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Influence of Process Parameters on the Extraction of Flavanones from Mandarin Peel

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Abstract

Flavanones are an important group of flavonoids that are characteristic for citrus. In the present work isolation of flavanones from mandarin peel was performed by conventional extraction using water, ethanol, acetone and aqueous solutions of acetone and ethanol. The extracts were analysed on the content and composition of flavanones. Furthermore the DPPH radical scavenging activity of extracts was determined. Finally, the influence of extraction parameters (particle size, extraction temperature, extraction time, material to solvent ratio, number of extraction stages and type of solvent) on the yield and the efficiency of extraction were determined by Plackett-Burman experimental design.

The results showed that 70% aqueous solution of acetone was the most efficient solvent for isolation of flavanones from mandarin peel. The main flavanones present in the obtained extracts were hesperidin (HES) and narirutin (NRT). The number of extraction stages influenced the yield of extraction, type of solvent influenced the hesperidin extraction efficiency and particle size of material influenced the narirutin extraction efficiency.

Keywords: Conventional extraction, Mandarin peel, Flavanone, Hesperidin, Narirutin, Plackett-Burman experimental design

1. Introduction

Citrus fruits contain several important nutrients, such as vitamin C, dietary fibres, carotenoids and flavonoids. Therefore they are an important part of a healthy diet.[1] Citrus fruits such as oranges (*C. sinensis*), mandarins (*C. reticulata*), lemons (*C. lemon*) and grapefruits (*C. paradisi*) are also important for the production of fruit juices and concentrates which are mainly used in food industry for obtaining fruit drinks.[2] Large amounts of citrus peels are produced as residues, which can be a potential source of pectin and natural flavonoids.[3,4] Citrus fruits have specific and characteristic composition of flavonoids. The main group of flavonoids present in citrus are flavanones. They are present as diglycosides (7-O-glycosylflavanones) mainly in the white part of peel (albedo).[5] There are two different types of glycosides: neohesperidosides with disaccharide neohesperidose (rhamnosyl-α-1,2 glucose) condensed on basic flavanone structure; and rutinosides that contain disaccharide rutinose (rhamnosyl-α-1,6 glucose). Neohesperidoside flavanones, naringin, neohesperidin and neoeriocitrin, have bitter taste and are present mostly in grapefruits and bitter orange. Rutinoside flavanones, narirutin, hesperidin and didymin, are without taste and they are present in orange, lemon and mandarin.[1,6] Another important group of flavonoids present in citrus are flavones. They are present as polymethoxylated derivates located mainly in the external coloured part of citrus peel (flavedo). Two often present polymethoxylated flavones in citrus are nobiletin and tan-

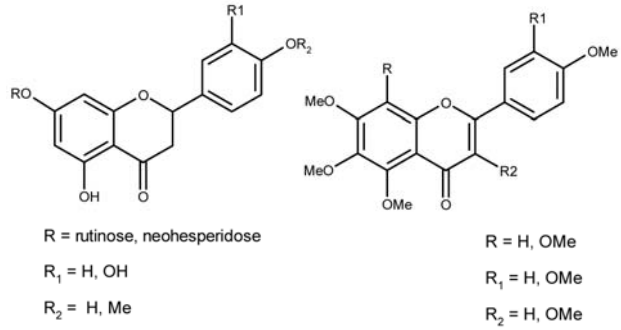

Figure 1: Chemical structure of flavanones (left) and polymethoxylated flavones (right).[5] Group R represent disaccharides rutinose (rhamnosyl-α-1,6 glucose) or neohesperidose (rhamnosyl-α-1,2 glucose). (Me – methyl group, OMe – methoxy group).

geretin. Figure 1 presents the basic structure of the main citrus flavonoids; flavanones (left) and polymethoxylated flavones (right).[5,6] Studies have shown that citrus flavonoids play an important role in the prevention of degenerative and infectious diseases. Due to their anticarcinogenic, antiatherogenic, antimicrobial and anti-inflammatory properties, flavanones and polymethoxylated flavones are interesting for pharmaceutical and food industry.[1,7,8,9]

Conventional extraction is the most commonly used method for isolation of valuable compounds from natural materials. Due to the simple procedure, it is used in basic investigations, to determine the optimal conditions for isolation of valuable compounds.[10,11] In the present work isolation of flavanones from mandarin peels was performed by conventional extraction. Mandarin peels contain high amount of hesperidin (HES) and some narirutin (NRT) (Figure 2). Didymin (DID) and some polymethoxylated flavones (PMF) such as tangeretin (TAN) and nobiletin (NOB) are also present but in smaller amount.[6,12,13]

