Journal of Chromatography A, 1218 (2011) 2505–2512

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Influence of extraction methods on stability of flavonoids

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ARTICLE INFO

Article history:
Received 30 October 2010
Received in revised form 24 January 2011
Accepted 23 February 2011
Available online 3 March 2011

Keywords:
Flavonoids
Extraction
Stability
LC–MS
Maize

ABSTRACT

The LC–MS/MS was applied for the determination of flavonoids' stability under four types of solvent extraction methods (reflux heating, sonication, maceration and microwave) from maize samples. The 11 flavonoids belong to different groups: flavonols (kaempferol, myricetin, rhamnetin, quercetin, rutin), flavanones (naringenin, naringin, hesperedin), flavones (apigenin, luteolin), isoflavones (genistein) were studied. The effect of the degradation of flavonoids depended on extraction mode and chemical structure. The smallest decomposition was observed by heated reflux extraction procedure within 30 min in water bath and by microwave assisted extraction under 160 W during 1 min. The decomposition for flavonoids depends on number of substituents in flavonoid molecule. The most unstable compound (recovery below 50%) in tested condition was myricetin. The higher number of hydroxyl groups promote degradation of flavonoids, whereas sugar moiety and methoxyl groups protect flavonoids of degradation during microwave and ultrasonic assisted extraction.

1. Introduction

Flavonoids are one of the most important groups of compounds occurring in plants, where they are widely distributed. The flavonoids of dietary significance exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic and antithrombotic actions [1,2]. The epidemiological studies point out to their possible role in preventing cardiovascular diseases and cancer [3–5]. Flavonoids behave as antioxidants by a variety of way including direct trapping of reactive oxygen species, inhibition of enzymes responsible for superoxide anion production, chelation of transition metals involved in processes forming radicals and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals [2].

Flavonoids are built upon C_6–C_3–C_6 flavone skeleton in which the three-carbon bridge between phenyl groups is commonly cyclized with oxygen (Fig. 1). According to the unsaturation and oxidation degrees of the three-carbon segment, several families of flavonoids could be distinguished such as flavonols, flavanones, flavones, anthocyanidins and isoflavones. The benzene ring B position is the basis for the categorization of the flavanoid class (position 2) and the isoflavonoid class (position 3). Additionally, a wide variety of derivatives are present in each family according to the number and nature of substituent groups attached to the flavonoid nucleus.

Extraction of compounds from plant materials is one of the most important steps prior to their determination by HPLC. Generally, it is a separation process where the distribution of analyte between two immiscible phases is made in order to appropriate distribution coefficient. Different solvents such as methanol, ethanol, acetone or their combination with water [6–12], ethyl acetate [13] and also through steam distillation [14] have been usually used for classical extraction. The optimum extraction conditions varied depending on the active compounds as well as kind of plant. Conventional extraction is usually performed at reflux temperature of 90 °C for several hours or maceration with solvent for days in room temperature. These methods, which have been used for many decades are time consuming and require relatively large quantities of solvents. In recent years, some novel extraction methods of flavonoids have been developed e.g., ultrasonic extraction (USE) [15–24] and microwave-assisted extraction (MAE) [21–33].

The extraction of bioactive compounds under ultrasound irradiation (20–100 kHz) can offer high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and temperature and lower energy input [15]. During sonication, the cavitation process causes the swelling of cells or the breakdown of cell walls, which allow high diffusion rates across the cell wall in the first case or a simple washing out of the cell contents in the second [17]. It has been suggested, that the improvement of USE is mainly due to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls. Better recoveries of cell contents can be obtained by optimizing ultrasound application factors including frequency, sonication power and time, as well as ultrasonic wave distribution [18]. The higher

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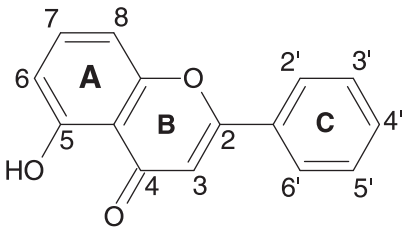
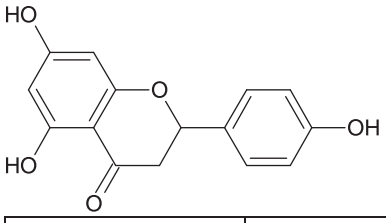
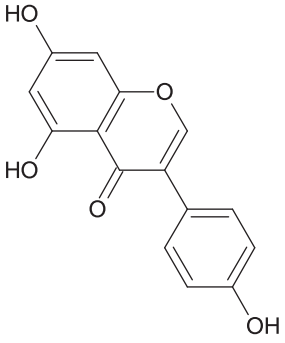
doi:10.1016/j.chroma.2011.02.059



	R3	R7	R3'	R4'	R5'
apigenin	H	OH	H	OH	H
luteolin	H	OH	OH	OH	H
kaempferol	OH	OH	H	OH	H
myricetin	OH	OH	OH	OH	OH
rhamnetin	OH	OCH_3	OH	OH	H
quercetin	OH	OH	OH	OH	H
rutin	O-rutinoside	OH	OH	OH	H



	R7	R4'	R5'
naringenin	OH	OH	H
naringin	O-rutinoside	OH	H
hesperedin	O-rutinoside	OCH_3	OH



genistein

Fig. 1. Chemical structures of the studied flavonoids.

temperature and pressure within a collapsing cavitation bubble produced by ultrasonic irradiation causes the formation of free radicals and various other species. The primary chemical effects are therefore the promotion and acceleration of reactions [20].

