Effect of abiotic stress factors on polyphenolic content in the skin and flesh of pear by UPLC-PDA-Q/TOF-MS

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Abstract

Growing social interest in foods with high biological quality results in the need to look for possibilities of increasing the biological quality of food products. The aim of this research was an attempt to estimate the increase of polyphenolic compounds (by UPLC-PDA-MS/MS) in the flesh and skin of pear under the influence of stress elicitors such as UVC radiation, L-EMF, H-EMF and US with various exposure times. The applied stress factors differentiated the content of phenolic acids, flavan-3-ols and flavonols both in flesh and in skin. In all cases, pear skin demonstrated a decrease in the sum of polyphenolic compounds; however, when it comes to flavan-3-ols and procyanidins, the concentration of the compounds increased in two cases: after a 30-min exposure to L-EMF (+18%) and after a 5-min exposure to H-EMF (+20%). Following a 30-min flesh exposure to US, the determined sum of polyphenolic compounds was +28% higher than in the control sample. It was observed that the level of flavan-3-ols and procyanidins in the flesh increased after a 60-min exposure to UVC radiation and low-frequency and high-frequency electromagnetic fields, and after a 20-min and 30-min exposure to ultrasounds. The presence of flavonols was only observed in pear skin. The research results show that it is possible to produce juice with a higher content of polyphenolic compounds, because juice is obtained mainly from the flesh; however, there is a need for further research to confirm the observed tendencies in the changes of polyphenolic compounds in fractions of pears.

Keywords *Pyrus communis* L. · Electromagnetic field · Microwave radiation · UV-C radiation · Polyphenolic compounds

Introduction

The health and nutritional properties of pears have been known for ages. The fruit originated in Asia, where it was used in Chinese folk medicine already 3000 years ago [1]. Two commonly available pear species are European pear (*Pyrus communis*) and Asian pear (*Pyrus pyrifolia*). The People's Republic of China is the global pear potentate. The differences between European and Asian pear include the form and texture, with the former being sweeter thanks to a higher content of fructose. The fruit is very nutritional and rich in glucose and fructose. In the case of European pear, the ratio of glucose to fructose is 0.12:0.52, whereas in the case of Asian pear, 0.39:0.59 [2]. Pears can be one of the major ingredients of a well-balanced diet. Their nutritional and health properties are mainly due to the result of antioxidant ingredients, which are scarce in the diet of the societies in developed countries. Therefore, it is important to search for possibilities to boost the antioxidant properties of the fruit and at the same time preserve the taste and nutritional value. According to a study conducted by Cebulak et al. [3]

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on chokeberries, it was observed that one of such factors can be exposure to particular stress elicitors.

Source literature shows that pears are particularly valued for their health benefits. They are rich in minerals: 100 g of dry mass contains 91.7 mg of potassium, 14.9 mg of calcium and 10.06 mg of magnesium, as well as 2.8 mg of fibre. Moreover, pear protein is rich in leucine and lysine [4]. The fruit also contains high amounts of biologically active ingredients, dominated by polyphenols, which determine the anti-diabetic, anti-inflammatory, antibacterial, anti-cancer properties and have a positive influence on the cardiovascular system [5, 6]. In research Simirgiotis et al. [7], and Wang et al. [8] conducted on diabetic mice [7, 8] observed that a daily intake of 500 mg of dried pear per kg b.w. for a period of 2 weeks had a significant hypoglycaemic effect. Pear skin contains a large amount of flavan-3-ols, polymeric procyanidins, flavonols and triterpenoids, whose content is positively correlated with the increased content of wax covering the skin [9]. The literature data indicate [10, 11] that the content of polyphenolic compounds in the local varieties of pear was higher than in commercial varieties, whereas significantly higher content was determined in the skins than in the flesh. However, the level of secondary metabolites depended on the degree of maturity, climatic factors, variety and cultivation technology applied.