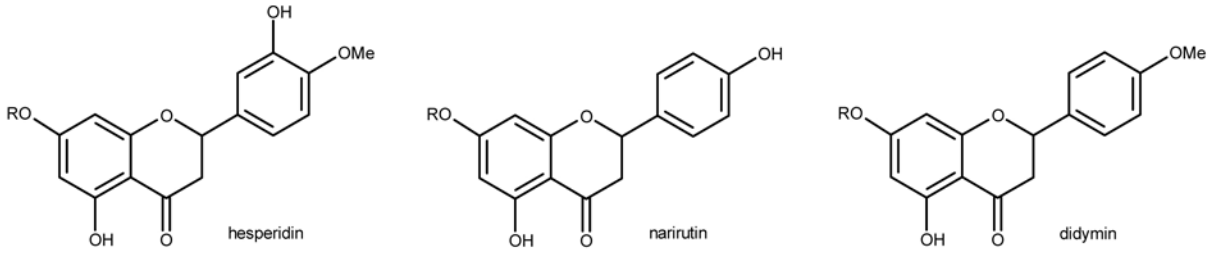

Figure 2: Chemical structure of flavanones hesperidin, narirutin and didymin. Group R represents disaccharide rutinose (rhamnosyl-α-1,6 glucose), Me represents methyl group.

To determine the optimal solvent, solutions of different concentration of acetone and ethanol in water were tested. Ethanol and acetone are both approved for use in food industry as volatile solvents for extraction of valuable compounds. Because of the low solubility of HES in water and polar protic solvents such as ethanol,[14,15,16] acetone was used as polar – aprotic solvent. No information about the use of acetone solutions for the isolation of flavanones from citrus peels was found by performing literature review. For extraction of flavanone HES from mandarin peels usually methanol and ethanol and their water solutions (70, 80%) were used.[3–5,10,17,18]

In the present work optimal extraction conditions for efficient isolation of flavanones from mandarin peel were determined. Influence of extraction parameters on the yield and efficiency of extraction were determined by Plackett-Burman (PB) experimental design (ED). PB experimental designs are factorial designs that examine up to *N*–1 factors in *N* experiments and are usually performed to study the effect of several factors or process parameters (e.g. solvent composition) on one or more responses (yield of extraction, efficiency, etc.).[19,20] PB design is often used for screening process parameters and for testing the robustness of analytical method validation.[20,21,22]

2. Materials and Methods

2. 1. Chemicals

Hesperidin (Cat.No. 52040) and didymin (Cat.No. 36814) were purchased from Fluka (Germany); narirutin (Cat.No. 1130 S), tangeretin (Cat.No. 1033) and nobiletin (Cat.No. 1348 S) were purchased from Extrasynthese (France). All standards were HPLC grade. Acetone, ethanol (96%), methanol and anhydrous acetic acid were provided by Merck (Germany). Milli Q water produced by Milli-Q plus apparatus was used for HPLC analysis.

2. 2. Preparation of Material

Mandarin peel was collected from fruits bought at the local supermarket in season 2010. Peels were dried by hot air flow (40–50 °C) and stored in a dark and cool place. Dried peels were ground before use. Average particle size after grinding was 0.65 mm. Moisture content was determined by thermo-gravimetric balance (Mettler Toledo HB43–S Halogen) and was in the range between 7.7 and 8.6% (w/w).

2. 3. Conventional Extraction

2. 3. 1. Determination of Optimal Solvent for Flavanone Isolation

Conventional extraction was performed using aqueous solutions of acetone and ethanol with different composition: 10, 35, 50, 70, 85% (v/v) and pure solvents acetone, water and 96% ethanol. Extractions for each solvent composition were performed in triplicate. 10 g of dry grinded mandarin peel were weighed in a glass flask and 200 mL of solvent were added. After 2 hours of mixing at room temperature, the solution was separated by vacuum filtration, the residual material was returned in the flask and the procedure was repeated with fresh solvent two more times. Extract solutions we collected in each step separately and the solvent was then removed by evaporation. Prepared extracts were saved in a cool place before

analysis by HPLC. Yield of extraction (y) was calculated by eq. 1

$$y = \frac{m_{extract}}{m_{material}} \cdot 100 \ [\%] \quad (1)$$

where $m_{extract}$ is the mass of dry extract (in g) and $m_{material}$ is the mass of dry mandarin peels used for extraction (in g).

