.56	179	323	1E-8	0.999	0.025	0.076
3	Ferulic acid	5.04	193	322	3E-8	1	0.012	0.036
Flavonoids								
4	Epicatechin	4.58	289	319	6E-8	0.998	0.025	0.076
5	kaempferol-3-O-rutinoside*	4.80	593	351	–	–	–	–
6	kaempferol-O-glucoside*	4.96	446	351	–	–	–	–
7	Apigenin	5.99	269	267, 335	1E-8	0.999	0.010	0.030
8	Kaempferol	6.12	285	319	3E-8	0.998	0.013	0.040

R_t retention time, $[M]^-$ (m/z) parent ion, a calibration slope, R^2 coefficient of determination, LOD limit of detection, LOQ limit of quantification

*Analytical parameters for kaempferol was used

of AGEs formation by a horseradish extract or aminoguanidine (AG) solution (1 mM) used as a positive control was calculated.

Statistical analysis

The data are presented as mean values \pm standard deviations of triplicate measurement. A one-way ANOVA analysed the differences between samples with Fisher's test ($p < 0.05$). Furthermore, data on TPC, TFC, individual polyphenols, the sum of polyphenols (SPC), AGEs and antioxidant capacity (DPPH and ABTS assays) were subjected to principal component analysis (PCA). The statistical analysis was performed using STATISTICA 13.0 (TIBCO Software Inc., Tulsa, OK, USA).

Results and discussion

Colour parameters

The effects of different cooking processes on the colour parameters of horseradish are presented in Table 2. It indicated a decrease in the lightness (L^*) of processed horseradish samples using different heating methods compared with fresh horseradish. Concerning the sous-vide and boiled sample, the L^* value which was observed in the present study was similar to that reported by Gonnella et al. [16].

Boiled, baked, and sous-vide samples showed less intense greenness than fresh horseradish, considering that the redness parameter (a^*) was increased in these samples. The yellowness (b^*) parameter was also observed with a

relatively equal augmentation in each cooked sample. The total colour difference (ΔE^*) in heating processed products was between 16.91 and 21.47, which implies that cooking procedures caused a distinct change in colour compared with the fresh raw root. Determining the overall colour difference (ΔE^*) of the tested horseradish samples, a very significant deviation was observed, which proves the colour difference recognizable by an inexperienced observer [17]. Also, similar observations were shown on cruciferous vegetables in the study by Danowska-Oziewicz et al. [12]. As presented in Table 2, the apparent degradation of BI was observed at sous-vide samples (0.28 ± 0.03), while the BI of baked samples was the highest. However, the baked and boiled samples were not statistically significantly different. Moreover, overall findings about the yellowness (b^*) parameter are reported by Lafarga et al. [18] after sous-vide cooking of *Brassica* plants, a genus of the Brassicaceae family.

TPC and TFC in horseradish products

The values of TPC and TFC are presented in Table 3. In horseradish samples, TPC concentration ranged from 1.51 to 1.80 mg GAE/g dm. Sous-vide and fresh horseradish extracts showed the highest TPC. The lowest content of TPC was detected in boiled samples. A considerable increase in the TPC value of sous-vide samples was noted in comparison with the other cooking processes. As demonstrated, the chemical oxidation of phenolic antioxidants occurs due to the high temperature [19]. In contrast, sous-vide, which translates to under vacuum cooking, is a method that involves the placement of raw or partially cooked food in a hermetically sealed plastic bag

Table 2 Colour parameters of horseradish products in CIE $L^*a^*b^*$ system

Products	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Total colour difference (ΔE^*)	Browning index (BI)
Fresh	86.28 ± 0.32^a	-0.45 ± 0.03^c	13.13 ± 0.57^b	nd	nd
Boiled	66.59 ± 0.62^d	1.41 ± 0.03^a	21.47 ± 0.26^a	21.47 ± 0.77^a	0.53 ± 0.01^a
Baked	68.76 ± 0.14^c	1.49 ± 0.10^a	21.85 ± 0.14^a	19.67 ± 0.36^b	0.56 ± 0.04^a
Sous-vide	71.60 ± 0.38^b	0.77 ± 0.08^b	21.42 ± 0.23^a	16.91 ± 0.66^c	0.28 ± 0.03^b

Data are a mean \pm standard deviation ($n = 6$). Different letters in the same column indicate statistical significance ($p < 0.05$)

nd non detected

Table 3 Results of TPC, TFC and TEAC (ABTS and DPPH) determined in horseradish products

