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PHENOLIC CONTENT OF APPLE POMACE AFFECTED BY THEIR MICROWAVE IRRADIATION

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ABSTRACT

Thermal degradation mechanisms and products of some polyphenols, such as quercetin and rutin, have been intensively observed. The structural changes of polyphenols caused by food processing, may lead to different changes. The aim of this work was to test the changes of TPC, selected individual phenols and antioxidant activity of apple pomace gained from producers and compared to the samples prepared in laboratory conditions caused by microwave irradiation. Antioxidant activity was determined by the DPPH method, total polyphenolic content with use of Folin-Ciocalteau reagent and individual phenolic substances by HLPC -DAD method. The control samples were treated by the microwave radiation for 1 min. in a microwave oven at 650 W. The results of DPPH method showed an increased inhibition of DPPH radical, so higher antioxidant activity, mainly at laboratory prepared samples of pomace was detected. At TPC evaluation, an increase in the content of total polyphenols was observed similarly, at 3 of 4 irradiated samples the significant increase was detected at the level of significance p=0.001. Several individual phenols were detected after irradiation of pomace in higher amounts as well. Application of microwave irradiation in the extraction process might lead to significant increase of the total phenolic content.

Keywords: polyphenols, antioxidant activity, apple pomace, microwave treatment, HPLC-DAD

INTRODUCTION

Food irradiation is a technology that improves the safety and extends the shelflife of foods by reducing or eliminating microorganisms **(Barkai-Golan and Follet, 2017)**. Consumers are interested in products minimally processed and formulated with no chemical preservatives. Food industry has responded by developing technologies such as high-pressure processing, pulsed electric field, ohmic heating **(Prakash, 2020)**.
In 2022, Europe produced approx.20 tons of apples. Most of them is consumed as fresh fruit, 20% is processed for various purposes. These include concentrates, beverages, ciders and other foods, such as jams, purees. During these processes a significant amount of the waste, such as apple pomace is produced, around 20-30% of the weight of the fresh apple. Apple pomace requires careful handling and disposal, as it represents potential risk and burden for the environment, including pollution, gas production and microbial contamination. Effective management strategies are crucial to reduce the risk of negative effect on the environment **(Garofalo et al., 2024)**. Agrifood industries generate large amounts of waste that may result in environmental problems, such as soil and water contamination. Therefore, proper waste management and treatment have become an environmental, economic, and social challenge. Most of these wastes are rich in polyphenols with potential applications in the food, cosmetic, and pharmaceutical industries. The recovery of polyphenols from agrifood waste is a way of circular bioeconomy, that contributes to the valorisation of waste. Unconventional extraction techniques at the industrial scale, such as microwave-assisted extraction has demonstrated its efficacy at the laboratory level for analytical purposes **(Tapia-Quirós et al., 2024)**.
Apples are popular among consumers and are known as a rich source of biologically active compounds. Several studies confirmed the effects of apples against chronic diseases, due to their high content of phenolics in plant tissues. Geographical origin and variety of apples affect the content of these bioactive compounds and are connected with their antioxidant activity fluctuation. The choice of polyphenol-rich raw material as well as proper processing are important to achieve high-quality fruit-based products with a high content of antioxidants after production. Processing of apples could significantly modify the content of phenolics in the finished products. A lot of methods have been used to monitor the phenolic content and antioxidant activity of apple samples, based on spectrophotometry, HPLC, LC-MS, and LC-MS/MS techniques for their identification **(Starowicz et al., 2020)**. Flavonoids with their anti-cancer, anti-inflammatory, and antioxidant properties, are utilized in the food, pharmaceutical, and cosmetic industries. However, traditional extraction methods for flavonoids showed limitations such as long extraction time and low yield. Microwave-assisted extraction is a widely used extraction technique that utilizes microwave energy to extract intracellular flavonoids **(Wang et al., 2025)**. The extraction of bioactive components, from natural sources has gained significant attention due to increasing demand for natural and functional components **(Bhadange et al., 2024)**.
Apple pomace, a by-product rich in nutritional substances such as polyphenolic compounds, is primarily composed of apple peel and unprocessed flesh (up to 95 %), with the remaining portion consisting of seeds and stems **(Ginni et al., 2020)**. By-products from the plant processing are still an interesting source of nutritionally valuable substances, such as individual phenols, fibre, proteins for food/feed purposes. They were tested into the food for special nutritional groups such as people suffering from celiac disease or/and diabetes. Apple pomace addition into cornbread was very good accepted by evaluators **(Fikselová et al., 2023)**.
The aim of this pilot study was to treat apple pomace by microwave irradiation in order to test the total polyphenol content and selected individual phenols as supporting parameter as well as antioxidant activity of different samples, which originated from 2 apple producers and from laboratory conditions.