2. 4. HPLC Analysis of Flavonoids

The composition and the content of flavonoids were determined by high performance liquid chromatography (HPLC) method. For HPLC analysis of flavonoids Agilent 1220 HPLC system with detector DAD and column Chromsep SS C-18 250 × 4.6 mm Microsorb 100 stationary phase with 5 μm particle size was used. The mobile phase consisted of two solvents: A – methanol, and B – 2% (v/v) acetic acid in Milli-Q water. The method started with linear gradient from 35% A to 70% A in 90 min and finished with isocratic for 10 min at 70% A. The flow rate was 0.85 mL/min and detection was performed at 282 nm and 330 nm. The standard solutions were prepared by dissolving standards in methanol. Extract solutions were prepared by dissolving 10 mg of extract in 10 mL of methanol, sonicated and filtrated before analysis. The quantification of flavanones HES and NRT was made with calibration curves obtained with HES and NRT standards. Identification of other flavanones (DID) and polymethoxylated flavones (NOB, TAN) was performed by comparing retention times of HPLC analysis of components and standards.

2. 5. Determination of Antiradical Activity of Extracts (DPPH Radical–Scavenging)

Antiradical activity of mandarin peel extracts was determined against stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. DPPH radical–scavenging activity of obtained extracts was determined spectrophotometrically, as described by Miliauskas et al.[23] and Majhenič et al.[24] The radical–scavenging activity was expressed as % inhibition of DPPH free radical.

2. 6. Determination of the Influence of Different Parameters on Extraction

To determine the influence of different parameters on extraction yield and efficiency two level Plackett-Burman experimental design (PB ED) was used. Parameters of extraction – factors used in experimental design were: particle size (X_1), extraction temperature (X_2), extraction time (X_3), material to solvent ratio (X_4), number of extraction stages (X_5), type of solvent (X_6). The proper Plackett-Burman ED for our system was ED with 8 experiments for 7 parameters. To assure confidence of PB ED, a 7th factor was introduced as blank factor (X_7), which has no influence on the tested system. Factors and their values at separate levels are presented in Table 1. The experiments performed by PB ED are presented in Table 2.

Table 1: Parameters of extraction (factors) and levels used in Plackett-Burman experimental design.

N. of Factor	Parameter of extraction	Levels of factor	
		+	–
1	Particle size range / μm	500–800	125–250
2	Extraction temperature / °C	60	20
3	Extraction time / min	120	60
4	R(material/solvent) / g/mL	1:50	1:20
5	No. of stages of extraction	3	1
6	Solvent	70% acetone	70% ethanol

Table 2: Plackett-Burman experimental design.

Experiment	Level of factor						
	X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	+	+	+	–	+	–	–
2	–	+	+	+	–	+	–
3	–	–	+	+	+	–	+
4	+	–	–	+	+	+	–
5	–	+	–	–	+	+	+
6	+	–	+	–	–	+	+
7	+	+	–	+	–	–	+
8	–	–	–	–	–	–	–

Factors influence was determined by statistic Student t-test (Microsoft Excel software was used). Test of influence of factor X_i on response Y determined for 8 experiments (N = 8) was calculated by the following equation:

$$t_{calc.} = \frac{\left|Influence\ X_i\right|}{\sqrt{S^2_{i\ pooled}\frac{4}{N}}} = \frac{\left|\overline{Y}^{i(+)} - \overline{Y}^{i(-)}\right|\sqrt{2}}{S_{i\ pooled}} \quad (2)$$

where $\overline{Y}^{i(+)}$ is average of responses on (+) level of factor X_i, $\overline{Y}^{i(-)}$ is average of responses on (–) level of factor X_i, $S_{i\ pooled}$ is pooled variance of influence of factor X_i on response Y, calculated by equation:

$$S^2_{i\ pooled} = \frac{\left(S_i^{(+)}\right)^2 + \left(S_i^{(-)}\right)^2}{2} \quad (3)$$

where $(S_i^{(+)})^2$ is variance of influence of factor X_i for level (+) and $(S_i^{(-)})^2$ is variance of influence of factor X_i for level (–). Variances of influence for factor X_i on both levels were calculated by equations

$$\left(S_i^{(+)}\right)^2 = \frac{\sum_{j=1}^{N/2}\left(Y_j^{i(+)} - \overline{Y}^{i(+)}\right)^2}{\frac{N}{2}-1} \quad (4)$$

