Application of principal component analysis for optimization of polyphenol extraction from alternative plant sources

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Summary

In the current study, principal component analysis (PCA) was used to optimize variables related to the extraction of polyphenols from plant materials including various solvents (water, methylene chloride and 80% aqueous solutions of methanol or ethanol) and pre-treatment parameters (homogenization and microwave processing). Moreover, the profile of phenolic compounds of tested fruits that demonstrated the highest content of those substances (elderberry, Japanese quince and cornelian cherry) was determined. Water and methylene chloride were not suitable for polyphenol extraction, while the best results were obtained when 80% methanol solution was used. Moreover, elderberry was proven to accumulate the highest quantities of cyanidin-3-*O*-sambubioside and it was the richest source of most of analysed phenolic compounds. Different homogenization speeds were required to enhance polyphenol recovery from selected plant materials (183.33 Hz for elderberry; 316.67 Hz and 400 Hz for Japanese quince). Microwave treatment decreased the recovery of those substances in both tested fruits. Overall, PCA appeared to be a useful tool for description of changes in the efficiency of polyphenol extraction, though indicating parameters that were not optimal for the whole process from the economical point of view.

Keywords

elderberry; Japanese quince; extraction method; solvent; polyphenol; principal component analysis

Polyphenols and other bioactive compounds are usually obtained from plant materials through extraction processes. Extraction yield depends on material disintegration, the size of extracted particles, solvent properties and its flow speed [1], contact time and the number of extraction cycles [2]. The key issue is to select an effective solvent, which should be based on its affinity to extracted compounds [3]. This may be a complicated matter due to the great variety of antioxidants in plant materials [4]. Each raw material has its own, unique antioxidant profile, varying both qualitatively and quantitatively one from another. When BOEING et al. [5] were examining different eluents for polyphenol extraction from berries, they proved that water or aqueous solutions of organic solvents (acetone, methanol, ethanol) were the most effective [5]. Moreover, acidified solutions of acetone were proven to decrease the recovery of phenolic compounds from elderberry [6] or lingonberry [7] fruits.

On the other hand, extracts of grape pomace obtained with 80% ethanol solution demonstrated the highest antioxidant activity (*AOX*) [8]. However, in industrial applications, 40% to 90% ethanol solutions are commonly used because they elute both volatile aroma compounds and substances with antioxidant properties [9]. Sometimes even lower concentrations could be sufficient, as it was reported for dry sage and red grape pomace, for which optimum ethanol concentration was 30% [10, 11]. Those variations could be caused by the differences in polyphenol profiles of materials used for extraction. Differences could be caused by extraction temperature, pressure and extraction time.

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Food components with antioxidant properties tend to get oxidized when subjected to external factors such as light, temperature and, most of all, atmospheric oxygen. Vitamins showing antiradical potential in fruits undergo degradation, e.g. vitamins A, C, E, and polyphenols undergo polymerization or transformation to less active glycosides [12]. It was previously shown that microwave treatment might effectively increase the recovery of phenolic compounds from tea leaves [13, 14], dried plums [15] or apple chips [16], providing the increase of antioxidant activity. That effect was attributed to inactivation of polyphenol oxidases that, in contact with oxygen, transform phenolic compounds to biologically inactive substances or to compounds demonstrating lower *AOX* [16].

Numerous studies focused on modelling polyphenol recovery were carried out so far [17–22]. The method, which was used the most frequently for that purpose, was response surface methodology. Other method was factorial design of Box-Behenken design. The methods have some limitations but, in previous studies, they were applied to choosing the optimum liquid-to-solid ratio [17, 20–22], the most efficient concentration of the organic solvent applied in the research [17, 18, 20–23] or other factors considered by researchers. Principal component analysis (PCA) shows the ranges of variables that influence the examined process and allows designing following research steps. The main advantage of the latter method is that it allows analysis of several samples simultaneously, i.e. different plant materials extracted with the same solvent at various concentrations. It is also worth mentioning that, according to our knowledge, it has not been applied for optimization of polyphenol recovery.

In order to optimize technological processes, PCA can be a very useful tool to indicate the variables that have a significant impact on the examined process and which are less important. It has been already applied for selecting preferable cultivation conditions to preserve phenolic compounds in lettuce leaves [24] or for classifying spices in terms of their antioxidant properties and total polyphenol content (*TPC*) [25]. Strong correlations were found when PCA was applied to assess relations between the occurrence of polyphenols in different grape hybrids and it was found that the most abundant substances were delphinidin-3-*O*-glucoside-5-*O*-glucoside, petunidin-3-*O*-glucoside-5-*O*-glucoside, petunidin-3-*O*-glucoside and procyanidin B2 [26]. Moreover, that analysis was found to be a good tool for assessing the origin of young red wines in Tenerife based on their *TPC* [27].

PCA was also applied in the current study to select the solvent that would allow efficient polyphenol recovery. It was also used for determining correlations in polyphenol profiles in extracts that demonstrated significant differences among each other, and to optimize homogenization and microwave pre-treatment conditions, which were applied to enhance the extraction efficiency. Moreover, according to our knowledge, insufficient data are available on polyphenol profiles and extraction efficiencies for all plant materials tested in the current study, so our study will also provide scientific information in support of their use as alternative sources of phenolic compounds.

MATERIALS AND METHODS

Fruits were obtained from experimental orchard of the University of Agriculture in Krakow, located in Garlica Murowana (near Krakow, Poland) or were purchased from ecological cultivations (Malopolska and Podkarpacie districts, Poland) in 2013. The following species were used for experiments: lingonberry (*Vaccinium vitis-idaea* L.), elderberry (*Sambucus nigra* L.), cornelian cherry (*Cornus mas* L.), black mulberry (*Morus nigra* L.), Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) and quince (*Cydonia oblonga* Mill.). Plant materials were selected on the basis of high polyphenol content and significant antioxidant capacity [28–30] and based on tunpublished preliminary studies. Fruits were washed, dried with paper towel and frozen at –80 °C to prevent changes of compounds with antioxidant activity.