MATERIAL AND METHODS

Material

In this pilot study there were used two samples of apple pomace obtained directly from apple producers (marked as K 6 and K7) and two samples of pomace were prepared in laboratory conditions (marked as K 4 and K 5) by the known procedure from apples as follows:
Pomace no. 4 (marked as K 4): in this case, apples from organic farming were obtained. The apples were processed into apple pomace. Production process was applied as follows: after washing the apples, they were juiced and obtained pomace were dried (Fruit Jerky Plus 9, Klarstein, Germany) at the temperature of 30 °C to its dry state and stored in the dark place until the analysis.
Pomace no. 5 (marked as K 5): variety of apples (Idared) was processed into apple pomace. Production process: after washing of the apples, they were juiced and obtained pomace were dried (Fruit Jerky Plus 9, Klarstein, Germany) at the temperature of 50 °C to its dry state and stored in the dark place until the analysis.

Pomace no. 6 (marked as K 6): fresh apple pomace originated from conventional agriculture was used. Variety Idared was obtained from the Slovak apple producing company, dried in a dryer at the temperature of 50 °C in a juice dryer (Fruit Jerky Plus 9, Klarstein, Germany) to its dry state and stored in the dark place until the analysis.
Sample no. 7 (marked as K 7): apple pomace from conventional agriculture from the retail in the Czech Republic was obtained in the form of granules, with description as follows: moisture content 8 %, protein 7%, vegetable fat 4.7%, fiber 18.3%, carbohydrates 12%, ash 4.3%. They were described as a product from dried apple pulp, without any other additives or ingredients (no details about their processing were provided).
The control samples (K4- K 7) were treated by the microwave irradiation (MI) as follows below (marked as M4-M7).

Methods

Sample preparation

Variant M (samples M4 – M7) were irradiated for 1 min. in a microwave oven (Whirpool W10691902, Italy) at 650 W. Extraction of 1 g of sample was performed by adding 10 mL of 80% methanol and shaken in a laboratory shaker (GFL Orbital Shaker 3005, Germany**)** for 20 hours. The extraction mixture was then centrifuged (Froilabo SW14, O.K. Servis, BIOPRO) for 10 min at 6000 rpm and extract was purified, filtration using a syringe filter (0.45µm, ø25mm Frisenette, Danmark). Clean extract was used for determination of total polyphenolic content (TPC) and total antioxidant activity (TAA). 200 µl of extract on TPC and 100 µl of extract on TAA were used for analysis.

Determination of total polyphenol content (TPC)

Total polyphenol content was determined by the modified method according to **Singleton & Rossi (1965)** using 20% Na_2CO_3 solution, Folin-Ciocalteau reagent and deionized water. Extract from apple pomace was pipetted in amount of 1 ml into 50 ml volumetric flasks in triplicates per pomace variant. The extract was diluted with 25 mL of deionized water, then 2.5 ml of Folin-Ciocalteau reagent (diluted 1:1 with deionized water) and after 3 minutes 1.5 mL of 20% Na_2CO_3 solution were pipetted. Total volume was unified to 50 mL by deionized water in the volumetric flasks. Blue-coloured complex was developed at laboratory temperature for 2 hours. Gallic acid calibration solutions were prepared in the same way with the calibration range 50 – 500 $mg.L^{-1}$. The absorbance of the sample solutions was measured against the blank solution at 765 nm at a microplate reader (Epoch 2 Microplate Reader, BioTek Instruments, Inc., Vermont, USA). The content of total polyphenols in the pomace extracts was calculated as the amount of gallic acid equivalent (GAE) in mg per kg of apple pomace (mg $GAE.kg^{-1}$).