Products	TPC [mg GAE/g dm]	TFC [mg QE/g dm]	ABTS [μ mol Trolox/g dm]	DPPH [μ mol Trolox/g dm]
Fresh	1.80 ± 0.04^a	1.23 ± 0.02^c	10.46 ± 0.53^a	12.16 ± 0.48^a
Boiled	1.51 ± 0.02^c	2.02 ± 0.02^a	3.74 ± 0.04^c	7.72 ± 0.34^b
Baked	1.70 ± 0.03^b	1.77 ± 0.07^b	3.17 ± 0.06^d	5.33 ± 0.14^d
Sous-vide	1.79 ± 0.06^a	1.84 ± 0.03^b	4.59 ± 0.05^b	6.02 ± 0.29^c

Data are a mean \pm standard deviation ($n = 3$). Different letters in the same column indicate statistical significance ($p < 0.05$)

and cooking it for an extended period at a lower temperature [20]. The protection of degradation of the phenolic compounds under vacuum conditions and lower temperatures could explain the limitation of oxidation and, consequently, the high TPC value of horseradish after sous-vide treatment. Also, a similar observation was noted by Chiavaro et al. [21]. It is noteworthy that recent studies have shown that sous-vide cooking is regarded as the most effective approach for producing food products with superior functional and technological qualities [22]. The TFC in horseradish extracts presented different results from 1.23 to 2.02 mg QE/g dm (Table 3). The highest TFC value was found in boiled samples, while the lowest was noted in fresh horseradish. At drying by hot-air drying method, according to Chunning et al. [23], the TFC of horseradish was lower than infrared-assisted hot-air drying, freeze drying and pulse-spouted infrared freeze drying. The primary reason for this result was documented by Xi et al. [24], due to enzymatic catalysis, the exposure of phenolic compounds to oxygen for a prolonged period causes oxidation. In the case of TFC, the mean value of available data for horseradish root extracts was relatively in line with those obtained by Calabrone et al. [25]. Processing conditions or methodological factors could explain the value differences between TPC and TFC. In addition, the differences obtained in this study and the results of other authors may result from different species of horseradish used in the research or different conditions of its cultivation.

Profile of phenolic compounds

In this study, eight compounds were detected; three compounds belong to phenolic acid and five to flavonoids

(Table 4). Phenolic acids presented higher concentrations than flavonoids, while the most abundant phenolic acid was syringic acid. Significantly higher content of phenolic acids, especially syringic and ferulic acids, were detected in boiled and sous-vide samples. Caffeic acid was also identified in horseradish products, and the highest concentration ($11.43 \pm 0.53 \mu\text{g/g dm}$) was observed in boiled samples. The results indicate that the primary compound of the five flavonoids was epicatechin, with an average percentage contribution of 45.9% of the sum of individual flavonoids. Less dominant compounds in horseradish products were kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside. Apigenin and kaempferol were also detected in meagre quantities and principally in boiled and baked extracts, respectively. The highest sum of individual flavonoid compounds contained in the present study was found in sous-vide products. In contrast, fresh samples of horseradish were characterized by the poorest concentration of individual flavonoids ($33.91 \mu\text{g/g dm}$). More specifically, findings about kaempferol are by the values of cooking kale reported by Sikora et al. [26]. The concentration of ferulic acid in the same brassica vegetable by blanching process also presented similar values to the current study. In the case of fresh horseradish, similar epicatechin results were also found [27]. Considering flavonoids, it was found that 13 plants of the Brassicaceae family contained lower amounts of flavonoids than phenolic acids [28]. The results obtained by Bajkacz et al. [28] are comparable to those obtained in this study.

Table 4 Concentration ($\mu\text{g/g dm}$) of phenolic acids and flavonoids detected in horseradish products

No	Compounds	Horseradish products			
		Fresh	Boiled	Baked	Sous-vide
Phenolic acids					
1	Syringic acid	108.01 ± 0.48^c	170.35 ± 1.63^a	142.00 ± 8.45^b	153.73 ± 6.93^b
2	Caffeic acid	1.81 ± 0.03^d	11.43 ± 0.53^a	9.30 ± 0.14^b	6.27 ± 0.32^c
3	Ferulic acid	10.25 ± 0.04^c	49.64 ± 2.96^b	52.37 ± 0.46^b	63.92 ± 3.38^a
Flavonoids					
4	Epicatechin	13.75 ± 0.74^c	25.36 ± 0.44^b	21.65 ± 1.45^b	33.77 ± 2.10^a
5	kaempferol-3- <i>O</i> -rutinoside*	9.97 ± 0.14^b	9.37 ± 0.36^b	8.14 ± 0.43^c	12.98 ± 0.39^a
6	kaempferol- <i>O</i> -glucoside*	10.19 ± 0.40^c	15.04 ± 0.03^b	16.92 ± 0.65^a	16.11 ± 0.08^a
7	Apigenin	nd	1.11 ± 0.04	nd	nd
8	Kaempferol	nd	nd	8.03 ± 0.31	nd
Sum of phenolic content		153.99 ± 0.27^c	282.30 ± 4.20^a	258.41 ± 6.15^b	286.78 ± 5.50^a