Interest in the role of bioactive compounds in human health has impacted research on content of these compounds impact in food and opportunities maintaining their content and even improving [12]. Recently shown the use of many treatments as high or low temperature treatments, altered gas composition, ultraviolet and gamma irradiation, can enhance polyphenolic compounds, and thus antioxidant activity [13]. UV-C irradiation has been tested as a treatment to increase ascorbic acid and total phenolic contents in plants [14]. According to Yu et al. [15], the UV irradiation and heat stress lead to changes in polyphenolic compounds content, the range of which depends on time and/or the power of use abiotic stress factors. Effective use of these factors could increase the content of bioactive compounds in finished products. That is why it is so important to check under optimal conditions their impact on the health values of plants and their increase. Moreover, to our knowledge there are no reports on effect abiotic stress factors to phenolic compounds in pear fractions. Therefore, the objective of this study was an attempt to estimate the increase of polyphenolic compound contents in the flesh and skin of pear under the influence of stress elicitors such as UVC radiation, low-frequency electromagnetic field (L-EMF), high-frequency electromagnetic field (H-EMF) and ultrasounds (US) with various exposure times.

Materials and methods

Reagents and standards

Methanol and formic acid were from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was from Merck (Darmstadt, Germany). Caffeic acid was purchased from Sigma-Aldrich (Steinheim, Germany). Kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, apigenin 7-*O*-glucoside, quinic, *p*-coumaric and ferulic acids, (+)-catechin and procyanidin B2 were purchased from Extrasynthese (Lyon, France).

The experimental material

The study was based on the fruit of the ‘Conference’ pear cultivar. It was acquired from a commercial orchard located in Dwikozy near Sandomierz (N 50°71′19.9060″, E 21°77′74.7726″). The fruit was harvested at the stage of technological ripeness in September 2017. Following initial processing involving washing and drying, the fruit was divided into 3-kg portions that were exposed to abiotic stress factors varying in terms of intensity and exposure time. The collected fruits were then subjected to abiotic stress factors: UV-C radiation using the NBV 30N lamp (UltraViol, Zgierz, Poland) with a radiation intensity of 2.3 W/m²; electromagnetic field (150 µT, 100 Hz); microwaves (100 W and 180 W) and ultrasound (800 W, 40 Hz). Different times of action were used for these stress factors. Exemplary codes with the appropriate stress factor and its duration of action are given below. (1) UVC UVC radiation for 20, 40 and 60 min; (2) L-EMF low-frequency electromagnetic radiation (150 µT) for 30 and 60 min; (3) H-EMF high-frequency electromagnetic radiation (40 µT) for 0.5, 2 and 5 min; (4) US ultrasounds for 20, 30 and 40 min. After the end of the elicitation process, the fruit was left to rest for 3 h. Then, the fruit was peeled and divided into smaller fragments, which were then frozen in liquid nitrogen. After freezing, the fruit fragments were ground in a lab grinder (IKA 11A) and lyophilised (Christ Alpha 1–4 LSC). Prior to the analysis, the lyophilisate was stored at –80 °C.

Extraction

Polyphenolic compounds were isolated by means of extraction supported by ultrasounds. First, 1 g of the finely ground material was placed in a Falcon centrifuge tube (50 mL), to which 50% aqueous methanol containing formic acid (1%) was poured. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry

was centrifuged at 19,000g for 10 min and the supernatant was filtered through a Hydrophilic PTFE 0.20 µm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The content of polyphenols in individual extracts was determined by means of the UPLC-PDA-MS/MS method [16]. All extractions were carried out in triplicate.

Identification and quantification of polyphenols by the UPLC-PDA-MS method

Qualitative and quantitative analyses of polyphenolic compounds were carried out with the use of ultra-performance liquid chromatography with a photodiode array detector (PDA) and a tandem mass detector measuring the quadrupole-time of flight (Q-TOF), based on a procedure described by Cebulak et al. [3]. Polyphenols were analysed in the negative-ion mode, while the remaining in their identification was done by comparing spectra of maximum UV-radiation absorption, molecular weight determined as the mass/charge ratio, retention times, as well as fragmentation spectra, with the available literature data (Table 1) [17, 18].

Phenolic compounds were identified by ultra-performance liquid chromatograph equipped with a binary solvent manager (BSM), a sample manager (SM) coupled with a PDA detector and a quadrupole-time-of-flight (Q-TOF) tandem mass detector (Waters, Manchester, UK). Separations were carried out on a 2.1 × 100 mm UPLC BEH C18 column containing 1.7 µm particles. Isocratic gradient elution was chosen as the elution mode, in which were used: 2% aqueous formic acid (A) and acetonitrile (B), at the flow of mobile phase velocity of 0.45 mL/min. Elution was initiated at 99% A for 1 min, then a linear gradient was applied to 75% B in 12 min. The column temperature was 30 °C and the volume of injections 5 µL. Operating parameters of the mass detector were as follows: capillary voltage of 2.5 kV and the sampling cone voltage of 30 V. Temperatures of the ion source and desolvation were 130 °C and 350 °C, respectively. Nitrogen, at a flow rate of 300 L/h, was used as carrier gas. Analyses were carried out in a full scan mode within the range 100–1500 *m/z*, upon the tolerance of 0.001 Da and resolution of 5000. The internal reference standards, leucine and enkephalin, were introduced continuously through lock-spray reference channel. The chromatograms were analysed employing the base peak (BPI) calibrated to 12,400 cps (100%). Data were collected and analysed using MassLynx v4.1 software (Waters).