The basis of microwave assisted extraction is improvement in the extraction kinetics provided by heating. Additionally, the use of closed systems reduces the risk of losses, and the fact that microwave irradiation reduces overheating problems could also minimize the degradation of analytes. Extraction using microwaves can result in a yield increase in shorter time at the same temperature using less solvent. The great advantage of microwave heating is that all of sample fluid is heated, allowing the extraction solution (solvent and sample) to reach the desired temperature more rapidly and avoiding a thermal gradient caused by conventional heating. The experimental results demonstrated that extraction time is dramatically reduced and the yields of flavonoids are effectively improved [21–24,28–33].

However, extraction techniques may either cause the degradation of the targeted compounds due to high temperature and long extraction times as in solvent extractions, or unwanted reac-

tion during microwave irradiation or sonication. Moreover the selectivity of these methods is low, and significant amounts of non-phenolic compounds are also extracted, what could cause degradation processes of the analytes. Thus, before proposing an extraction method in the determination of polyphenols, it is necessary to verify that these compounds are stable under the extraction conditions. The process of degradation can be originated by several factors such as light, air, time and temperature. The temperature of extraction should be high enough in order to minimize the process duration. The enzymes presented in samples, principally oxidative enzymes, that are released during non specific extraction can also promote degradation reaction. Also free radicals can occur during extraction and cause the degradation.

The aim of this study was the determination of stability of selected flavonoids in maize samples in the different extraction techniques. The conventional liquid-solid extraction techniques such as heating reflux (HR), maceration (MR) or more innovative techniques: ultrasonic assisted extraction (USE) or microwave assisted extraction (MAE) were compared from the point of view degradation of flavonoids by matrix effects. In spite of the known beneficial effects of MAE and UAE (higher efficiency, shorter extraction time, less solvent usage), some aspects related to the stability of the extracted compounds have been poorly studied in literature and should therefore be considered.

2. Materials and methods

2.1. Chemicals and reagents

The commercial standards of flavonoids as well as the rest of the chemicals were purchased from Sigma (Steinheim, Germany). Methanol and acetonitrile were of HPLC gradient grade from Merck (Darmstadt, Germany). Ultra pure water from Milli-Q system (Millipore, Bedford, MA, USA) with a electrical resistivity of 18 MΩ*cm was used in all experiments. Stock solutions of flavonoids were prepared in methanol. Diluted mix standards were prepared with water. All solutions were filtered through 0.45 μm membranes (Millipore) and degassed prior to use. Ears of maize were purchased on local market. The kernels were collected from ears, dried at 30 °C in dark. Dried samples were ground in an electrical grinder to obtain fine powder and were stored in dark, closed container until analysis.

2.2. LC–MS/MS conditions

Chromatographic analysis was performed with a Shimadzu LC system consisted of binary pumps LC20-AD, degasser DGU-20A5, column oven CTO-20AC, autosampler SIL-20AC, detector UV SPD 20A connected to 3200 QTRAP Mass spectrometer (Applied Biosystem/MDS SCIEX). A MS system equipped with electrospray ionization source (ESI) operated in negative-ion mode. ESI conditions were following: capillary temperature 450 °C, curtain gas at 0.3 MPa, auxiliary gas at 0.3 MPa, negative ionisation mode source voltage −4.5 kV. Nitrogen was used as curtain and auxiliary gas. For each compound the optimum conditions of multiple reaction mode (MRM) were determined in infusion mode (Table 1). Standard solutions were infused into the electrospray source via a 50 μm i.d. PEEK capillary using a Harward Apparatus pump at 10 μL/min. Continuous mass spectra were obtained by scanning *m*/*z* from 50 to 650.

Compounds were separated on Kinetex™ (Phenomenex) C-18 column (100 mm × 2.1 mm, 2.6 μm) with precolumn at 30 °C. 8 mM formic acid (pH 2.8) as eluent A and acetonitrile as eluent B were used. The mobile phase was delivered at 0.2 mL/min in gradient mode: 0–5 min. 20% B, 10–15 min 25% B, 20–25 min 30% B, 30–31 min 90% B, 32 min 20% B. Compounds were identified by comparing retention time and *m*/*z* values obtained by MS and MS^2 with the mass spectra from standards tested under the same conditions. Quantification of compounds was done from the calibration curves obtained in MRM mode [34].

Table 1
LC/MS/MS characteristics of studied compounds in negative ion mode.

Compound	Retention time, (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP, V	CE, V
Rutin	3.55	609	300	−65	−56
Naringin	5.49	579	271	−80	−52
Hesperedin	5.75	609	164	−85	−72
Myricetin	8.23	317	151	−20	−26
Luteolin	14.46	285	133	−60	−44
Quercetin	14.72	301	151	−40	−30
Naringenin	18.71	271	119	−45	−34
Genistein	19.41	269	133	−75	−52
Apigenin	20.62	269	117	−55	−42
Kaempferol	21.82	285	151	−45	−25
Rhamnetin	28.86	315	300	−35	−24

2.3. Extraction procedures

Prior to the extraction, the dried maize corns were blended with home mixer. The samples were extracted with 60% methanol/water (v/v) solvent. The concentration of methanol was optimized for heating reflux extraction and then used for other extraction modes. For each extraction mode the three parallel samples of maize, standards and maize with standard addition were analyzed in three repetitions were analyzed. After extraction step the samples were filtered through a PTFE membrane filters a 0.45 μm and then injected to LC-ESI-MS/MS for analysis.