$$\left(S_i^{(-)}\right)^2 = \frac{\sum_{j=1}^{N/2}\left(Y_j^{i(-)} - \overline{Y}^{i(-)}\right)^2}{\frac{N}{2}-1} \quad (5)$$

where $Y_j^{i(+)}$ is value of j response on (+) level of factor X_i and $Y_j^{i(-)}$ is value of j response on (+) level of factor X_i. Factor X_i has influence on response Y_j, if

$$t_{calc.} \geq t_{tab.}(\alpha, N-2) \quad (6)$$

where $t_{calc.}$ is calculated t-value from experimental data, $t_{tab.}$ is t-value read from statistic tables at probability α and degree of freedom $N - 2$. α is the probability that all responses are out of the confidence interval and N is the number of measured responses (number of experiments).[25]

3. Results and Discussion

3. 1. Yield of Extraction

Extractions of mandarin peels were performed by conventional procedure in tree stages. To simplify the presentation of results and discussion abbreviated labelling of extracts is introduced for different stages of extraction: E1 – 1st stage of extraction, E2 – 2nd stage of extraction and E3 – 3rd stage of extraction. Different compositions of acetone or ethanol water solutions were used as extraction solvents. Extractions were performed in triplicate and extraction yields are presented in Figures 3 and 4.

Figure 3 presents extraction yield obtained in separate stages of extraction depending on solvent composition. The main amount of extract was obtained in the first stage of extraction. In the case of acetone extraction yields increased with increasing water content in the solvent solution. The yield varied between 3.3% (100% acetone) and 43.7% (10% aqueous acetone solution). High increase of extraction yield with small addition of water to acetone is a consequence of solvent properties. Acetone is polar – aprotic solvent in comparison to ethanol that has similar properties to water, both being protic polar solvents. Because of that composition of acetone solution has higher influence on extraction yield than composition of ethanol solution.

In the case of ethanol solutions extraction yield in E1 was more constant and varied between 30.9% and 37.7%, except for 96% ethanol solution (16.4%). The highest yield of extraction in E1 was determined for pure water, 45.9%. Extraction yields in E2 were much lower and varied from 6.5 to 11.0% for both systems, except for 10% solution of acetone (18.0%) and pure acetone (2.2%). In E3 yields varied in the range from 1.5–3.0%. From the presented results it can be concluded that composition of solvent influences the extraction yield in E1.

Influence of solvent composition on total extraction yields for both solvents is presented in Figure 4. It can be concluded that in the case of acetone total yield of extraction increases with decreasing content of organic solvent while in the case of ethanol total extraction yield increases with decreasing concentration from 96 to 70% and stays approximately constant whit further decreasing of solvent concentration. The increase of extraction yield with increasing water content in solvent is probably due to higher content of water soluble compounds such as pectin in extracts.[17] The highest total yield of extraction, 64.2 ± 1.6%, was determined for 10% aq. acetone solution.