Determination of total antioxidant activity
Total antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay described by Brand-Williams et al. (1995). Methanol solution of DPPH (250 $\mu g.L^{-1}$) was pipetted in amount 3.9 mL into a 12-well microplate and initial absorbance was measured at 516 nm on a microplate reader (Epoch 2). Then 0.1 ml of sample extract was added, mixed and left to react for 10 minutes. After 10 minutes, the absorbance was measured at 516 nm and the antioxidant activity was expressed as % inhibition of DPPH radical. All analyses were done in triplicate.

HPLC-DAD analysis of phenolic compounds

Selected individual phenolic substances such as rutin, quercetin, chlorogenic acid, caffeic acid, ferulic acid, syringic and gallic acid were observed in the control samples and in their treated samples of pomace after microwave treatment as well. Selected flavonoids were extracted from the samples as follows: into the flasks was weighed 1 g of sample and 5 mL of extract solvent was added (80% water extract of methanol). The content was stirred in orbital mixer at 200 rpm for 10 min., then for 10 min. in ultrasonic bath at laboratory temperature and finally centrifuged at 10 000 rpm at 20 °C for 10 min. Aliquot part was filtered using a syringe filter with cellulose membrane (pore size 0.45 µm) and analysed. Flavonoid content was determined by the HPLC-DAD method **(Belajová, 2012)**.

HPLC equipment

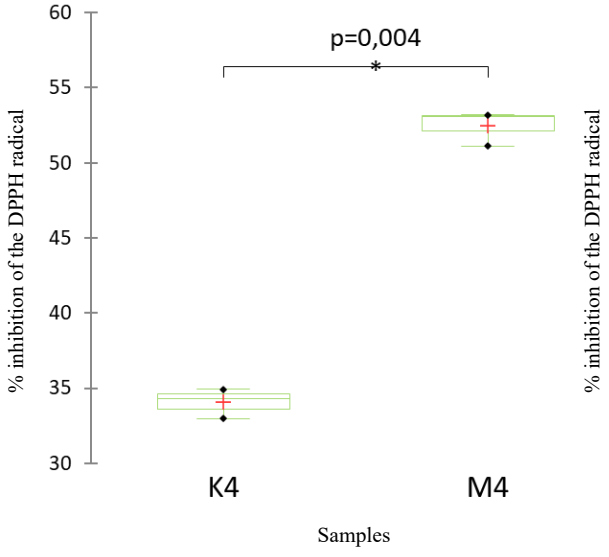
The separation was performed using the liquid chromatography instrument Agilent Technologies 1100 Series equipped with a diode array detector (DAD), quartenary pump, degasser, column thermostat and autosampler (Agilent Technologies, Waldbronn, Germany). Chromatographic separations were performed at the Purospher STAR RP-18 endcapped column (250 x 4.6 mm i.d., 5 µm particle size, Merck) at 30 °C. The linear gradient program was as follows: 0–1 min 100% B, 1–5 min 80% B, 5–10 min 75% B, 10–12 min 65% B, 12–18 min 50% B, 18–26 min 50% B, 26–28 min 100% B. Solvent A was 100% methanol and solvent B was the mixture of 0.01 mol.l-1 orthophosphoric acid + methanol (95 + 5, v + v). The flow rate of the mobile phase was 1.3 ml.min-1 and samples were injected in amount of 20 µl. Detection was performed by DAD at 256, 280, and 305 nm. The compounds were identified by the comparison of their retention times with those of pure standards. Phenolics were monitored with DAD in a range of 200–400 nm for the spectral identification. Measured data were processed by the chromatographic program Agilent ChemStation. For calibration, linear regression diagnostics was performed by the Excel XP software (Microsoft, Redmont, Washington, USA).
Phenolic acids were determined after extraction of samples by the following procedure: 1 g of sample was taken, and 10 mL of extraction solution was added (80 % water solution of methanol) and the content was stirred for 20 minutes at laboratory temperature. The sample was centrifuged at 4700 rpm, for 10 minutes at temperature of 25 °C. After centrifugation was sample analysed, the content of phenolic acids was determined by the HPLC-UV (Daško et al., 2012) measurement. Phenolic acids were separated and quantified with the HPLC system (Agilent Technologies, Palo Alto, California, USA). Samples were injected by an autosampler cooled to 5 °C. Variable wavelength detector was used for quantification at 280 nm wavelength for all phenolic acids. Quaternary pump was used for gradient formation. Zorbax SB-C18 (Agilent Technologies) 4.6 x 250 mm column with 5 mm particles was used for separation. A binary gradient was used for separation: A: 0.01 $mol.l^{-1}$ water solution of phosphoric acid; B: methanol – start with 100% A, linear gradient to 95% A in 1.5 min, stable up to 2nd minute, linear decrease to 83% A in 3rd minute, stable up to 23rd minute, linear decrease A to 74% up to 30th minute, stable up to 35th minute, linear decrease A to 30% in 45th minute. Column was flushed for 7 min with methanol and afterwards equilibrated for 10 min with 100% A. External standard procedure was used for phenolic acids quantification.