2.3.1. Heated reflux extraction (HR)

The maize corn materials (0.15 g) were weighted, mixed with 5 mL of methanol/water solvent (60/40 v/v) and placed in screwed glass vial (12 × 2 cm) and placed in boiling water bath (temp 95 °C), for 30 min. In case of standard addition the appropriate amount of standards in methanol were added instead of methanol solution [11].

2.3.2. Microwave-assisted extraction (MAE)

Household microwave oven (Whirpool) with power alternatively set to160; 350; 500 W was used for extraction. The microwave oven was equipped with a rotor on which a maximum of 12 a 100 ml Teflon digestion vessels can be placed. Rotation of vessels guaranteed the homogenize microwave energy absorption. The vessels were fitted with a pressure-relief valve and were sealed in a capping station. The microwave oven allows both time and energy to be programmed. The maize corn materials (0.15 g) were weighted, placed in Teflon vessels, mixed with 5 mL of methanol/water solvent (60/40 v/v), put in rotor and irradiated with appropriate microwaves and time.

2.3.3. Ultrasonic extraction (USE)

The maize corn materials (0.15 g) were weighted, mixed with 5 mL of methanol/water solvent (60/40 v/v) and placed into screwed glass vial (12 × 2 cm) and placed in an ultrasonic bath (Branson, Danbury, USA) for 30 min.

2.3.4. Maceration extraction

The maize corn materials (0.15 g) were weighted, mixed with 5 mL of methanol/water solvent (60/40 v/v) and placed into screwed glass vial (12 × 2 cm) and placed in a dark place at room temperature for 24 h.

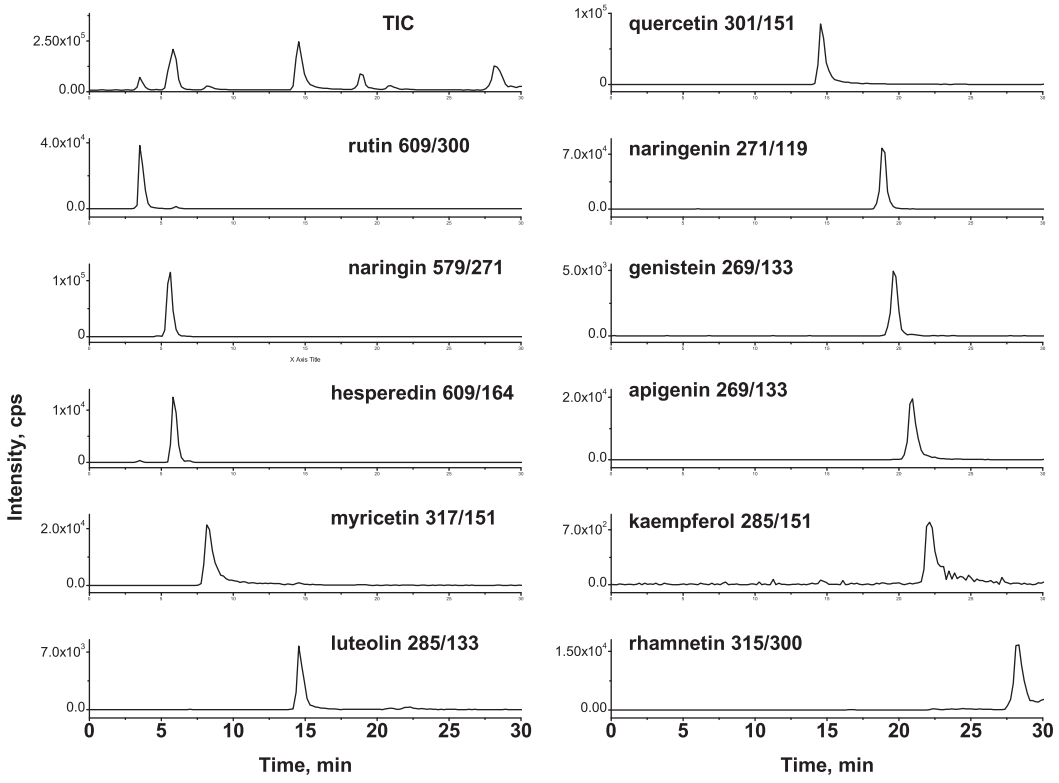


Fig. 2. Chromatograms of total ion current of all MRM (TIC) for maize sample spiked with standards (heated reflux extraction).

2.4. Scanning electron microscopy (SEM)

All analyses of dried samples were done using LEO 435VP scanning electron microscope. After evaporation of solvent in room conditions, the maize samples were fixed on an adhesive carbon tape and the sputtered with gold-palladium 4-5 nm layer. All samples were examined with SEM under high vacuum conditions at an accelerating voltage of 15 kV (20 μm, 2000× magnification).