Statistical analysis

Statistical calculations were based on a significance test for means and cluster analyses, procedures available in the statistical analytics software Statistica 12 (Kraków, Poland).

The significance for means was evaluated by a Student's *t* test of independent samples with the intensity level of $\alpha=0.05$. Cluster analysis provided an image of interfactorial relations of the influence of abiotic stress factors on the differentiation of polyphenol content in the skin and flesh of pear.

Results

The influence of abiotic stress factors: UV-C radiation, ultrasounds [US], low-frequency electromagnetic radiation 150 µT [L-EMF] and high-frequency electromagnetic radiation of 40 mT [H-EMF], on the level of polyphenolic compounds in the flesh and skin of pear is presented in Tables 2, 3, 4, 5. The identified polyphenolic compounds in pear fruits subjected to abiotic stress factors are given in Table 1. Interrelations between the abiotic stress factor and the changes of polyphenol content in the skin and flesh of pear are presented in Figs. 1, 2, 3, 4, 5. The concentration of phenolic acids, flavan-3-ols, procyanidins and flavonols was calculated on the basis of standardised benchmark substances.

The highest concentration of polyphenolic compounds in the analysed skins was found in the control sample (3.19 mg/100 g dry matter) (Table 2), and the use of stress elicitors decreased the sum of polyphenolic compounds in all skin samples, with a slight increase in the flesh. The determined level of the sum of polyphenolic compounds in the flesh was significantly lower than in the skin (Tables 2, 3, 4, 5). The observed decrease of polyphenolic compound concentration in the skin ranged from –3% (3.09 mg/100 g dry matter) after exposure to H-EMF for 5 min, to 2.37 mg/100 g dry matter in the skin exposed to H-EMF for 2 min, which corresponds to a decrease of –25% of polyphenolic compounds. The concentration range of polyphenolic compounds in the flesh subject to stress factors ranged from 98.20 mg/100 g (–24%) in the case of H-EMF-2, to 165.30 mg/100 g DM (+28%) in the case of US-30, with a concentration level of 129.20 g/100 g dry matter of the control sample.

Phenolic acid

Cluster analysis determined the intergroup relations between the abiotic stress factors and the concentration of phenolic acids in the skin and flesh of pear (Figs. 1, and 2). In the case of pear skin, the factors grouped in cluster area 1 (H-EMF-2, UV-C-60, H-EMF-0.5, L-EMF-60, L-EMF-30) on average decreased the concentration of phenolic acids by –20.7%; cluster area 2 (H-EMF-5, US-30, US-20) included stress elicitors that resulted in an average decrease of phenolic acid concentration by –13.8%, whereas cluster area 4 contained

Table 1 Identification of phenolic compounds in pear (*Pyrus communis* L.) fruits by ultra-performance liquid chromatography and photodiode detector-quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/ToF-MS/MS)