Statistical processing of the results

Data analysis was done by the XLSTAT® Cloud (version 2018.5.52280). The significance level was fixed at $p < 0.05$. Data are presented as the mean of three determinations. PCA analysis of individual phenols and paired T test for the antioxidant activity and the total polyphenol content (TPC) of apple pomace were prepared.

RESULTS AND DISCUSSION

Phenolic compounds are secondary metabolites in plants. The chemical structure of phenolic compounds is responsible for their key properties, including antioxidant activity and the ability to chelate metal ions. Phenolic compounds can be classified into flavonoids and non-flavonoids. Various subgroups belong to the category of flavonoids: flavones, flavanones, flavanonols, flavanols, flavonols, anthocyanins and chalcones. Non-flavonoid phenolics include phenolics acids, lignans, and stilbenes, with phenolic acids as the most represented. The phenolic content of apple pomace is presented by chlorogenic acid, caffeic acid, catechins, rutin, and quercetin glycosides. In seed and stems, mostly di-hydrochalcones can be found, for example phlorizin, while chlorogenic acid and flavonol glycosides are important compounds found in flesh **(Barreira et al., 2019)**.



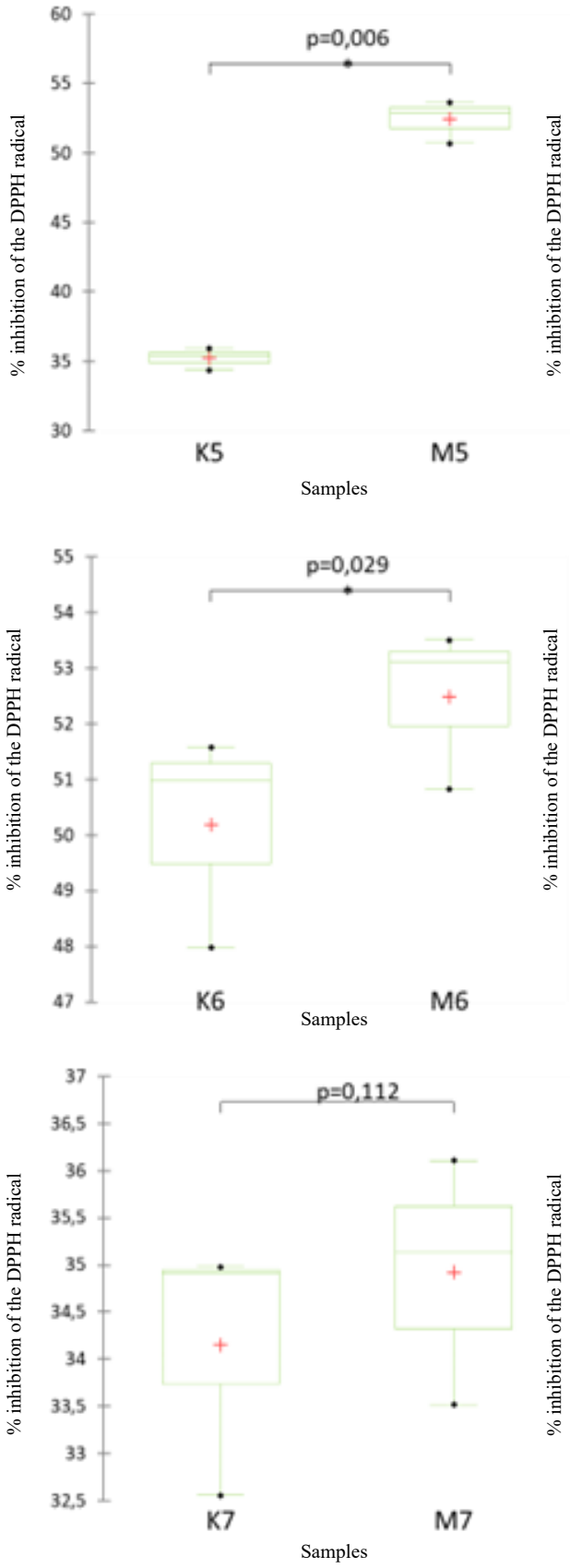


Figure 1 Antioxidant activity (% inhibition of the DPPH radical) in observed apple pomace samples. **Legend**: K4-K7 are pomace before the treatment, samples M4 – M7 are pomace after MI

By applying the microwave irradiation process, it was possible to observe different changes in the percentage inhibition of the DPPH radical at individual analysed samples. Figure 1 represents the results of the test between individual control materials (K4- K7), which were compared to the same sample exposed to microwave radiation (M4-M7).