3. Results and discussion

3.1. HPLC analysis

The LC–MS method previously used [33] was modified to be compatible with new column Kinetex™ C-18 instead of Atlantis C-18 (50mm × 2.1 mm, 3 μm). The longer column and smaller shell core particles (100mm × 2.1 mm, 2.6 μm) allowed the separation of all 11 flavonoids (Fig. 1) studied in less than 30 min using gradient elution of acetonitrile (ACN). The application of Kinetex column allowed to improve resolution and sensitivity in compare to conventional 3 μm particle column. Efficiency calculated as number of theoretical plates for meter for Kinetex column was at least 2 times higher than for conventional fully porous C-18 3 μm column used previously. The combination of the small particle size, narrow size distribution, and significantly shorter diffusion path results in higher column efficiency. The increased efficiency provided a benefit on sensitivity (narrower and taller peaks).

The elution sequence of flavonoids is directed by their structural characteristics. Generally, the retention times followed the expected reversed-phase pattern: O-glycosides < flavonoid aglycones (Table 1). Among flavonols, hydroxylation decreases retention owing to increasing polarity (hydrogen bond formation ability) and the elution pattern is affected by the number of OH-groups in B ring. Myricetin has three OH-groups at 3′, 4′ and 5′ position and it is eluted as first followed by quercetin (two OH groups) and kaemferol (one group) in group of flavonols. Similar situation was observed for flavons: luteolin (two OH groups) is eluted before apigenin (one OH group). Methylation in ring A caused the increase of retention time kaempferol/rhamnetin-flavonols) and naringin/hesperedin (flavonon O-glycosides). Also position of B ring 2 in case of apigenin and 3 in case of isoflavon–genistein had influence on retention time, slightly shorter retention time for genistein.

The linearity of the detector response was determined by the square correlation coefficients of the calibration curves generated by three repeated injections of standard solutions at six concentration levels (0.005–10 mg L^{-1}). All the compounds showed a good linearity with regression coefficients ≥0.999. Limits of detection (LODs) were estimated by decreasing the concentration of the analyte down to the smallest detectable peaks and then this concentration was multiplied by three. LODs were ranged between 0.5 and 10 ng L^{-1}. The reproducibility of method was evaluated by six consecutive injection of the standard solution. The relative standard deviations of intra-daily and inter-daily were below 5%.

3.2. Extraction of flavonoids

Samples were extracted under different extraction methods and the extracts were analyzed by HPLC-ESI-MS/MS method. Fig. 2 shows the example chromatograms obtained for heated reflux extraction of maize sample spiked with standards. Peak identity was established by both the retention time and the characteristic transitions (precursor and product ion pair).

The concentration of some relevant flavonoids found in the maize after 30 min heated reflux extraction at water bath with different concentration of methanol (MeOH) in the range of 40–80% are presented in Table 2. It is well known that flavonoids are better soluble in methanol than in water, but extraction of these compounds by pure solvent was worse than in water-alcoholic solution [11,23,27,32]. Kaempferol, myricetin, rhamnetin and hesperedin

Table 2
Contents of flavonoids found in maize samples versus methanol concentration in solvent by heated reflux extraction. Values are expressed in ng/g.

MeOH %	Naringenin	Quercetin	Apigenin	Luteolin	Rhamnetin	Naringin	Rutin
40	7.8	136.0	nd	nd	nd	5.4	5.5
50	14.5	523.0	1.1	1.9	nd	nd	3.8
60	14.5	1500.0	2.4	3.8	nd	nd	1.6
70	20.3	1450.2	2.9	5.9	0.9	nd	1.7
80	33.1	1420.1	3.8	4.5	1.2	nd	2.1

nd: not detected.

were not found in maize samples in any methanol concentration. The extraction yield of quercetin increased with increasing percentage of methanol up to 60%. This was probably due to the relative polarity, increase in effective swelling of the plant sample by water, which helped the increase the surface area for solute–solvent contact and higher solubility of flavonoids in methanol than in water. As one can see from results presented in Table 2 the different flavonoids require different concentration of MeOH in solvent to obtain the maximum of extraction. Because quercetin was the main flavonoid in maize samples the solvent containing 60% of methanol was chosen for all other extraction methods.

The comparison of standards stability (expressed as recovery of initial concentration) in methanol solution in tested extraction conditions is presented in Fig. 3. All flavonoid standards were stable during heating reflux in water bath for 30 min and maceration for 24 h (recovery above 95%). Rhamnetin and myricetin were slightly decomposed within 5 min microwave irradiation under 500 W (recovery 89 and 88% respectively). Application of sonication caused degradation of all tested compounds. The highest decomposition was observed for myricetin (40%), followed by hesperedin (30%).

Fig. 4 presents the recovery of standards addition obtained in different extraction modes in maize samples. The lowest degradation below 20% was observed for heated reflux extraction for all standards except myricetin (40%).

The smallest recoveries (the highest degradation) were observed for ultrasound assisted extraction. Application of USE within the same time (30 min) as in HR caused the significant degradation of all compounds. The lowest recoveries were obtained in order myricetin, quercetin, kaempferol and rhamnetin, which can be compared with number of hydroxyl groups in flavonoid moiety.