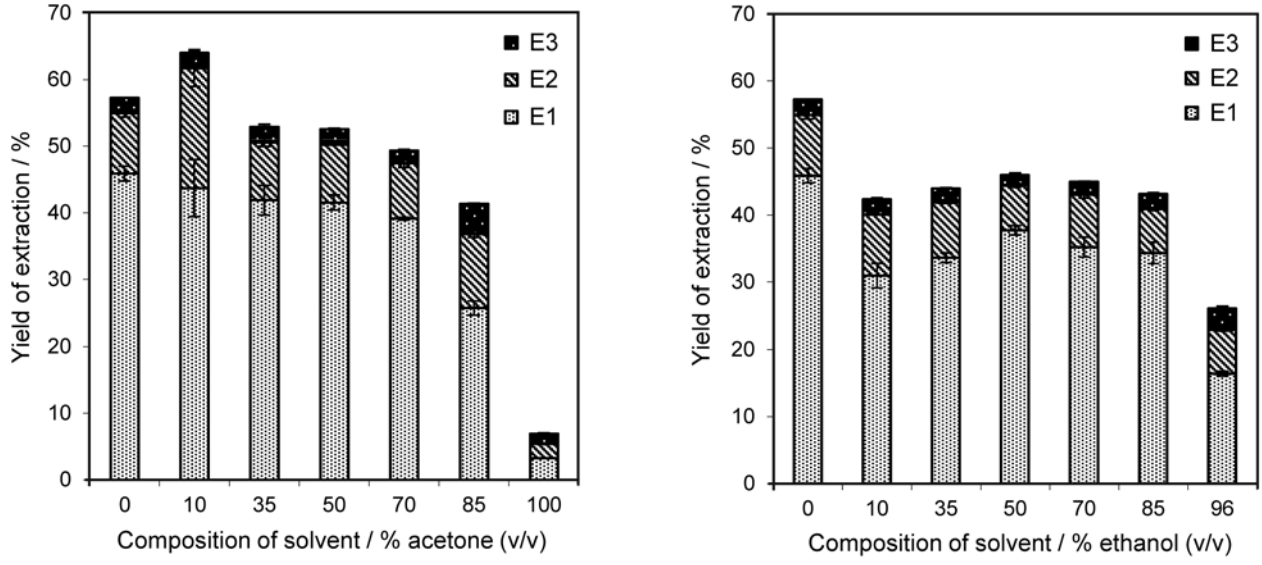

Figure 3: Influence of solvent composition on extraction yield: aqueous solutions of acetone (left), aqueous solutions of ethanol (right).

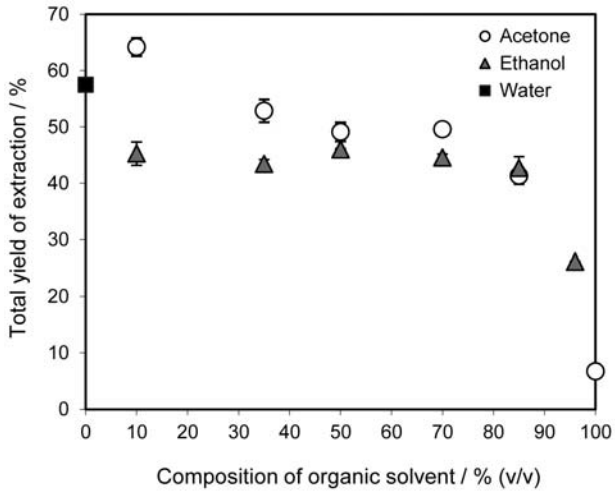

Figure 4: Influence of organic solvent composition on total extraction yield.

By increasing the content of water, jelly like extracts was obtained, and removing of solvent by evaporation becomes difficult.

3. 2. Composition and the Content of Flavonoids

Composition of flavanones in mandarin peel extracts was determined by HPLC method and was compared with the literature data. The main flavanones present in mandarin peel were hesperidin (HES) and narirutin (NRT). Didymin (DID) was also identified. Polymethoxylated flavones (PMF) were also detected and identified but they were not quantitatively determined. Figure 5 presents the chromatogram of HPLC analysis of extract. Tables 3 and 4 present the amounts of HES and NRT extracted with different aqueous solutions of acetone and ethanol.

In Table 3 the amount of HES extracted from 1 g of material – dry mandarin peels, is presented. The highest amount of HES was obtained in the 1st stage of extraction, for both solvent solutions. Generally higher HES amounts were obtained by using acetone solutions in comparison to ethanol solutions in all three stages of extraction. By extraction with acetone solutions between 6 and 32 mg HES/g material were isolated from 1 g of dry mandarin peel. The highest amount, 32.87 mg HES/g material, was obtained with 70% acetone solution. High amount, 31.41 mg HES/g material, was obtained also with 85% acetone solution. By

Table 3: Amount of HES extracted by different acetone and ethanol solution expressed in mg HES/g material.

Composition of solvent / (v/v) %	Acetone			Ethanol		
	E1	E2	E3	E1	E2	E3
10	6.45 ± 0.93	4.38 ± 0.27	2.38 ± 0.64	4.76 ± 0.40	3.52 ± 0.69	1.92 ± 0.19
35	16.43 ± 2.03	6.22 ± 1.51	3.32 ± 0.45	7.04 ± 0.85	3.28 ± 0.66	2.45 ± 0.30
50	23.19 ± 1.62	5.63 ± 0.99	4.47 ± 1.02	12.63 ± 0.45	3.16 ± 0.30	2.74 ± 0.18
70	32.87 ± 0.86	5.31 ± 0.66	2.77 ± 0.74	16.13 ± 0.35	3.59 ± 0.16	2.86 ± 0.89
85	31.41 ± 0.40	5.35 ± 1.09	2.47 ± 0.21	14.01 ± 1.83	2.62 ± 0.27	1.68 ± 0.19
100	14.24 ± 0.29	5.64 ± 0.36	2.98 ± 0.80	* 9.36 ± 0.25	*3.14 ± 0.28	*1.85 ± 0.26

* 96% ethanol was used instead of 100% absolute ethanol solution.