The result of the analysis shows small changes in the percentage inhibition of the DPPH radical in sample M7 (form of granules directly from producer), this change could be observed as an increased inhibition. These changes did not represent a deviation in DPPH inhibition of more than 1 percentage point. Significant increase in DPPH inhibition could be observed after irradiation by an average of more than 2 percentage in sample M6 and more than 15 percentage points in samples M4 and M5 (laboratory prepared).

Polyphenols have attracted attention due to their health benefits. Thermal processing, irradiation, fermentation, high pressure, microwave, and drying are several popular food processing methods. However, polyphenols are instable in food processing, they degrade and react with other components because of their polyhydroxy characteristic. The main factors affecting the stability of polyphenols are pH, temperature, light, oxygen, enzymes, metal ions, and macromolecules. Thermal processing showed their degradation. Thermal degradation products of some polyphenols, such as quercetin and rutin, were proven. Structural changes of polyphenols caused by food processing, may lead to different bioactivities from the obtained results based on unprocessed polyphenols **(Toldra et al., 2022)**.

At TPC evaluation, an increase in the content of total polyphenols was observed at our samples as well (Fig. 2). In samples M4, M5 and M6 the significant increase was detected even at the level of significance p=0.001. At M7 sample, the significance level was less than $p < 0.05$, also only small changes in the percentage inhibition of the DPPH radical was detected in sample M7.

Determination of individual phenolic substances showed, that in the sample 4, the content of rutin slightly increased by microwave treatment (191 vs. 200.48 $mg.kg^{-1}$) compared to the control, the content of quercetin increased from 4.9 to 7.16 $mg.kg^{-1}$, similarly, chlorogenic acid increased from 21.38 to 36.35 $mg.kg^{-1}$. Caffeic acid was detected only in the irradiated sample in the content of 2.35 $mg.kg^{-1}$.