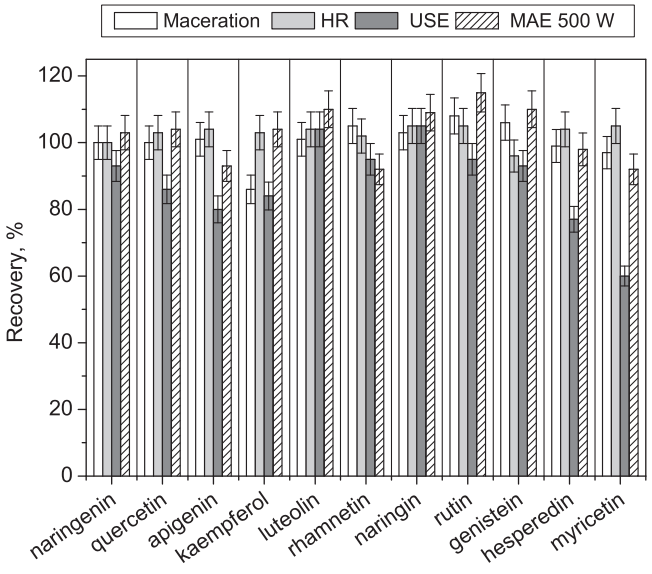


Fig. 3. Stability of standards with different extraction modes.

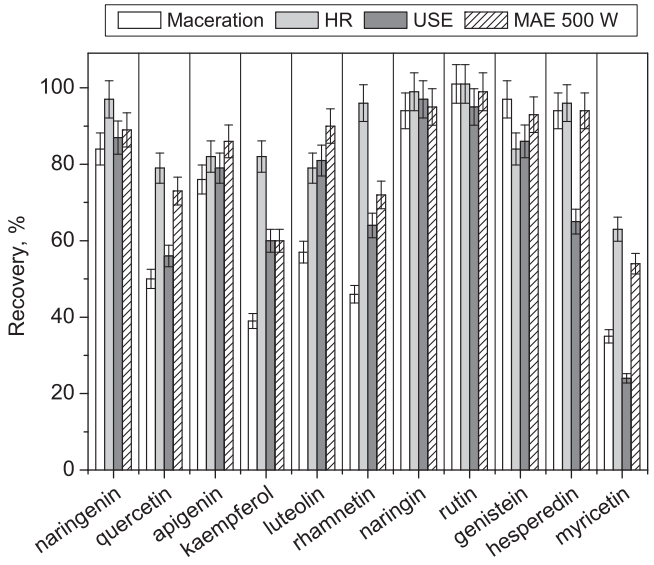


Fig. 4. Recovery of flavonoids from spiked maize samples for different extraction modes: maceration for 24 h, heated reflux in water bath for 30 min., USE for 30 min, MAE for 5 min under 500 W.

The comparison of degradation of kaempferol and luteolin showed that presence of hydroxylic group in 3 position enables degradation. The decomposition of genistein and apigenin was similar; it can suggest that position of B ring have no influence on degradation. The slight degradation was observed for rutin, naringin and hesperedin. These three compounds possess sugar moiety, which can protect molecules against degradation. Degradation of rutin by USE was previously described by Paniwnyk et al. [21]. The authors explained this phenomena by the reaction with high reactive hydroxyl radicals formed during sonication in solvent containing water, but under tested condition glycosides were stable. Comparison of USE and MAE for luteolin and apigenin described by Liu et al. [19] in pigeon pea leaves showed significantly better results for USE than for microwave extraction due to the effect of temperature. During sonication the temperature was lower than during MAE, which was advantageous for the prevention of degradation and oxidation of tested flavonoids. The results presented in Fig. 4 do not confirm their observation about stability, however, for some compounds the differences in stability between USE and MAE are small.

Maceration of maize samples within 24 h gave significant lower recoveries for myricetin, kaempferol, rhamnetin, quercetin and luteolin. During such long time some unknown e.g. enzymatic reactions, especially oxidation can occur causing decomposition of these compounds. The degradation order was compatible with the number of substituents in analyte moiety, especially hydroxylic groups. The disorder observed for kaempferol might be explained with fact that this compound was just degraded in standard solution (Fig. 3) during standing for 24 h in room temperature. No decomposition was observed for glycosides (rutin, naringin, hesperedin).

The recovery of standard addition was determined under different microwave power 160, 350 and 500 W and duration 1, 3, 5, and 10 min, respectively. The results presented in Figs. 5 and 6 indicate the influence of power and time microwave irradiation on flavonoids stability. Increasing of microwave power and time caused higher degradation of these compounds. The influence of time of irradiation was shown for 500 W in Fig. 6. The smallest degradation was observed for rutin and naringin. The significant decomposition was observed for myricetin, kaempferol, rhamnetin, and quercetin. Fig. 5 shows the results obtained for recovery of these unstable compounds at different power. The