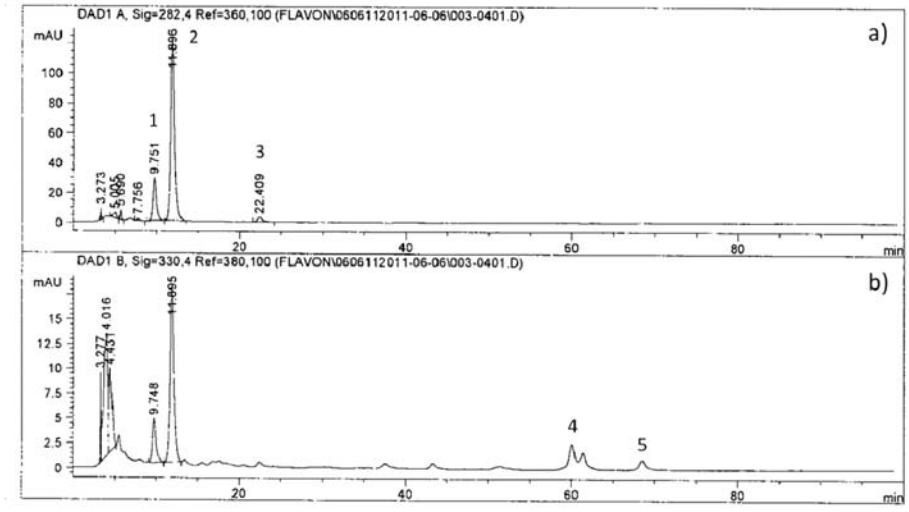

Figure 5: Chromatogram of HPLC analysis of mandarin peel extracts: a) 282 nm for determination of flavanones (0–50 min) and b) 330 nm for determination of polymethoxylated flavones (50–100 min). Present flavanones: 1 – narirutin (NRT); 2 – hesperidin (HES); 3 – didymin (DID); 4 – nobiletin (NOB) and 5 – tangeretin (TAN).

Table 4: Amount of NRT extracted by different acetone and ethanol solution expressed in mg NRT/g material.

Composition of solvent / (v/v) %	Acetone E1	Acetone E2	Acetone E3	Ethanol E1	Ethanol E2	Ethanol E3
10	2.73 ± 1.17	1.25 ± 0.11	0.38 ± 0.03	2.49 ± 0.78	1.44 ± 0.46	0.40 ± 0.06
35	3.57 ± 1.10	1.20 ± 0.16	0.40 ± 0.13	3.60 ± 1.43	1.36 ± 0.30	0.35 ± 0.08
50	4.54 ± 1.44	1.00 ± 0.17	0.37 ± 0.05	4.49 ± 1.98	1.02 ± 0.02	0.33 ± 0.01
70	6.26 ± 0.42	0.95 ± 0.08	0.28 ± 0.08	6.27 ± 0.13	1.17 ± 0.05	0.36 ± 0.06
85	5.88 ± 0.90	1.17 ± 0.06	0.38 ± 0.03	5.91 ± 0.45	0.92 ± 0.07	0.31 ± 0.05
100	2.15 ± 0.12	0.76 ± 0.08	0.36 ± 0.07	*2.76 ± 0.09	*0.95 ± 0.15	*0.45 ± 0.06

* 96% ethanol was used instead of 100% absolute ethanol solution.

extraction with ethanol solutions lower HES amount was achieved (7–22 mg HES/g material). The best ethanol solution for effective isolation of HES was 70% solution where 16.13 mg HES/g material were obtained in E1.

Results show also that with acetone solutions (70 and 85% solution) high total amount of HES is obtained, 42.46 and 40.72 mg HES/g material, respectively. Total amount of HES extracted with ethanol solutions was generally lower; the highest was 22.58 mg HES/g material for 70% ethanol solution.

Table 4 presents NRT amount obtained by using different acetone and ethanol solutions. As already noted, E1 was the most efficient for NRT isolation also. In comparison to HES, amount of extracted NRT was much lower and similar for both solvent systems. In E1 between 2 and 6 mg NRT/g material were obtained.

Total amount of extracted NRT with ethanol solutions is somewhat higher than with acetone solutions. The highest total amount of NRT was obtained with 70% ethanol solution, 7.98 mg NRT/g material. With 50, 70 and 85% ethanol solution similar total amounts of NRT were obtained (7–8 mg NRT/g material).