Assigned identity	Rt (min)	[M–H] [–] (m/z)	Fragment ions (m/z)	Absorbance maxima (nm)	Peel	Flesh
Caffeic acid and derivatives						
Caffeoyl- <i>N</i> -tryptophan B-type procyanidin dimer	3.1962.882	365,577	229,289	326,280	x	xx
1- <i>O</i> -caffeoylquinic acid	3.376	353	191/179	324		x
3- <i>O</i> -caffeoylquinic acid	3.558	353	191/179	324	x	x
Caffeoylhexose	3.948	341	179	324	x	
5- <i>O</i> -caffeoylquinic acid	4.333	353	191	324	x	
<i>Cis</i> -3- <i>O</i> -caffeoylquinic acid	4.604	337	173	324	x	
Caffeoylhexose	4.833	341	179	324	x	
Caffeoyl- <i>l</i> -malic acid	4.987	295	179	326	x	
3,5-di- <i>O</i> -caffeoylquinic acid	6.502	515	515	326	x	
Caffeoyl- <i>p</i> -coumaroylquinic acid	6.906	499	337/163	313		x
3,4-di- <i>O</i> -caffeoylquinic acid	7.276	515	515	326	x	x
<i>p</i>-coumaric acid and derivatives						
<i>p</i> -coumaroyl hexose (isomer 1)	3.473	325		314		x
<i>Cis</i> -4- <i>p</i> -coumaroylquinic acid	5.340	337	173	309	x	x
<i>Cis</i> -4- <i>p</i> -coumaroylquinic acid	6.091	279	163	309		x
<i>Cis</i> -5- <i>p</i> -coumaroylquinic acid	6.094	337	191	326	x	
3,5-di- <i>O</i> -caffeoylquinic acid	6.502	515	515	326	x	
Caffeoyl-3,4-di- <i>O</i> -caffeoylquinic acid	7.276	515	515	326	x	x
Monomeric catechins						
(+)-Catechin	3.220	289	245	280	x	x
(–)-Epicatechin	4.644	289	245	280	x	x
Procyanidins						
B-type procyanidin dimer	2.882	577	289	280	x	x
B-type procyanidin dimer	2.917	577	290	280	x	x
B-type procyanidin dimer	3.068	577	291	280	x	x
B-type procyanidin tetramer	3.140	1153	577/287	280	x	x
Caffeoyl- <i>N</i> -tryptophan	3.196	365	229	326		x
1-caffeoylquinic acid	3.376	353	191/179	324		x
B-type procyanidin trimer	3.404	865	577/287	280	x	x
B-type procyanidin dimer	3.689	577	289	280	x	
B-type procyanidin trimer	3.858	865	577/287	280	x	
B-type procyanidin dimer	4.149	577	289	280	x	x
B-type procyanidin trimer	4.855	865	577/287	280	x	
B-type procyanidin tetramer	5.177	1153	577/287	280	x	
Quercetin derivatives						
Quercetin 3- <i>O</i> -rutinoside	5.817	609	301	350	x	
Quercetin 3- <i>O</i> -galactoside	5.991	463	301	355	x	
Quercetin 3- <i>O</i> -glucoside	6.194	463	301	355	x	
Quercetin-acylated-hexoside	6.691	505	463/301	340	x	
Isorhamnetin derivatives						
Isorhamnetin 3- <i>O</i> -rutinoside	6.861	623	315	351	x	
Isorhamnetin 3- <i>O</i> -galactoside	6.971	477	315	350	x	
Isorhamnetin 3- <i>O</i> -glucoside	7.101	477	315	350	x	
Isorhamnetin-acylated hexoside	7.687	519	315	340	x	
Isorhamnetin-acylated hexoside	7.771	519	315	340	x	
Kaempferol derivatives						
Kaempferol-3- <i>O</i> -6-acetylglucoside	7.614	489	285	340	x	

Table 2 Influence of low-frequency electromagnetic field (L-EMF) on the content of polyphenolic compounds in pear fruits, mg/100 g dm dry matter ($n = 3$)

Groups of polyphenolic compounds	Control		Time of exposure to low-frequency electromagnetic field (L-EMF) (min) duration of action of the abiotic stress factor in min			
			30		60	
	Components					
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Phenolic acids						
Caffeic acid and derivatives	1344.0±1.5**	49.0±0.1**	1055.0±2.3*	39.0±0.2*	1057.0±3.3*	42.0±0.1
<i>p</i> -coumaric acid and derivatives	108.0±0.8**	2.2±0.0**	81.0±1.1*	1.8±0.0*	96.0±0.7	2.0±0.0*
Sum	1452.0±1.4**	51.2±11.0**	1136.0±1.4*	40.8±15.0*	1153.0±1.37*	44.0±13.0
Flavon-3-ol and procyanidins						
Monomeric catechins	697.0±0.3**	24.0±0.0**	732.0±0.3	19.0±0.0*	484.0±0.2*	26.0±0.1
Polymeric procyanidins	289.0±0.3**	54.0±0.1**	430.0±0.1*	51.0±0.2	381.0±0.5*	62.0±0.1
Sum	986.0±158.0**	78.0±0.5**	1162.0±186.0*	70.0±0.7	865.0±108.0	89.0±0.5*
Flavonols						
Quercetin derivatives	175.0±0.0**	nd	104.0±0.5*	nd	111.0±0.3*	nd
Isorhamnetin derivatives	482.0±0.0**	nd	317.0±0.2*	nd	386.0±0.1*	nd
Kaempferol derivatives	94.0±0.0*	nd	42.0±0.1*	nd	53.0±0.0*	nd
Sum	751.0±0.3**	nd	422.0±0.2*	nd	498.0±0.2*	nd
Total sum	3190.0±3.3**	129.2±0.5**	2720.0±2.8*	110.8±0.7*	2516.0±2.6*	133.0±0.6