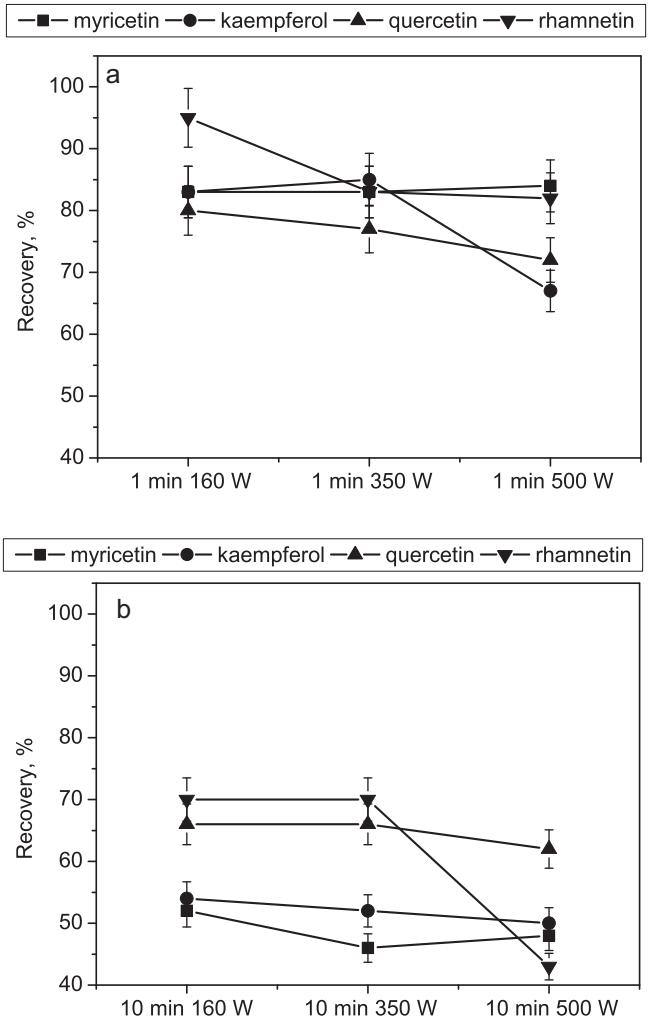


Fig. 5. The influence of microwave power on recoveries of myricetin, kaempferol, quercetin, rhamnetin (a) 1 min irradiation, (b) 10 min irradiation.

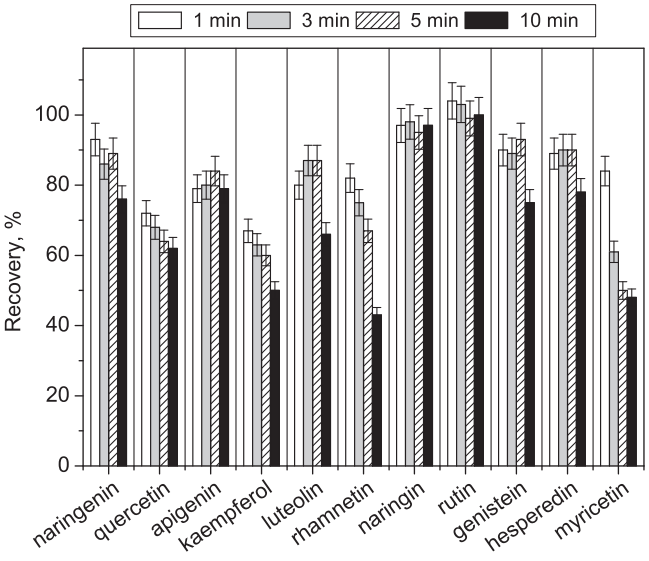


Fig. 6. The influence of duration of microwave irradiation under 500 W on recoveries of all tested flavonoids for spiked maize samples.

highest decomposition was observed for higher irradiation power. Fig. 6 presents the influence of time on the decomposition of all tested flavonoids in maize samples under 500 W. Longer extraction time and microwave power in closed vessels caused the extraction process to be performed at higher pressure and acceleration increasing the temperature. The evaluated temperature would result in improvement of extraction efficiency but also in degradation of termolabile compounds. Increasing time with the same microwave power significant decreased the recovery of myricetin, kaempferol, quercetin and rhamnetin. Presented results are in good correlation with data presented by Trusheva et al. [25] for total flavonoids amount in propolis samples. Unfortunately, they determined the sum of flavonoids by spectrophotometric method, so there was no information about decomposition of individual flavonoids.

The observed degradation of flavonoids during MAE occurred in order glycosides (rutin, naringin, hesperedin), flavanone (naringenin), isoflavone (genistein), flavones (apigenin, luteolin) and flavonols (quercetin, kaempferol, rhamnetin and myricetin). Stability of myricetin and kaempferol using MAE from grape skin and seeds was described by Liazid et al. [28]. Authors observed the decomposition of these two compounds under microwave irradiation. They suggested that the greater degree of substitution (hydroxylic groups) translated into reduced stability of these compounds. In addition, when two compounds have an equal number of substituents in the ring, the hydroxylates will be more easily degradable. In presented study the degradation comparison between quercetin (5 OH groups) and rhamnetin (4 OH groups 1 methoxylic group) confirmed their hypothesis. Similar observation obtained for naringin (3 substituents) and hesperedin (4 substituents) also confirmed their suggestion about influence of number of substituent on degradation of compounds.

The comparison of stability between kaempferol and luteolin (both compounds have the same number of OH groups) shows that the position of hydroxylic group can be important in stability of flavonoids. The presence of hydroxyl group in 3 position–kaempferol (flavonols) caused higher degradation than in case of luteolin (flavonons), which has OH groups in ring A and B. The recovery comparison of apigenin and genistein showed no significant influence of position B ring on stability of these compounds.

Stabilization of flavonoids by sugar moiety was also observed for pairs naringin-naringenin, rutin-quercetin. Hesperedin has 4 substituents (sugar molecule, 2 hydroxilic groups, one methoxylic group) like kaempferol and luteolin but is more stable during microwave extraction than these aglycones. Hydrolysis of glycosides to aglycones (rutin-quercetin, naringin-naringenin, hesperedin-hesperetin) was not observed in tested conditions.

3.3. SEM observation

In order to study the structural alteration during the different extraction techniques and to understand the extraction mechanism the maize samples were examined by SEM. Different extraction methods produced distinguishable physical changes. Fig. 7 shows the images of raw material (a), HR (b), USE (c) and MAE (d) under 500 W within 5 min.