Figure 6 presents the results of total flavanones amount extracted with acetone and ethanol solutions. For both solvents the total amount was strongly dependent on solvent composition. It can be concluded that acetone solution especially 70, 85 and 50% are very good solvents for successful isolation of flavanones; the total amount was 50.52, 48.48 and 41.08 mg of flavanones per g of mandarin peel, respectively. Ethanol solutions were less efficient for flavanones isolation. The best results were obtained with 70% ethanol solution, where 30.38 mg flavanones were extracted per g of material. Similar total amount of flavanones was obtained with 35% acetone solution. Pure solvents acetone, ethanol and water were also less effective solvents. With 100% acetone 26.68 mg of flavanones were extracted, while with 96% ethanol and water only 17.79 mg and 15.39 mg of flavanones per g of material were obtained, respectively.

3. 3. DPPH Radical–Scavenging Activity of Extracts

Table 5 presents DPPH radical–scavenging activity

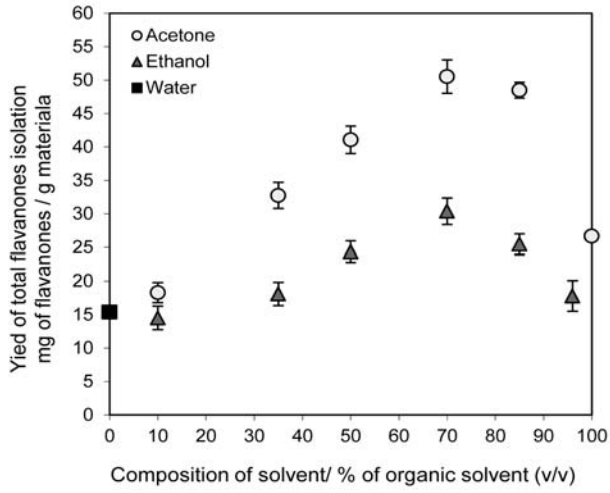

Figure 6: The yield of total flavanones (HES and NRT) isolation depending on solvent composition: aqueous solutions of acetone and ethanol.

Table 5: Antiradical activity of extracts obtained by different acetone and ethanol solutions expressed as % of DPPH radical inhibition.

Composition of solvent / (v/v) %	Acetone E1	Acetone E2	Acetone E3	Ethanol E1	Ethanol E2	Ethanol E3
10	1.51 ± 0.86	3.76 ± 0.08	4.49 ± 0.21	5.04 ± 0.06	5.62 ± 0.15	5.30 ± 0.16
35	4.36 ± 0.45	5.06 ± 0.30	0.98 ± 0.49	4.62 ± 0.16	4.86 ± 0.24	4.71 ± 0.21
50	6.59 ± 0.71	6.11 ± 0.89	3.78 ± 0.83	7.32 ± 0.47	9.17 ± 0.50	5.21 ± 0.71
70	5.60 ± 0.42	6.94 ± 0.32	10.77 ± 0.06	9.66 ± 0.76	9.68 ± 0.24	10.17 ± 0.83
85	4.76 ± 0.02	3.87 ± 0.77	4.47 ± 0.41	3.74 ± 0.38	3.74 ± 0.37	4.77 ± 0.25
100	7.30 ± 0.25	4.71 ± 0.26	1.64 ± 0.59	*4.35 ± 0.17	*4.07 ± 0.31	*3.99 ± 0.42

* 96% ethanol was used instead of 100% absolute ethanol solution.

of extracts obtained by different aqueous acetone and ethanol solutions. The highest radical–scavenging activity, 10.77% inhibition of DPPH radical, was determined for 70% acetone extract, obtained in E3. Similar activity, 10.17% inhibition was determined for 70% ethanol obtained from E3. The range of radical–scavenging activity of acetone solution extracts was between 0.89 and 10.77%, while ethanol extracts show similar activity, between 3.74 and 10.17% inhibition of DPPH radical.

Table 6: Plackett-Burman experimental design: Results of extraction experiments.

Experiment	Extraction yield	Amount of extracted flavanone mg/g material		
	%	HES	NRT	Total
1	50.6	31.02	5.89	36.91
2	46.8	54.25	10.11	64.36
3	49.9	32.90	7.87	40.78
4	53.6	41.06	6.67	47.72
5	49.9	58.12	10.45	68.57
6	47.2	31.95	5.90	37.86
7	46.7	26.28	6.08	32.35
8	39.5	16.61	7.81	24.41

3. 4. Influence of Operating Parameters on Extraction by Plackett-Burman Experimental Design

Results of extraction experiments designed by Plackett-Burman experimental design are presented in Table 6. The highest yield of extraction, 53.6%, was obtained in experiment 4 (particle size: 500–800 μm, 20 °C, 60 min, ratio material/solvent 1:50, 3 stages of extraction and 70% acetone solution). The highest amount of the main flavanones HES and NRT was obtained in experiment 5 (particle size: 500–800 μm, 20 °C, 60 min, ratio material/solvent 1:50, 3 stages of extraction and 70% acetone solution).