*Statistically significant differences compared to the control at significance level $\alpha = 0.05$ **Table 3** Influence of UV-C radiation (UV-C) on the content of polyphenolic compounds in pear fruits, mg/100 g dry matter ($n = 3$)

Groups of polyphenolic	UV-C radiation					
Groups of polyphenolic compounds	Time of exposure to UV-C radiation duration of action of the abiotic stress factor (in min)					
	20		40		60	
	Components					
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Phenolic acids						
Caffeic acid and derivatives	1238.0±0.2*	36.0±0.1*	1359.0±0.3	45.0±0.0	1076.0±0.1*	34.0±0.1*
p-Coumaric acid and derivatives	94.0±0.0	1.8±0.0	95.0±0.1	2.3±0.0	78.0±0.2*	1.7±0.1*
Sum	1332.0±708.0	37.8±12.0*	1454.0±726.0	47.3±10.0	1154.0±854.0	35.7±12.0*
Flavon-3-ol and procyanidins						
Monomeric catechins	616.0±0.1*	17.0±0.0*	536.0±0.1*	13.0±0.0*	634.0±0.0	22.0±0.1
Polymeric procyanidins	229.0±0.0	53.0±0.0	230.0±0.1*	43.0±0.1*	290.0±0.2	60.0±0.1*
Sum	845.0±355.0*	70.0±0.5	766.0±240.0*	56.0±0.3*	924.0±263.0	82.0±0.3
Flavonols						
Quercetin derivatives	157.0±0.0	nd	154.0±0.4	nd	81.0±0.0*	nd
Isorhamnetin derivatives	425.0±0.2	nd	420.0±0.1*	nd	223.0±0.2*	nd
Kaempferol derivatives	79.0±0.1	nd	78.0±0.1*	nd	36.0±0.0*	nd
Sum	662.0±0.1*	nd	654.0±0.0*	nd	341.0±0.2*	nd
Total	2839.0±367.0*	107.8±0.5*	2874.0±255.0*	103.3±0.2*	2419.0±463.0*	117.7±0.0

*Statistically significant differences compared to the control at significance level $\alpha = 0.05$

Table 4 Influence of ultrasounds (US) on the content of polyphenolic compounds in pear fruits, mg/100 g dry matter ($n=3$)

Groups of	Ultrasound US					
Groups of polyphenolic compounds	Time of exposure to ultrasound US (min)duration of action of the abiotic stress factor in min					
	20		30		40	
	Components					
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Phenolic acids						
Caffeic acid and derivatives	1140.0±0.1*	48.0±0.1	1189.0±0.5*	49.0±0.1	1294.0±0.2	44.0±0.0
<i>p</i> -Coumaric acid and derivatives	77.0±0.3*	2.4±0.2	80.0±0.2*	2.3±0.0	92.0±0.0	2.3±0.0
Sum	1217.0±742.0	50.4±10.0	1269.0±651.0	51.3±10.0	1386.0±716.0	46.3±15.0
Flavon-3-ol and procyanidins						
Monomeric catechins	579.0±0.3*	25.0±0.1	620.0±0.3	37.0±0.0*	671.0±0.1	30.0±0.1*
Polymeric procyanidins	259.0±0.0	65.0±0.2*	309.0±0.1	77.0±0.2*	268.0±0.1	60.0±0.0
Sum	838.0±156.0*	90.0±0.5	929.0±257.0	114.0±0.5*	939.0±255.0	90.0±0.3
Flavonols						
Quercetin derivatives	114.0±0.0*	nd	90.0±0.1*	nd	147.0±0.0	nd
Isorhamnetin derivatives	320.0±0.2*	nd	249.0±0.1*	nd	376.0±0.1*	nd
Kaempferol derivatives	57.0±0.2*	nd	45.0±0.2*	nd	68.0±0.0*	nd
Sum	492.0±0.0*	nd	385.0±0.3*	nd	591.0±0.1*	nd
Total	2547.0±368.0*	140.4±0.1*	2583.0±355.0*	165.3±0.2*	2916.0±356.0	136.3±0.2