Extraction of polyphenols by maceration

Frozen material (10 g ± 0.1 g) was weighed, disintegrated by cutting with knife, combined with 90 ml of solvent (hot water, 100% methylene chloride or 80% aqueous solutions of methanol and ethanol) and then subjected to extraction on magnetic stirrer (6 h, 3.33 Hz, laboratory temperature). Concentrations of tested solvents were selected on the basis of literature research [5, 8, 10, 31, 32] and preliminary studies. Extracts demonstrating the highest *TPC* were examined regarding the presence of selected phenolic compounds. In the next step, various ethanol concentrations in aqueous solutions were used to select optimum solvent load: 40 %, 60 %, 80 %, and 96 % (v/v). When extraction was finished, extracts were filtered through paper filtration disks (type 388; Munkell-Filtrak, Bärenstein, Germany) and their volume was made up to 100 ml with adequate sol-

vent in the first research step or with ethanol solutions in further experiments.

Extraction of polyphenols by homogenization

Frozen material (10 g ± 0.1 g) was combined with 90 ml of 80% ethanol and extracted using a high-speed homogenizer (UltraTurrax T25 Basic; IKA, Staufen, Germany) at various combinations of speeds and operation times: 183.33 Hz, 316.67 Hz, 400 Hz and 2 min, 5 min, 10 min, 15 min, respectively. Obtained extracts were filtered and their volume was made up to 100 ml with the solvent.

Extraction of polyphenols by homogenization and microwave pre-treatment

Biological material (10 g ± 0.1 g) was initially disintegrated, subjected to microwave radiation by microwave generator Mars Xpress (CEM, Kamp-Lintfort, Germany) at a power of 50 W, 100 W, 200 W, 300 W or 600 W, for 30 s or 60 s, combined with 90 ml of 80% ethanol and then extracted with the use of a high-speed homogenizer (UltraTurrax T25 Basic; 316.67 Hz; 5 min). Control samples in the experiment involving microwave treatment were extracts prepared by homogenization (316.67 Hz; 5 min) with 80% ethanol solution. Extracts were filtered and made up to 100 ml with 80% ethanol.

Determination of total polyphenol content and antioxidant activity

TPC was assessed by the Folin-Ciocalteu method according to the protocol described previously [33] and it was expressed as grams of (+)-catechin per kilogram of fresh weight (FW) on the basis of a calibration curve.

AOX of fruit extracts was determined using active cation-radical of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS; Sigma Aldrich, St. Louis, Missouri, USA) by the method described by TARKO et al. [33]. *AOX* was calculated on the basis of calibration curve, prepared each time from synthetic vitamin E ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox; Sigma Aldrich) and expressed as grams of Trolox per kilogram of FW of the plant material.

HPLC analysis of polyphenols

The analysis of polyphenols was carried out by high-performance liquid chromatography (HPLC) using Flexar instrument (Perkin-Elmer, Waltam, Massachusetts, USA) equipped with UV-Vis detector. The Synergi Fusion RP-80A column (200 mm × 4.6 mm; pore size 4 μm; Phenomenex, Torrance, California, USA), thermostated at 30 °C was used for all analyses. A 2.5% aqueous solution of acetic acid (solution A) and acetonitrile (solution B) were used as a mobile phase.

Gradient programme for the analysis of gallic acid, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, epigallocatechin gallate, phloridzin, hesperetin, hesperidin, procyanidins B1 and B2 was as follows: linearly from 5% B to 20% B for 30 min, then linearly from 20% B to 100% B for 3 min, isocratically for 7 min 100% and linearly 100% B to 5% B within 4 min (flow rate 0.5 ml·min^{-1}, detection at 280 nm). After each separation, the column was washed with 5% B.

The gradient programme for the determination of caffeic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, vitexin, isovitexin, apigenin and resveratrol was carried out isocratically with 20% B (15 min), linearly 20% B to 100% B within 30 min, isocratically 100% B for 4 min, linearly 100% B to 20% B within 3 min time (flow rate 0.5 ml·min^{-1}, detection at 325 nm) and then washed with 20% B.

In case of quercetin, quercetin-3-*O*-glucoside, rutin, kaempferol (flow rate 1 ml·min^{-1}, detection at 360 nm) and hippuric acid, protocatechuic acid, ellagic acid, daidzin, daidzein and genistein (flow rate 1 ml·min^{-1}, detection at 250 nm) the following programme was applied: linearly from 5% B to 20% B within 20 min, then linearly 20% B to 100% B within 10 min, isocratically for 3 min 100% B, linearly 100% B to 5% B within 4 min and washing the column with 5% B.

Anthocyanins (myrtillin, ideain, kuromanin, keracyanin, cyanidin-3-*O*-arabinoside, callistephin, peonidin-3-*O*-glucoside, cyanidin-3-*O*-sambubioside, cyanin) were analysed (flow rate 0.5 ml·min^{-1}, detection at 520 nm) using the following programme: linearly from 5% B to 20% B within 30 min, then linearly to 100% B within 3 min, isocratically for 4 min 100% B and linearly 100% B to 5% B within 4 min.

For quantitative analyses, standard curves were prepared for the following standards: ferulic acid, caffeic acid, chlorogenic acid, (+)-catechin, quercetin (all Sigma Aldrich), phloridzin, (–)-epicatechin, procyanidins B1 and B2, cyanidin-3-*O*-galactoside (ideain), cyanidin-3-*O*-rutinoside (keracyanin), cyanidin-3-*O*-sambubioside, cyanidine-3,5-O-diglucoside, quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-galactoside (all Extrasynthese, Genay, France). Polyphenols not detected in any experimental variant were not included in tables or figures.