Quercetin in plants, is a part of several glycosides. Its molecular structure, consisting from three rings and five hydroxyl groups, is responsible for antioxidant properties. It seems that quercetin possesses anticarcinogenic activity as well **(Osman et al., 2016)**.

Sample of pomace 5, showed a slight increase of chlorogenic acid from 13.47 to 13.94 $mg.kg^{-1}$ and gallic acid from 2.14 to 3.27 $mg.kg^{-1}$ of the monitored components.

At the sample 6 an increase was detected at content of chlorogenic acid from 106.9 to 135.6 $mg.kg^{-1}$, ferulic acid from 2.7 to 3.24 $mg.kg^{-1}$ and syringic acid from 3.13 to 3.43 $mg.kg^{-1}$.

Chlorogenic acid is another important phenolic compound, a product from the esterification of caffeic and quinic acid. It is known for its anti-inflammatory and antioxidant properties. It has obtained significant attention due to its effects on cardiovascular and cerebrovascular system, as well as its potential benefits for diabetes **(Huang et al., 2023)**.

Sample 7 after its irradiation demonstrated an increase in the content of rutin from 116.17 to 123.6 $mg.kg^{-1}$, quercetin from 33.9 to 41.07 $mg.kg^{-1}$, ferulic acid from 4.96 to 5.17 $mg.kg^{-1}$, and caffeic acid was detectable only in the sample of pomace after irradiation at the content of 2 $mg.kg^{-1}$.

Caffeic acid is a natural polyphenolic compound, its structure includes an aromatic core with a substituted unsaturated three-carbon side chain and two hydroxyl groups which allows its antioxidant and chelating properties. As donor of hydrogen for free radicals, it acts as a primary antioxidant, and by metal chelation by hydroxyl groups as a secondary antioxidant. Caffeic acid activity has been proven for its antiproliferative effect, and in cancer cell inhibition. It has shown potential in treating some neurodegenerative and brain-related illnesses and an important antimicrobial and antiviral properties **(Pavlíková, 2023)**.