*Statistically significant differences compared to the control at significance level $\alpha=0.05$ **Table 5** Influence of high-frequency electromagnetic field (H-EMF) on the content of polyphenolic compounds in pear fruits, mg/100 g dry matter ($n=3$)

Groups of polyphenolic	High-frequency electromagnetic field (H-EMF)					
Groups of polyphenolic compounds	Time of exposure to hight-frequency electromagnetic field (H-EMF) (min) duration of action of the abiotic stress factor in min					
	0.5		2		5	
	Components					
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Phenolic acids						
Caffeic acid and derivatives	1091.0±4.2*	51.0±0.0	1057.0±3.5*	34.0±0.0*	1168.0±4.8	47.0±0.1
p-Coumaric acid and derivatives	84.0±0.6*	2.7±0.0*	76.0±0.6*	2.2±0.1	100.0±0.8	2.1±0.0
Sum	1175.0±2.1*	53.7±10.0	1133.0±1.89*	36.2±9.0*	1269.0±1.3	49.1±10.0
Flavon-3-ol and procyanidins						
Monomeric catechins	642.0±0.3	29.0±0.1	485.0±0.3*	15.0±0.0*	742.0±0.3	22.0±0.0
Polymeric procyanidins	271.0±0.5	69.0±0.1*	216.0±0.3*	47.0±0.3*	438.0±0.1*	62.0±0.0*
Sum	913.0±110.0	99.0±0.6*	701.0±187.0*	62.0±0.5*	1181.0±143.0*	84.0±0.3
Flavonols						
Quercetin derivatives	75.0±0.1*	nd	126.0±0.2*	nd	152.0±0.2	nd
Isorhamnetin derivatives	225.0±0.5*	nd	417.0±0.3*	nd	497.0±0.3	nd
Kaempferol derivatives	32.0±0.0*	nd	57.0±0.2*	nd	78.0±0.1*	nd
Sum	300.0±0.0*	nd	543.0±0.2*	nd	649.0±0.3	nd
Total sum	2388.0±2.36*	152.7±0.7*	2377.0±2.5*	98.2±0.6*	3099.0±1.3	133.1±0.3

*Statistically significant differences compared to the control at significance level $\alpha=0.05$

Fig. 1 The influence of abiotic stress factors (UV-C, H-EMF, L-EMF, US) on the content of phenolic acids in peel of pear fruits (1—cluster area –20.7% to control sample, 2—cluster area –13.8% to con-

trol sample, 3—cluster area statistically insignificant, 4—cluster area –6.2% to control sample)

Fig. 2 The influence of abiotic stress factors (UV-C, H-EMF, L-EMF, US) on the content of phenolic acids in flesh of pear fruits (1—cluster area statistically insignificant, 2—cluster area –20% to control sample)

the factors that decreased the content of phenolic acids by –6.2% on average.

Cluster area 3 involved the relation between the control sample and factor US-40, which did not result in a statistically significant change in the concentration of phenolic acids. In the case of flesh (Fig. 2), exposure to the same abiotic stress elicitors as in the skin contributed to the formation of two areas: the first one including stress elicitors

(US-20, US-30, UV-C-40, H-EMF-5, H-EMF-0.5) related to the control sample, which caused changes in the concentration of phenolic acid that were not statistically significant. Cluster area 2 consisted of stress factors (UV-C-20, H-EMF-2, UV-C-60, L-EMF-30, L-EMF-60, US-40) that resulted in an average decrease of the phenolic acid level in the flesh by –20%.

Fig. 3 The influence of abiotic stress factors (UV-C, H-EMF, L-EMF, US) on the content of flavan-3-ols and procyanidins in peel of pear fruits (1—cluster area statistically insignificant, 2—cluster area – 8% to control sample, 3—cluster area + 19% to control sample)

Fig. 4 The influence of abiotic stress factors (UV-C, H-EMF, L-EMF, US) on the content of flavan-3-ols and procyanidins in flesh of pear fruits (1—cluster area statistically insignificant, 2—cluster area

– 23% to control sample, 3—cluster area + 20% to control sample, 4—cluster area + 48% to control sample)

Flavan-3-ols and procyanidins

Changes in the concentration of flavan-3-ols and procyanidins in the skin and flesh of pear under the influence of stress elicitors are presented in Tables 2, 3, 4 and illustrated in Figs. 3, 4. The span of the determined flavan-3-ols and procyanidins in the skin ranged from 701 mg/100 g dry matter,

when using H-EMF for 2 min, to 1181.00 mg/100 g dry matter for the same factor used for 5 min, which corresponded to concentration changes from – 29% to + 20% as compared to the control sample.