Statistical analysis

All experiments were performed at least in three replicates and results were presented as

arithmetic mean ± standard deviation. Normality of distribution was assessed by Shapiro-Wilk test and significance of differences between means was assessed by one-way variance analysis (ANOVA) with post hoc Tukey's test. PCA with varimax rotation was applied to assess correlations among variables. All statistical analyses were carried out using R (The R Foundation for Statistical Computing, Vienna, Austria), a language and environment for statistical computing, version 3.1.3. ANOVA was carried out using linear model (lm) function and Tukey's test was done using honest significant difference (HSD) test function in 'agricolae' package. PCA was carried out according to the scripts formulated by BEAUMONT [34] and it was carried out in 'psych' package. Cortest-Bartlett test was carried out in 'psych' package as well. The data demonstrated normal distribution so their transformation was not necessary. Correlations between loads and scores were considered

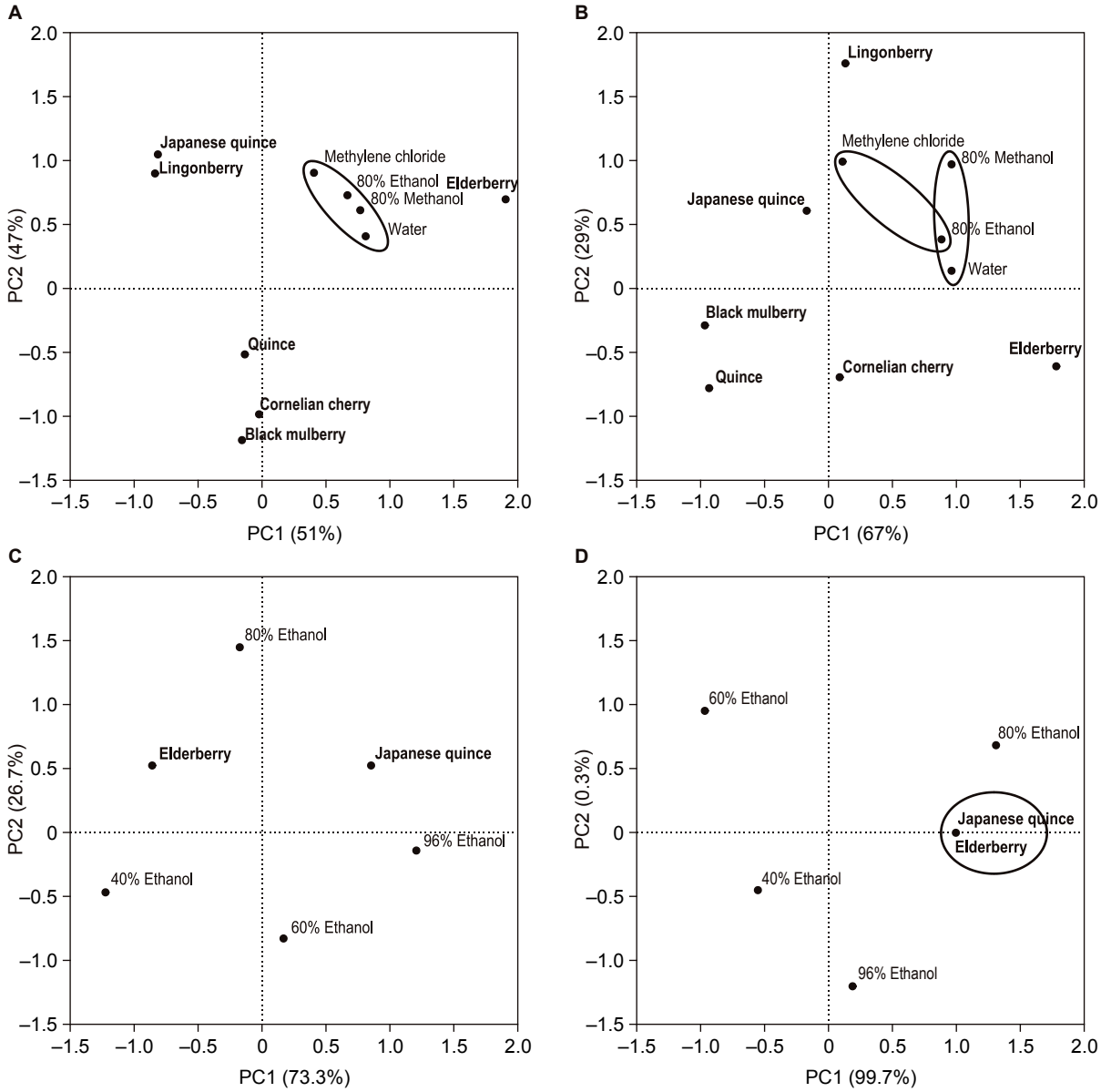

Fig. 1. Principal component analysis describing the influence of the applied solvent on total polyphenol content and antioxidant activity of fruit extracts.

A – Total polyphenol content (loads) in the extracts obtained with four different solvents (scores); B – Antioxidant activity (loads) in the extracts obtained with four different solvents (scores); C – Total polyphenol content (loads) obtained at various ethanol concentrations (scores); D – Antioxidant activity (loads) obtained at various ethanol concentrations (scores).
Points within circles indicate correlation between loads.

Tab. 1. Influence of solvent on total polyphenol content and antioxidant activity of fruit extracts.