In heated reflux the solvent transfers into the samples and extracts the compounds by permeation and solubilisation under higher temperature. Hence little destruction of microstructure of sample occurs and a few slight ruptures took place on the surface of sample. In this extraction process the surface of maize was not considerably different than in raw material.

After USE, the mechanical effects of ultrasound provided a greater penetration of solvent into cellular materials, via cavitation effects, and improved the release of chemical substances into the solvent. The ultrasounds induced a subsequent changes in the sur-

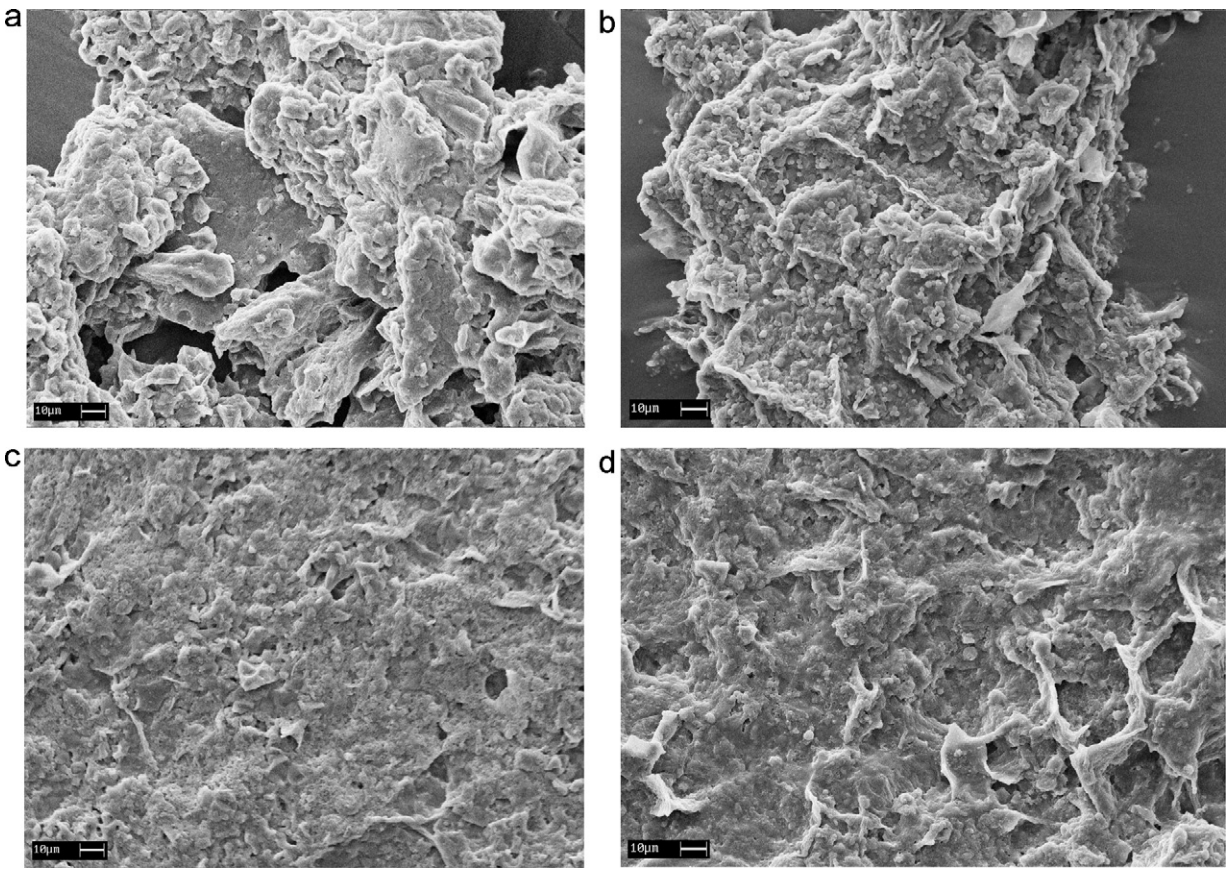


Fig. 7. SEM images of maize samples for raw material (a) and after HR (b) (30 min 95 °C), USE (c) (30 min), MAE (d) (500 W 5 min).

face, number of pits appeared on the plant surface. These changes could cause the plant to crumble and rupture more readily. The surface of the sample after USE was obviously destroyed.

After MAE the surface is much more destroyed than during heated reflux extraction process but less than after USE. MAE affects the structure of samples through the sudden temperature rise and the internal pressure increase. During the USE and MAE process, all chemical substances within the cell are rapidly released into surrounding solvents, what could enhance the degradation of flavonoids.

4. Conclusions

In presented study the stability of eleven flavonoids were tested under four extraction modes. Microwave-assisted extraction and ultrasonic assisted extraction have been considered as a potential alternative to traditional heated reflux and maceration solid–liquid extraction for the isolation of flavonoids from maize samples. The significant decomposition of myricetin, kaempferol, rhamnetin and quercetin was observed by USE, MAE and maceration. The compounds can be affected by elevated temperatures created by microwaves in closed vessels and they were partially decomposed, in order to number of substituents (hydroxylic and methoxylic groups). Regarding the relationship between the chemical structure and the degradation process, it has been found that number and type of substituents as well position of hydroxyl group influence on stability. Smaller number of substituents affected higher stability of flavonoids. Also sugar moiety stabilized the flavonoids during extraction process. Other chemical reaction, which can possibly occur such as hydrolysis (e.g. rutin to its aglycone quercetin) was not observed, but decomposition of myricetin by unknown matrix effects was very significant during maceration extraction. The best stabilities of tested flavonoids were obtained for traditional heated reflux in water bath and for MAE within 1 min under 160 W..