Data are a mean \pm standard deviation ($n=3$). Different letters in the same line indicate statistical significance ($p < 0.05$)

nd non detected

*The concentration of the compounds was calculated from the kaempferol calibration curve

Determination of antioxidant capacity

Significant differences were also found in the analysed horseradish products regarding the TEAC by ABTS and DPPH (Table 3). Extract from fresh horseradish noted the highest value of TEAC determined by ABTS and DPPH assays (10.46 and 12.16 $\mu\text{mol Trolox/g dm}$, respectively), while the extract obtained from baked samples was characterized by the lowest antioxidant capacity (3.17 and 5.33 $\mu\text{mol Trolox/g dm}$, respectively). Moreover, the boiled horseradish roots were characterized by 64% (ABTS assay) and 37% (DPPH assay) lower antioxidant capacity values than fresh samples. In the case of the sous-vide samples, this treatment decreased antioxidant capacity by about 50%.

Values by DPPH obtained from fresh horseradish root ($12.16 \pm 0.48 \mu\text{mol Trolox/g dm}$) were comparable to those of Tomsone et al. [27]. Also a similar tendency in the results was also detected on fresh horseradish roots measured by ABTS assay [29]. On the other hand, lower values of antioxidant activity determined by the ABTS test for fresh horseradish roots were determined by Ku et al. [30].

Inhibition of AGEs of horseradish products

A heterogeneous set of chemicals and related adducts known as advanced glycation end products (AGEs) are created when macromolecules (protein, lipid, and DNA) are glycosylated by the Maillard reaction [31]. Inhibitors of AGEs could be derived from natural dietary products [32]. Moreover, polyphenols are known to exert mitigating effects on

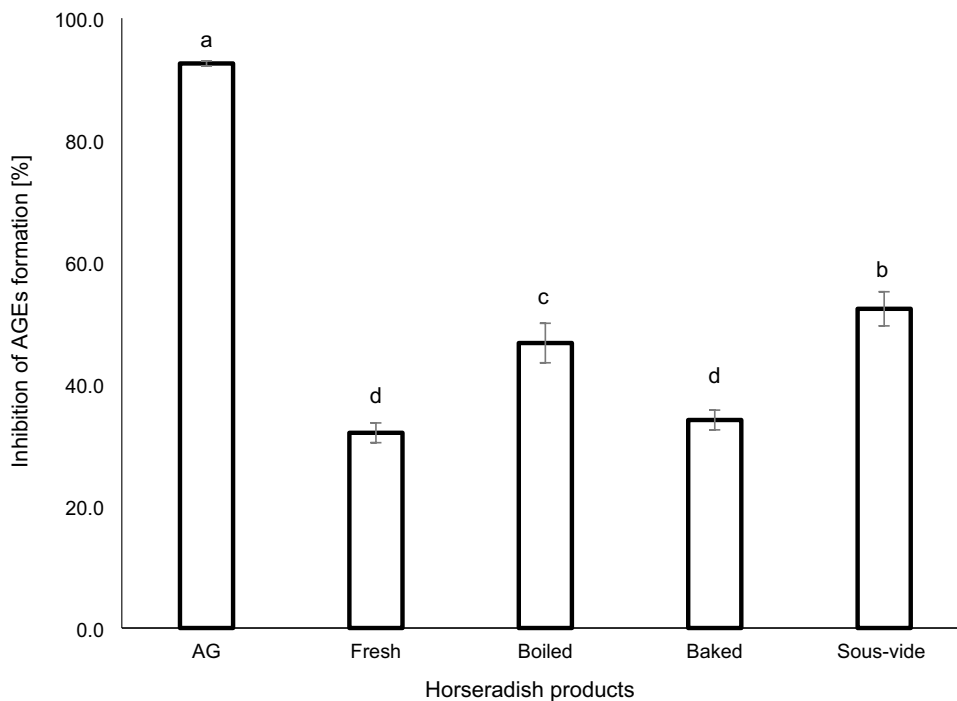
the harmful consequences of AGEs [33]. According to the results of AGEs, the inhibition effect of sous-vide extract was higher than the other samples and reached 52.34% (Fig. 1). This data can be related to the high content of phenolic compounds in the horseradish roots after sous-vide treatment. The percentage of inhibition of AGEs in horseradish products of this study in decreasing order was: sous-vide > boiled > baked = fresh. Horseradish after oven-baked treatment noted a lower value (34.11%) than boiled (46.73%), which could also be explained due to the polyphenolic content, as mentioned above. Polyphenols inhibit AGEs by capturing precursor active α -dicarbonyl compounds [34]. In vitro models have demonstrated the anti-AGEs capabilities of a wide range of phenolic substances [35]. According to Arfin et al. [36], the mechanism involves the following aspects: scavenging free radicals, chelating transition metals, eliminating active carbonyl intermediates and interacting with proteins to form complexes.