Plant material	Methanol 80%	Ethanol 80%	Water	Methylene chloride
Total polyphenol content [g·kg^{-1}]				
Lingonberry	19.56 ± 0.79^{a}	22.52 ± 1.32^{a}	5.54 ± 0.16^{b}	0.31 ± 0.02^{c}
Elderberry	53.42 ± 1.29^{a}	41.06 ± 0.71^{b}	24.30 ± 1.24^{c}	0.48 ± 0.09^{d}
Cornelian cherry	16.36 ± 0.37^{a}	10.33 ± 0.38^{b}	4.63 ± 0.47^{c}	0.07 ± 0.00^{d}
Black mulberry	8.45 ± 0.28^{a}	6.90 ± 0.55^{b}	4.61 ± 0.12^{c}	0.05 ± 0.01^{d}
Japanese quince	27.03 ± 0.20^{a}	19.46 ± 0.92^{b}	5.61 ± 0.09^{c}	0.35 ± 0.02^{d}
Quince	19.50 ± 0.68^{a}	12.96 ± 0.16^{b}	5.39 ± 0.30^{c}	0.14 ± 0.01^{d}
Antioxidant activity [g·kg^{-1}]				
Lingonberry	11.32 ± 0.12^{a}	19.32 ± 0.16^{b}	8.11 ± 0.13^{c}	0.02 ± 0.00^{d}
Elderberry	28.29 ± 0.80^{a}	22.11 ± 0.55^{b}	16.88 ± 0.88^{b}	0.01 ± 0.00^{c}
Cornelian cherry	15.77 ± 0.33^{a}	9.76 ± 0.15^{b}	3.43 ± 0.15^{c}	0.01 ± 0.00^{d}
Black mulberry	1.61 ± 0.03^{a}	1.11 ± 0.00^{b}	0.91 ± 0.02^{c}	0.01 ± 0.00^{d}
Japanese quince	14.48 ± 0.20^{a}	7.14 ± 0.34^{b}	5.26 ± 0.08^{c}	0.02 ± 0.00^{d}
Quince	1.61 ± 0.04^{a}	0.91 ± 0.04^{b}	0.27 ± 0.00^{c}	0.00 ± 0.00^{d}

a-d – the same letters indicate the lack of statistically significant differences between results obtained for raw material extracted with different solvents (verse) at $p < 0.05$, $n = 3$.
Total polyphenol content is expressed as grams of (+)-catechin per kilogram of fresh weight. Antioxidant activity is expressed as grams of Trolox per kilogram of fresh weight.

strong when values obtained in the correlation matrix exceeded 0.3.

RESULTS AND DISCUSSION

Solvent selection

In order to select the solvent, which would provide the highest efficiency of polyphenol extraction, PCA was applied to assess correlations between *TPC* or *AOX* (loads) and tested plant materials (scores).

In case of *TPC*, it was found that there were strong correlations among all tested solvents (Fig. 1A). This phenomenon might be explained by the fact that the same tendencies were found when comparing quantities of phenolic compounds detected in fruit extracts prepared with different solvents, i.e. the highest *TPC* was obtained for elderberry fruits in case of all solvents, while the lowest *TPC* was found in black mulberry in all variants as well. There were no significant differences for extraction rates of polyphenols from lingonberry when 80% ethanol or 80% methanol were used. Methylene chloride (100%) proved to be a very poor eluent for polyphenols (Tab. 1) so it was not used in further experiments.

On the other hand, water has been proven to be a very efficient solvent in case of several plant materials [10, 31, 32]. However, their polyphenol profiles were different than in case of fruits tested in the current study. In most of cited papers authors focused on phenolic acids and betacyanins, which are water soluble. Phenolic compounds that were found in plant materials tested in the current study demonstrate a higher affinity to organic solvents, which may explain the fact that yields obtained using 80% methanolic and 80% ethanolic extracts were significantly higher than in case of water.

Similar levels of polyphenols were released from both Japanese quince and lingonberry in case of all applied solvents. Another correlation of *TPC* might be observed between cornelian cherry and black mulberry; however, it must be underlined that even though very similar *TPC*s were found in water and methylene chloride extracts, the differences between alcohol-based (methanol or ethanol) solutions were very significant at $p < 0.05$. This indicates that cornelian cherry was a better source of phenolic compounds. All these findings prove that the type and composition of the plant material selected for polyphenol extraction has a more significant impact on their recovery than the type of solvent used for this process.

When the results of *AOX* were examined (Tab. 1), it was found that there were strong correlations among alcohol-based extracts and water (Fig. 1B). When those solvents were used, it was noted that the same tendencies occurred with all tested plant materials, i.e. elderberry held the highest *AOX* and quince demonstrated the lowest *AOX* in case of all three extracts. In the case of

Tab. 2. Polyphenol profiles of methanolic, ethanolic and water extracts obtained from the fruits of elderberry, Japanese quince and Cornelian cherry.