Statistical processing of data spaces with the use of cluster analysis determined three groups of abiotic stress factors that influenced the variability of the content of flavan-3-ols

Fig. 5 The influence of abiotic stress factors (UV-C, H-EMF, L-EMF, US) on the content of flavonols in peel of pear fruits (1—cluster area statistically insignificant, 2—cluster area –43% to control sample)

and procyanidins in the skin of the fruit in question (Fig. 3). Cluster area 1 included abiotic stress factors (US-20, UV-C-40, H-EMF-2) and the control sample whose result differences were statistically insignificant. The aggregation of stress elicitors in cluster area 2 (UV-C-20, L-EMF-60, UV-C-60, US-30, H-EMF-0.5, US-40) resulted in an average decrease of the analysed compounds by –8% in comparison to the control sample, whereas the stress factors grouped in cluster area 3 (L-EMF-30 and H-EMF-5) increased the concentration of the analysed compound groups of flavan-3-ols and procyanidins by +19% on average.

The concentration of flavan-3-ols and procyanidins in pear flesh under the influence of abiotic stress factors is presented in Fig. 4. The stress elicitors caused a significant variability of the concentration of flavan-3-ols and procyanidins in the flesh of the fruit in question. The use of cluster analysis as a tool ordering the data area enabled us to separate four groups differing in terms of the influence of stress elicitors on the content of flavan-3-ols and procyanidins in the flesh of pears. The first area comprised of stress factors (UV-C-60, H-EMF-5, UV-C-20, L-EMF-2) that resulted in changes in the content of the analysed compounds those were statistically insignificant as compared to the control sample. Group 2 included factors (UV-C-40, H-EMF-2) that caused an average decrease of –23% in the concentration of flavan-3-ols and procyanidins.

The aggregation of data in groups 3 and 4 involved stress elicitors that increased the concentration of the analysed compounds.

Cluster 3 included the effects of low-frequency electromagnetic field (L-EMF-60), ultrasounds (US-20, US-40) and high-frequency electromagnetic field (H-EMF-0.5), which

caused an increase of the concentration level of flavan-3-ols and procyanidins by +20% as compared to the control sample. Single element group 4 included the stress factor (US-30) that increased the concentration of the analysed compounds by +48% on average. The observed changes of flavonol concentration applied only to pear skin, whereas the determined levels in the flesh were below detection limit. The concentration of flavonol in pear skins under the influence of abiotic stress factors is graphically presented in Fig. 5 and Tables 2, 3, 4, 5.

The variability of flavonol concentration in pear skins under the influence of stress elicitors ranged from 751.00 mg/100 g dry matter for the control sample to 300.00 mg/100 g dry matter for skins of fruit exposed to high-frequency electromagnetic field (H-EMF-0.5). In every case, the procedures resulted in a statistically significant decrease of the concentration of flavonol compounds in pear skins.

Discussion

The concentration changes of polyphenolic compounds were mostly caused by heat stress whose effects can be observed during exposure to electromagnetic field.

Vibrations of the electromagnetic field increase the temperature inside cells and activate antioxidant protection mechanisms. According to Shabrangi et al. [19], by changing the electromagnetic field power and exposure time, various biological results can be achieved. Low-frequency fields have a more destructive effect on mitochondria by disrupting calcium channels and thus, reducing the synthesis of

antioxidant compounds [20]. The effects of electromagnetic field also depend on the content of dry mass. According to Nabizadeh et al. [21], exposure of dry and soaked pumpkin seeds to electromagnetic field of 2 mT for 15 and 30 min resulted in changes of the determined parameters. Exposure to the field for 15 and 30 min contributed to a decrease in the content of protein and the activity of peroxidase and dismutase in both dry and soaked pumpkin seeds. In the case of catalase, its activity increased only in dry seeds exposed to electromagnetic field for 15 min.