Principal component analysis

Principal component analysis (PCA) was used to provide an overview of the source of activity variation in horseradish products, and the plot is depicted in Fig. 2.

The cumulative input from the first and the last principal compound, presented in the biplot collectively, reached 83.46%. Component PC1 accounted for 63.29%, while the second PC2 predicted a lower variation of 20.17%. As results from the diagram (Fig. 2), the observed positive correlation between ABTS and DPPH assays could be further confirmed

Fig. 1 The inhibitory effects of horseradish products against AGEs formation. Data are a mean \pm standard deviation ($n=3$). AG—aminoguanidine; **a, b, c, d**—different letters indicate significant differences ($p < 0.05$)



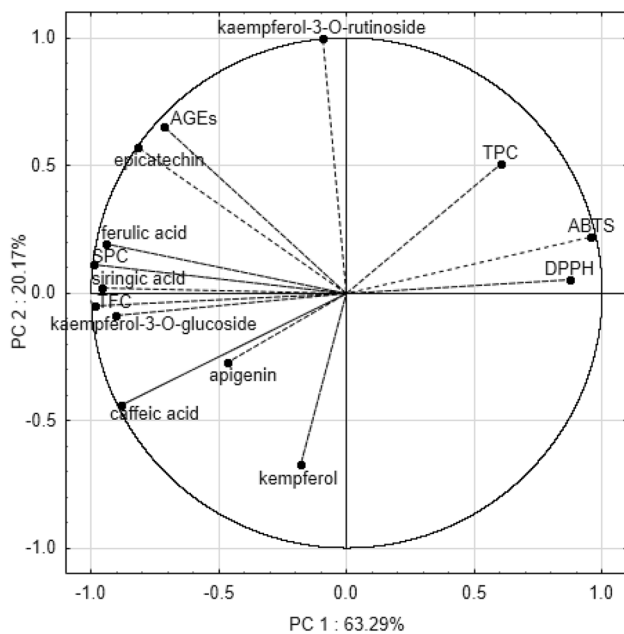


Fig. 2 Principal component analysis of TPC, TFC, individual polyphenols, sum of polyphenols (SPC) and antioxidant capacity (DPPH and ABTS assays)

due to a similar radical scavenging reaction between the two assays [37]. The sum of polyphenols (SPC) and the antioxidant activities assays projected on the delimited plane are far apart, suggesting that these two result from two distinct clusters. Outcomes about TPC and antioxidant activity assays showed that the arrow of TPC creates an acute angle with DPPH and ABTS, indicating that these assays are responsible for the antioxidant activity. Notably, the loadings representing apigenin and kaempferol were relatively short, indicating the low contribution of these variables to the total variance in the plot. Subsequently, it was observed clustering of variables in two main beams, one of which, with direction to the upper left quadrant, points to the centroid of AGEs, while the second one appears to be orthogonal to the first. The arrow of AGEs and epicatechin are close, forming a slight angle, indicating that this compound is positively correlated with inhibitory AGE formation. Kim et al. [38] showed that epicatechin, a major dietary monomeric flavonoid, could destroy antigenic AGEs both in vitro and in vivo. There is evidence that glycation inhibition of flavonoids, such as epicatechin, ensues due to the binding of α -dicarbonyl compounds [39].

Conclusion

This was the first work devoted to the effects of different processing methods on the profile of polyphenolic compounds and the health-promoting potential of horseradish

(*Armoracia rusticana*) roots, including antioxidant and anti-AGEs activity. The TPC, TFC values and concentrations of individual polyphenolics varied significantly among the four horseradish products. Syringic acid constituted the dominant phenolic acid, while in the field of flavonoids, epicatechin was noted as the most abundant concentration. The results indicated that cooking procedures caused a distinct change in colour compared with the respective fresh samples. Moreover, both radical scavenging assays indicated similar values, and the analysis showed higher values of fresh horseradish.

Furthermore, horseradish after sous-vide treatment pointed out the highest percentage of AGEs inhibition due to the high concentration of phenolics, as mentioned above. According to the PCA plot, epicatechin was positively correlated with inhibitory AGE formation. These data, consistent with research, may be a promising aspect of further use or consumption of horseradish. Obtained data can be helpful in the industry field for developing new products rich in natural substances with antioxidant properties and inhibiting the formation of advanced glycation end products (AGEs).

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Data availability All data included in this manuscript are available upon request by contacting with the corresponding authors.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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