Compound	Elderberry [g·kg^{-1}]			Japanese quince [g·kg^{-1}]			Cornelian cherry [g·kg^{-1}]		
	80% Methanol	80% Ethanol	Water	80% Methanol	80% Ethanol	Water	80% Methanol	80% Ethanol	Water
Phloridzin	nd	nd	nd	0.002 ± 0.001^{a}	0.002 ± 0.000^{b}	0.001 ± 0.000^{c}	nd	nd	nd
(–)-Epicatechin	0.802 ± 0.041^{a}	0.779 ± 0.012^{a}	0,497 ± 0.054^{b}	0.387 ± 0.080^{a}	0.279 ± 0.054^{b}	0.056 ± 0.010^{c}	0.230 ± 0.043^{a}	0.145 ± 0.041^{b}	0.017 ± 0.007^{c}
(+)-Catechin	0.367 ± 0.182^{a}	0.599 ± 0.093^{b}	0.263 ± 0.086^{a}	0.068 ± 0.003^{a}	0.195 ± 0.090^{b}	0.069 ± 0.012^{a}	0.062 ± 0.014^{a}	0.044 ± 0.008^{b}	0.007 ± 0.001^{c}
Procyanidin B1	0.389 ± 0.038^{a}	0.243 ± 0.049^{b}	0.217 ± 0.037^{b}	0.015 ± 0.001^{a}	0.006 ± 0.001^{b}	0.004 ± 0.001^{c}	0.302 ± 0.069^{a}	0.097 ± 0.000^{b}	0.009 ± 0.001^{c}
Procyanidin B2	0.504 ± 0.008^{a}	0.949 ± 0.074^{b}	0.832 ± 0.049^{c}	0.072 ± 0.002^{a}	0.343 ± 0.068^{b}	0.071 ± 0.021^{a}	0.019 ± 0.004^{a}	0.074 ± 0.014^{b}	0.030 ± 0.002^{c}
Quercetin	0.009 ± 0.000	nd	nd	0.018 ± 0.000^{a}	0.008 ± 0.001^{b}	0.001 ± 0.000^{c}	0.003 ± 0.002^{a}	0.001 ± 0.000^{b}	nd
Quercetin-3-*O*-rutinoside	0.266 ± 0.064^{a}	0.146 ± 0.007^{b}	0.117 ± 0.008^{c}	0.002 ± 0.001^{a}	0.004 ± 0.001^{b}	0.002 ± 0.000^{a}	0.003 ± 0.001^{a}	0.001 ± 0.000^{b}	nd
Quercetin-3-*O*-galactoside	0.034 ± 0.013^{a}	0.026 ± 0.003^{ab}	0.016 ± 0.005^{b}	0.032 ± 0.001^{a}	0.037 ± 0.010^{a}	0.007 ± 0.002^{b}	0.003 ± 0.002^{a}	0.006 ± 0.001^{b}	nd
Cyanidin-3-*O*-rutinoside	nd	nd	nd	0.003 ± 0.000^{a}	nd	0.004 ± 0.001^{b}	0.351 ± 0.033^{a}	0.164 ± 0.028^{b}	0.040 ± 0.024^{c}
Cyanidin-3-*O*-galactoside	0.540 ± 0.085^{a}	0.321 ± 0.085^{b}	0.168 ± 0.012^{c}	nd	nd	nd	0.056 ± 0.009^{a}	0.047 ± 0.009^{a}	0.002 ± 0.001^{b}
Cyanidin-3,5-*O*-diglucoside	1.517 ± 0.150^{a}	0.561 ± 0.127^{b}	0.826 ± 0.183^{c}	nd	nd	nd	nd	nd	nd
Cyanidin-3-*O*-sambubioside	8.067 ± 0.744^{a}	5.237 ± 0.066^{b}	5.469 ± 0.486^{b}	nd	nd	0.003 ± 0.000	nd	nd	nd
Caffeic acid	0.035 ± 0.006^{a}	0.075 ± 0.013^{b}	0.048 ± 0.006^{c}	0.008 ± 0.001^{a}	0.012 ± 0.001^{b}	0.002 ± 0.000^{c}	0.001 ± 0.000^{a}	0.009 ± 0.003^{b}	0.002 ± 0.001^{a}
Ferulic acid	0.002 ± 0.001	nd	nd	nd	nd	nd	0.003 ± 0.001	nd	nd
Chlorogenic acid	0.760 ± 0.143^{a}	0.406 ± 0.035^{b}	0.226 ± 0.074^{c}	0.207 ± 0.066^{a}	0.213 ± 0.008^{a}	0.074 ± 0.015^{b}	0.101 ± 0.023^{a}	0,089 ± 0.013^{a}	0.026 ± 0.008^{b}

Values are expressed per kilogram of fresh weight. a-c – the same letters indicate a lack of statistically significant differences at $p < 0.05$ for each plant material extracted with different solvents, $n = 3$.
nd – not detected, the concentration of the compound was below the method sensitivity.

quince, *AOX* did not increase as significantly as *TPC* when solvents were changed from water, through ethanol to methanol (Tab. 1). This is why PCA did not detect a strong correlation between *TPC* and *AOX* for quince extracts. Ethanolic extract of lingonberry demonstrated higher *AOX* in comparison to other tested solvents (19.32 g·kg^{-1} of FW, expressed as grams of Trolox) and this can be explained by the highest *TPC* among all lingonberry extracts. Only in case of methylene chloride, there was no correlation between *TPC* and *AOX* of extracts, even if the examined plant material contained more polyphenols than others, it did not demonstrate the highest *AOX* among other tested extracts. For example, the highest *TPC* among methylene chloride extracts was determined in case of elderberry fruits, whereas the highest *AOX* was noted in the case of lingonberry fruits. Fruits of elderberry, Japanese quince and cornelian cherry were proven to be the richest sources of compounds with a high *AOX*. Moreover, although *TPC* of lingonberry and quince with 80% methanol was similar (19.56 g·kg^{-1} FW and 19.50 g·kg^{-1} FW, respectively, expressed as grams of (+)-catechin), the lingonberry extract had 7-fold higher antioxidant potential than those of quince (11.32 g·kg^{-1} FW vs 1.61 g·kg^{-1} FW, expressed as grams of Trolox). In general, compounds extracted from quince had very low *AOX*, in case of water-soluble compounds their *AOX* was even 20-fold lower in comparison to the aqueous extract obtained from Japanese quince. Another conclusion that might be derived from PCA is that most of extracted phenolic compounds demonstrated higher affinity towards polar solvents, which is related to their chemical structure [35].

Polyphenol profiles

At the next research stage, polyphenol profiles of extracts produced from fruits, which demonstrated the greatest variety of anthocyanins and flavonoids (elderberry,

Japanese quince and cornelian cherry), were examined by PCA (Tab. 2). Only phenolic compounds that were common to all extracts were considered: (+)-catechin, procyanidins B1 and B2, caffeic acid, chlorogenic acid and (–)-epicatechin (Tab. 2). Chosen fruits accumulated polyphenols selected for PCA differently but it seems that the occurrence of (–)-epicatechin and (+)-catechin, caffeic acid and procyanidin B2, both procyanidins and caffeic acid were linked (Fig. 2). Those correlations could be explained by the fact that procyanidins are dimers of (+)-catechin and (–)-epicatechin [36] and chlorogenic acid is an ester of caffeic acid [37]. Moreover, it was shown that there were no correlations between concentrations of selected phenolic compounds in methanolic extracts of elderberry and cornelian cherry, but strong correlations were noted in case of water extracts of cornelian cherry and Japanese quince or ethanolic extract of cornelian cherry and methanolic extract of Japanese quince. This means that the occurrence of tested polyphenols was not specific for the species of tested fruits.