Abiotic stress factors stimulate cells to produce ROS (reactive oxygen species) as well as ABA (abscisic acid), SA (salicylic acid), JA (jasmonic acid), ET (ethylene), which are then responsible for the synthesis of MAF kinases, which directly induce the synthesis of genes, MYC, MYB, NAC, ZF, HFS, responsible for initiating the synthesis pathways of secondary metabolites. A too intense stress signal leads to the generation of high concentration of kinases, thus damaging the cell's genetic material, which determines the synthesis of secondary metabolites [22, 23]. Stress elicitors initiate in plant cells the mechanisms responsible for modifying metabolic pathways towards increased synthesis of secondary metabolites whose main task is to scavenge free radicals [24, 25]. Plant metabolic response to stress conditions is a dynamic process corresponding to the intensity of the stress factor. Adaptation to stress conditions requires extra energy necessary to synthesise protein for antioxidant protection [26, 27]. Many authors [24, 28, 29] showed, that exposure to UVC radiation results in an increased synthesis of polyphenolic compounds, which is a defence response related to increased temperature in plant cells. An increase of temperature inside plant cells also occurs under the influence of waveform factors, i.e. appropriate stimulation with ultrasounds or electromagnetic radiation, leading to the initiation of antioxidant protection mechanisms [30–33]. Gill and Tuteja [24] showed that a number of flavonoid biosynthesis genes are induced in stress conditions, in particular, when exposed to UV radiation. The most thoroughly analysed abiotic stress factor has been the influence of UV radiation and ultrasounds on the amount of polyphenolic compounds in plants. A research conducted by Chang-Hong et al. [13] showed that after exposure to various doses of UV radiation, tomatoes stored for 35 days demonstrated an increase in the content of gallic acid by 21%, catechins by 26%, chlorogenic acid by 14%, caffeic acid by 37%, quercetin by 38% and p-coumaric acid by 36%. Exposure to ultrasounds causes cavitation in cell organelles, leading to increased permeability of cell membranes by activating calcium channels, which contributes to an increase of enzymatic activity, which probably stimulates an increase of the synthesis of secondary metabolites [34]. Wu and Lin [33] showed that a 2-min exposure to 38.5 Hz US (ultrasound) in a water bath resulted in an increase of the level of polyphenolic compounds in the in vitro cell culture of ginseng suspension and at the same time caused increased

activity of enzymes (PPO) and (PO) and decreased water content in cells. Similar results were arrived at by Santos et al. [18] who used 25 kHz ultrasounds for 30 min on freshly sliced mango and achieved a general increase in the polyphenol content by approximately 20%. In turn, Yu et al. [29] used 25 kHz 26 W ultrasounds on romaine lettuce leaves for 1, 2 and 3 min and observed the highest increase of phenolic compounds after 60 h of storage, by 22.5%, 16.3% and 17.9%, respectively. The variability of flavan-3-ols concentration in skins depended on the stress factor. The influence of abiotic stress factors, including ultrasounds, activates the synthesis of secondary metabolites (polyphenolic compounds). The impact, time and frequency are the factors modelling the intensity of enzyme synthesis of the flavonoid pathway. In the discussed case, the observed phenomenon presented in Table 3, related to an increase and then a slight decrease of the polyphenol content, can be a result of changes in the enzyme system of the flavonoid pathway. The direct cause can be the depletion of substrates for enzyme production, but due to insufficient research, further studies are required to establish the reason of such changes.

Conclusion

Pear fruit is valued for its high amount of polyphenolic compounds. The use of abiotic stress factors such as UVC radiation, low-frequency electromagnetic field (L-EMF), high-frequency electromagnetic field (H-EMF) and ultrasounds (US) for various periods resulted in differences in the content of phenolic acids, flavan-3-ols, and procyanidins, and flavonols. Changes in the concentration of phenolic acids in the skin and flesh of pears depended on the abiotic stress factor. The effects of abiotic stress factors differentiated the content of polyphenolic compounds in the skin and flesh of the fruit in question. The presence of flavonols was only observed in pear skin and the level of changes was similar to phenolic acids. The stress used in fractions of pear in all cases lowered the sum of polyphenolic compounds in the peel, while the effect of US noticed the increase in the total polyphenols content in the flesh of pears. The research results show that it is possible to produce juice with a higher content of polyphenolic compounds, because juice is obtained mainly from the flesh; however, there is a need for further research to confirm the observed tendencies in the changes of polyphenolic compounds in fractions of pears.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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