Acknowledgements

The author would like to thank the Structural Research Laboratory (SRL) at the Department of Chemistry of University of Warsaw for using HPLC–MS. SRL has been established with financial support from European Regional Development Fund in the Sectorial Operational Programme “Improvement of the competitiveness of Enterprises, years 2004–2005” project no: WPK_1/1.4.3./1/2004/72/72/165/2005/U. Special thank is given to Phenomenex Inc. for the donation of Kinetex column.

References

[1] L.H. Yao, Y.M. Jiang, J. Shi, F.A. Tomas-Barberan, N. Datta, R. Singanusong, S.S. Chen, Plant Foods Hum. Nutr. 59 (2004) 113.
[2] N.C. Veitch, R.J. Grayer, Nat. Prod. Rep. 25 (2008) 555.
[3] E. Szliszka, Z.P. Czuba, K. Jernas, W. Krol, Int. J. Mol. Sci. 9 (2008) 56.
[4] M. Blonska, J. Bronikowska, G. Pietsz, Z.P. Czuba, S. Scheller, W. Krol, J. Ethnopharmacol. 91 (2004) 25.
[5] H.P. Hoensch, W. Kirch, Int. J. Gastrointest. Cancer 35 (2005) 187.
[6] G. Romanik, E. Gilgenast, A. Przyjazny, M. Kamiński, J. Biochem. Biophys. Methods 70 (2007) 253.
[7] M. Biesaga, K. Pyrzynska, Crit. Rev. Anal. Chem. 39 (2009) 95.
[8] B.A. Silva, F. Ferreres, J.O. Malva, A.C.P. Dias, Food Chem. 90 (2005) 157.
[9] V. Exarchou, Y.C. Fiamegos, T.A. van Beek, C. Nanos, J. Vervoot, J. Chromatogr. A 1112 (2006) 293.
[10] T. Sun, C. Ho, Food Chem. 90 (2005) 743.
[11] A. Wach, K. Pyrzynska, M. Biesaga, Food Chem. 100 (2007) 699.
[12] S. Kallithraka, C. Garcia Viguera, P. Bridle, J. Bakker, Phytochem. Anal. 6 (1995) 265.
[13] P. Avato, F. Raffo, G. Guglielmi, C. Vitali, A. Rosato, Phytother. Res. 18 (2004) 230.

[14] S. Cavero, M.R. Garcia-Risco, F.R. Marin, L. Jaime, S. Santoyo, F.J. Senorans, G. Reglero, E. Ibanez, J. Supercrit. Fluid 38 (2006) 62.
[15] M.K. Khan, M. Abert-Vian, A.S. Fabiano-Tixier, O. Dangles, F. Chemat, Food Chem. 119 (2010) 851.
[16] M.C. Herrera, M.D.L. de Castro, Anal. Bioanal. Chem. 379 (2004) 1106.
[17] F. Vinatoru, Ultrason. Sonochem. 8 (2001) 303.
[18] L. Wang, C.L. Weller, TIFS 17 (2006) 300.
[19] W. Liu, Y. Fu, Y. Zu, Y. Kong, L. Zhang, B. Zu, T. Efferth, J. Chromatogr. A 1216 (2009) 3841.
[20] M.D.L. de Castro, F. Priego-Capote, Anal. Chim. Acta 583 (2007) 2.
[21] L. Paniwnyk, E. Beaufoy, J.P. Lorimer, T.J. Mason, Ultrason. Sonochem. 8 (2001) 299.
[22] A.A. Casazza, B. Aliakbarian, S. Mantegna, G. Cravotto, P. Perego, J. Food Eng. 100 (2010) 50.
[23] Z. Liu, L. Ding, H. Zhang, X. Hu, F. Bu, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 719.
[24] M.A. Rostagno, A. Villares, E. Guillamon, A. García-Lafuente, J.A. Martinez, J. Chromatogr. A 1216 (2009) 2.
[25] B. Trusheva, D. Trunkova, V. Bankova, Chem. Cent. J. 1 (2007) 13.
[26] X. Pan, G. Niu, H. Liu, Chem. Eng. Process 42 (2003) 129.
[27] L. Ding, X. Luo, F. Tang, J. Yuan, Q. Liu, S. Yao, J. Chromatogr. B 857 (2007) 202.
[28] A. Liazid, M. Palma, J. Brigui, C.G. Barroso, J. Chromatogr. A 1140 (2007) 29.
[29] C. Proestos, M. Komaitis, LWT 41 (2008) 652.
[30] J.H. Wang, X.H. Xiao, G.K. Li, J. Chromatogr. A 1198–1199 (2008) 45.
[31] W. Xiao, L. Han, B. Shi, Sep. Purif. Technol. 62 (2008) 614.
[32] M. Gao, C.Z. Liu, World J. Microbiol. Biotechnol. 21 (2005) 1461.
[33] M. Biesaga, K. Pyrzynska, J. Chromatogr. A 1216 (2009) 6620.