In the case of tested fruits, methanol (80% v/v) was a more efficient solvent for flavonols, anthocyanins, (–)-epicatechin and chlorogenic acid. On the other hand, ethanol was more favourable for the recovery of procyanidin B2 from all tested fruits and (+)-catechin from elderberry and Japanese quince (Tab. 2). Methanolic extract of elderberry fruits was dominated by anthocyanins, mainly cyanidin-3-*O*-sambubioside, and demonstrated the highest content of those compounds. In case of Japanese quince, such tendencies were not observed. Extracts obtained from cornelian cherry (Tab. 2) contained much lower quantities of tested phenolic compounds than other fruits and 80% methanol was the most efficient solvent for most of tested polyphenols. Extraction with 80% ethanol solution proved to be more effective only

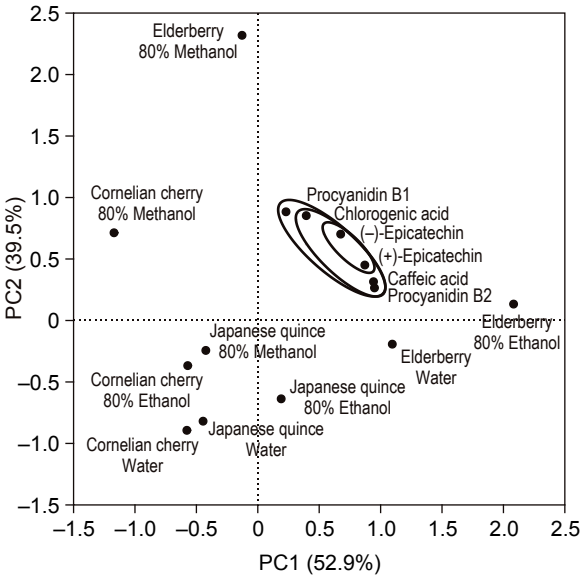

Fig. 2. Principal component analysis of polyphenol profiles of fruit extracts

PCA of selected polyphenols (loads), determined in extracts of elderberry, Japanese quince and cornelian cherry fruits obtained with water and aqueous solutions of 80% methanol and 80% ethanol (scores).
Points within circles indicate correlations between loads.

for procyanidin B2 and caffeic acid. Polyphenol recovery from plant matrices is believed to increase with solvent polarity up to its value exceeding about 6.0. Further growth of solvent polarity does not support extraction of phenolic compounds. On the other hand, polyphenols with sugar molecules within their structure are recovered more efficiently with solutions that have a high water content [5, 38]. Our findings support those hypotheses.

Due to the fact that ethanol is allowed for human consumption [39] and it provided satisfactory recovery of phenolic compounds, it was used in further research steps. On the other hand, extrac-

Tab. 3. Influence of ethanol concentration on total polyphenol content and antioxidant activity of selected fruit extracts.

Ethanol	Elderberry		Japanese quince	
	TPC [g·kg^{-1}]	*AOX* [g·kg^{-1}]	*TPC* [g·kg^{-1}]	*AOX* [g·kg^{-1}]
40%	37.93 ± 4.50^{a}	19.14 ± 0.80^{ab}	16.26 ± 0.21^{a}	6.24 ± 0.29^{a}
60%	32.36 ± 2.80^{b}	18.50 ± 1.84^{a}	18.54 ± 1.87^{ab}	6.09 ± 0.69^{a}
80%	38.27 ± 2.15^{a}	21.53 ± 2.64^{c}	20.55 ± 1.16^{bc}	7.09 ± 0.55^{a}
96%	30.20 ± 2.35^{b}	20.17 ± 0.66^{bc}	21.37 ± 3.18^{c}	6.55 ± 0.23^{a}

a-c – the same letters within the analysed parameter and raw material (columns) for each treatment method indicate a lack of statistically significant differences ($p < 0.05$), $n = 3$.
TPC – total polyphenol content expressed as grams of (+)-catechin per kilogram of fresh weight, *AOX* – antioxidant activity expressed as grams of Trolox per kilogram of fresh weight.

tion with methanolic solutions should be considered for assessing quantitative profiles of tested plants because, in the presence of methanol, those substances were recovered more effectively. Fruits of elderberry and Japanese quince were selected for further experiments. Cornelian cherry demonstrated lower levels of phenolic compounds and lower antioxidant capacities, so it was not further considered. Moreover, it is the fruit that is less popular and less available on the market.

Optimizing ethanol concentrations for the extraction of phenolic compounds

After selecting the solvent, it was necessary to optimize the extraction process by choosing optimum ethanol concentration in aqueous solution. It was shown that none of tested ethanol concentrations correlated with *TPC* and radically different tendencies were noted for each fruit, elderberry and Japanese quince (Fig. 1C). In case of elderberry (Tab. 3), *TPC* was firstly decreasing with