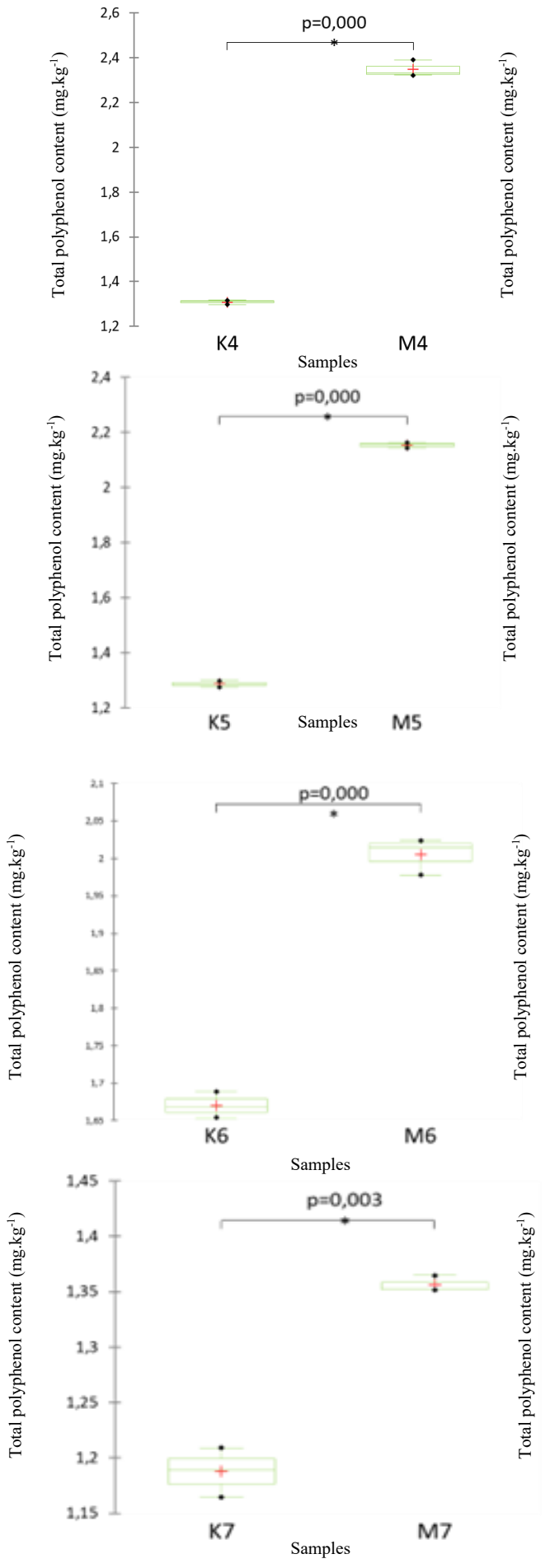


Figure 2 Total polyphenol content (mg.kg^{-1}) at observed apple pomace samples
Legend: K4-K7 are pomace before the treatment, samples M4 – M7 are pomace after MI

By the PCA analysis of individual phenols, it can be observed that changes in samples occurred in different ways: sample 4 showed some change that corresponds to the rutin vector. Sample no. 5 showed a very small change after its irradiation. Sample no. 6 showed an observable change in the area of chlorogenic acid concentration and epicatechin content. Sample no. 7 changed the quercetin values due to its microwave treatment.
Microwave irradiation accelerates the movement of polar molecules by causing them to rapidly rotate and rubbing against each other, that leads to producing of the heat, and the heat generated inside transfers outward. Application of microwave irradiation in the extraction process might lead to significant increase of the total phenolic content. Microwave irradiation-induced rapid heating promotes the release of polyphenols as a defence mechanism against external factors, such as air oxidation. In some cases, some polyphenols are heat-sensitive, and degradation can occur **(Hu et al., 2021)**.

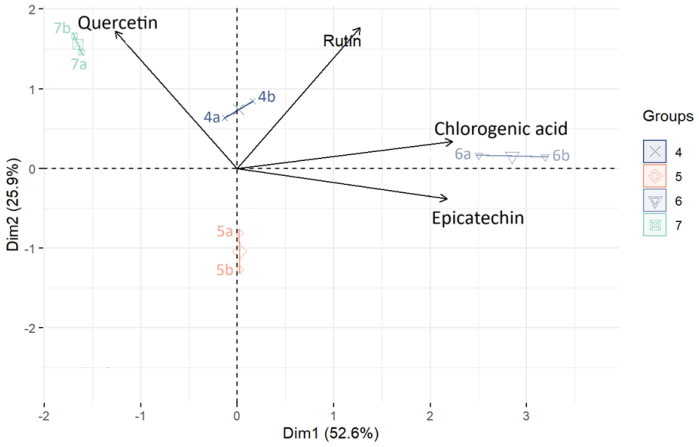


Figure 3 PCA analysis of selected individual phenols in samples of pomace

CONCLUSION

The results of DPPH method showed higher antioxidant activity, mainly in laboratory prepared samples of pomace. At TPC evaluation, an increase in the content of total polyphenols was observed similarly, at three irradiated samples the statistical significant increase even at the level of significance p=0.001 was detected. Several individual phenols were detected after MI of pomace in increased amounts, such as chlorogenic acid, gallic acid, rutin, ferulic and syringic acids, quercetin and caffeic acid. Microwave irradiation accelerates the movement of polar molecules by causing them to rapidly rotate and rubbing against each other, that leads to producing of the heat, and the heat generated inside transfers outward. Application of microwave irradiation in the extraction process might lead to significant increase of the total phenolic content.

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