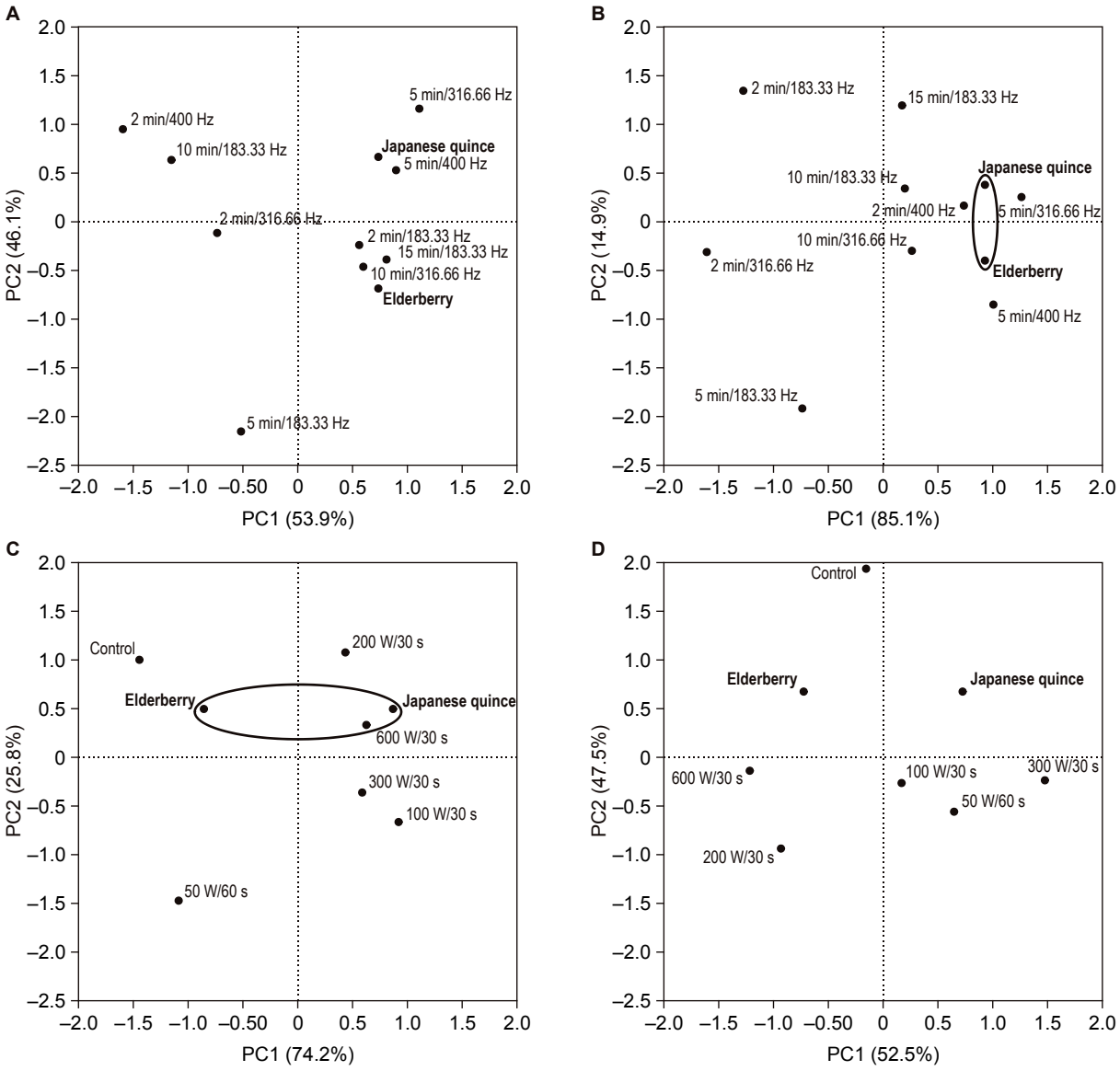

Fig. 3. Principal component analysis determining the influence of homogenization parameters and microwave treatment on total polyphenol content and antioxidant activity of selected fruit extracts.

A – Total polyphenol content (loads) in the extracts obtained at different homogenization parameters (scores); B – Antioxidant activity (loads) in the extracts obtained at different homogenization parameters (scores); C – Total polyphenol content (loads) under different conditions of microwave pre-treatment (scores); D – Antioxidant activity (loads) under different conditions of microwave pre-treatment (scores).
Points within circles indicate correlations between loads and scores.

Tab. 4. Influence of homogenization parameters on total polyphenol content and antioxidant activity of selected fruit extracts.

Homogenization (80% ethanol solution)		Elderberry		Japanese quince	
Time [min]	Rotational frequency [Hz]	*TPC* [g·kg^{-1}]	*AOX* [g·kg^{-1}]	*TPC* [g·kg^{-1}]	*AOX* [g·kg^{-1}]
2	183.33	54.80±4.51^{ab}	22.35±1.78^{a}	27.47±2.26^{ab}	11.62±0.93^{a}
	316.67	51.81±4.26^{ac}	23.88±1.91^{a}	25.43±2.09^{a}	8.35±0.67^{b}
	400.00	47.86±3.94^{c}	33.09±2.64^{bc}	25.64±2.11^{a}	16.44±1.31^{c}
5	183.33	56.30±4.63^{ad}	30.49±2.43^{bd}	22.51±1.85^{c}	8.99±0.72^{b}
	316.67	53.22±4.28^{ac}	35.17±2.81^{ce}	30.69±2.52^{d}	18.19±1.45^{d}
	400.00	54.00±4.69^{bd}	36.03±2.87^{e}	29.29±2.41^{bd}	15.91±1.27^{ce}
10	183.33	49.41±4.06^{c}	30.45±2.43^{bd}	25.90±2.13^{a}	14.95±1.19^{efg}
	316.67	55.33±4.55^{ab}	31.88±2.54^{b}	27.18±2.23^{ab}	14.30±1.14^{f}
15	183.33	55.65±4.58^{ab}	28.83±2.30^{d}	27.68±2.28^{e}	16.00±1.28^{cg}

a-g – the same letters within the analysed parameter and raw material (columns) for each treatment method indicate a lack of statistically significant differences ($p < 0.05$), $n = 3$.
TPC – total polyphenol content expressed as grams of (+)-catechin per kilogram of fresh weight, *AOX* – antioxidant activity expressed as grams of Trolox per kilogram of fresh weight.

Tab. 5. Influence of microwave treatment on total polyphenol content and antioxidant activity of selected fruit extracts.

Microwave treatment (80% etanol solution)		Elderberry		Japanese quince	
Power [W]	Time [s]	*TPC* [g·kg^{-1}]	*AOX* [g·kg^{-1}]	*TPC* [g·kg^{-1}]	*AOX* [g·kg^{-1}]
50	60	50.72±4.17^{b}	23.89±1.91^{b}	28.00±2.30^{b}	15.05±1.20^{b}
100	30	48.60±4.00^{c}	26.94±2.15^{c}	34.02±2.80^{c}	14.85±1.18^{b}
200	30	50.70±4.16^{b}	28.25±2.25^{c}	35.33±2.91^{c}	11.15±0.89^{c}
300	30	49.32±4.06^{c}	22.01±1.76^{b}	33.65±2.77^{c}	17.31±1.38^{a}
600	30	49.82±4.10^{c}	31.98±2.55^{d}	34.75±2.86^{c}	12.10±0.97^{c}
Control		53.22±4.28^{a}	30.69±2.52^{a}	35.17±2.81^{a}	30.69±2.52^{a}

Control samples in experiment involving microwaves were extracts prepared by homogenization (316.67 Hz; 5 min) with 80% ethanol solution.
a-c – the same letters within the analysed parameter and raw material (columns) for each treatment method indicate a lack of statistically significant differences ($p < 0.05$), $n = 3$.
TPC – total polyphenol content expressed as grams of (+)-catechin per kilogram of fresh weight, *AOX* – antioxidant activity expressed as grams of Trolox per kilogram of fresh weight.

the increase of ethanol concentration, then it increased again, reaching a maximum, when 80% solution was used (38.27 g·kg^{-1} FW, expressed as grams of (+)-catechin) and lowered in 96% ethanol solution (30.30 g·kg^{-1} FW, expressed as grams of (+)-catechin). A different pattern was observed for Japanese quince, for which *TPC* was increasing with the increase of ethanol concentration, reaching a maximum at 96% ethanol (21.37 g·kg^{-1} FW, expressed as grams of (+)-catechin), but there were no statistically significant differences between results obtained at 80% and 96% of that solvent.

Different tendencies were observed in case of *AOX* (Fig. 1D), which was increasing till the ethanol concentration reached 80%. There were no significant differences between results obtained for 80% and 96% ethanol solutions in both tested fruits (Tab. 3). Possible explanation to that phenomenon might be that when the water content decreased, the elution of water-soluble compounds with *AOX* decreased as well.

Overall, considering results obtained for both *AOX* and *TPC*, it was concluded that 80% ethanol solution provided the best effects.

Optimizing homogenization parameters

Due the fact that the size of particles of plant materials that are used for polyphenol extraction has a significant impact on the efficiency of that

process, this issue was addressed in the current study. Elderberry contained twice more phenolic compounds than Japanese quince and elution of those substances was performed according to different patterns (Fig. 3A). It seemed that there were few combinations of homogenization parameters that did not influence *TPC* in both tested fruits: 2 min/400 Hz, 10 min/183.33 Hz, 2 min/316.67 Hz and 5 min/183.33 Hz. On the other hand, there might be some correlation between *TPC* of Japanese quince and 5 min processing at 400 Hz or *TPC* of elderberry extracts obtained at 15 min/183.33 Hz and 10 min/316.67 Hz. Overall, it might be stated that homogenization parameters had no significant impact on the elution of phenolic compounds (Tab. 4), on the contrary to *AOX*. That parameter reached maximum values for both plants during 5 min at 316.67 Hz, 35.17 $g \cdot kg^{-1}$ FW and 18.19 $g \cdot kg^{-1}$ FW, expressed as grams of Trolox, for elderberry and Japanese quince, respectively. On the other hand, it seemed that antioxidant potential of elderberry was related to homogenization parameters 5 min/400 Hz and, in case of Japanese quince, the parameters were 2 min/316.67 Hz (Fig. 3B).

Presented results lead to the conclusion that low rates of rotation speed provided better elution of phenolic compounds and/or other substances with antioxidant properties when elderberry fruits were considered, however, extending extraction time did not seem to influence the recovery of polyphenols. Moreover, short homogenization time at high speeds was more preferable for eluting substances with a high *AOX* (Fig. 3B). For Japanese quince, different tendencies were noted for *TPC* and *AOX*, as high rotation speeds increased both of those parameters (Fig. 3A, Fig. 3B). However, from the practical point of view, it was concluded that rotation speed 316.67 Hz during 5 min time was optimal to elute antioxidants from tested materials, and those parameters were applied in further research steps (Tab. 4). Applying those parameters reduced the exposure of phenolic compounds to light or oxygen so more of them survived the homogenization process.

Applying microwave pre-treatment for the extraction of bioactive compounds

At the last research stage, microwave pre-treatment was applied to homogenates (316.67 Hz, 5 min, 80% ethanol solution) of elderberry and Japanese quince fruits (Tab. 5). Microwave treatment influenced the recovery of *TPC* differently among the tested fruits, but parameters 600 W (power) and 30 s (time) seemed to affect that process similarly (Fig. 3C). That phenomenon was caused by the fact that microwave treatment decreased *TPC* in elderberry and significantly increased *TPC* in Japanese quince (Tab. 5).

In case of *AOX*, no correlations were observed in both tested plant materials (Fig. 3D). Presented data suggest that thermolabile compounds occur in elderberry and that the composition of two fruits significantly differed. Another conclusion might be that those thermolabile substances contributed to the antioxidant properties of fruits because, when exposure to microwaves was extended to 60 s, *AOX* in both cases decreased significantly. Moreover, it might be stated that both tested fruits were poor sources of polyphenol oxidase.

CONCLUSIONS

PCA proved to be a very useful statistical tool for describing mechanisms of the processes that take place during various operations involved in the recovery of polyphenols from plant materials. On the other hand, parameters which were indicated in PCA were not always optimal from economical or practical point of view, e.g. 80% methanol was indicated as more efficient solvent for polyphenol extraction, while this substance is not allowed for human consumption. In this regard, 96% ethanol solution provided maximum recovery of phenolic compounds from Japanese quince but there were no statistically significant differences between extracts made by 80% and 96% solutions. Low homogenization speed was more preferable for extracting phenolic compounds from elderberry, while high homogenization speed was more efficient in the case of Japanese quince, whereas rotation speed 316.67 Hz and 5 min processing time were sufficient parameters for obtaining highest *TPC*.

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