Table 7 presents the factors' influence on extraction yield calculated by statistic *t*-test. To confirm the influence of a single factor on extraction yield $t_{calc.}$ was calculated and compared to t_{tab} value (t_{tab} (0.05, 5) = 2.571)) obtained from Excel *t*-table at 95% confidence and 6 degrees of freedom. Only factor X_5 had higher $t_{calc.}$ value then t_{tab} value (2.908 > 2.571), confirming that the number of extraction stages influences the extraction yield. Higher number of extraction stages results in higher yield of extraction.

Tables 8 and 9 present calculations of factors influence on the amount of extracted HES and NRT. Only factor X_6 (solvent) influences the amount of extracted HES (2.789 > 2.571). For effective isolation of HES it was important to determine whether 70% acetone or 70% ethanol solution should be used as solvent. That confirms the dependence of extracted HES amount on solvent composition. The amount of extracted of NRT was dependent on particle size (X_1, 4.006 > 2. 571). Because concentration of NRT in material was low, extraction was controlled mostly by diffusion of NRT from material to solvent.

4. Conclusion

Isolation of flavanones from mandarin peels was performed by conventional extraction. Using different aqueous solutions of acetone and ethanol, 70% aqueous solution of acetone was determined as the most efficient solvent for extraction and isolation of flavanones from mandarin

Table 7: Influence of factors on extraction yield.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
$\overline{Y}^{i(+)}$	49.510	48.484	48.608	49.245	50.994	49.369	48.416
$\overline{Y}^{i(-)}$	46.526	47.552	47.427	46.791	45.042	46.666	47.620
$\lvert\overline{Y}^{i(+)} - \overline{Y}^{i(-)}\rvert$	2.984	0.932	1.181	2.454	5.952	2.703	0.796
$(S_i^{(+)})^2$	10.448	4.155	3.619	10.697	3.140	9.909	2.979
$(S_i^{(-)})^2$	23.991	35.641	35.826	25.662	13.614	25.594	36.973
$S_{i\ pooled}$	4.150	4.461	4.441	4.264	2.894	4.213	4.469
$t_{calc.}$	1.017	0.295	0.376	0.814	**2.908**	0.907	0.252

Table 8: Influence of factors on the amount of extracted HES.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
$\overline{Y}^{i(+)}$	32.577	42.416	37.531	38.622	40.774	46.346	37.313
$\overline{Y}^{i(-)}$	40.469	30.630	35.515	34.424	32.272	26.701	35.733
$\lvert\overline{Y}^{i(+)} - \overline{Y}^{i(-)}\rvert$	7.892	11.786	2.016	4.198	8.502	19.645	1.580
$(S_i^{(+)})^2$	38.148	259.002	124.831	145.104	152.694	145.382	200.948
$(S_i^{(-)})^2$	376.067	104.123	328.194	298.884	254.849	53.061	253.123
$S_{i\ pooled}$	14.391	13.475	15.050	14.899	14.275	9.961	15.068
$t_{calc.}$	0.776	1.237	0.189	0.398	0.842	**2.789**	0.148

Table 9: Influence of factors on the amount of extracted NRT.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
$\overline{Y}^{i(+)}$	6.135	8.132	7.444	7.681	7.722	8.282	7.578
$\overline{Y}^{i(-)}$	9.061	7.064	7.751	7.515	7.474	6.913	7.618
$\lvert\overline{Y}^{i(+)} - \overline{Y}^{i(-)}\rvert$	2.926	1.068	0.307	0.166	0.247	1.369	0.041
$(S_i^{(+)})^2$	0.132	6.175	4.016	3.173	3.983	5.435	4.470
$(S_i^{(-)})^2$	2.001	0.905	3.762	4.650	3.817	1.156	3.370
$S_{i\ pooled}$	1.033	1.881	1.972	1.978	1.975	1.815	1.980
$t_{calc.}$	**4.006**	0.803	0.220	0.119	0.177	1.066	0.029

peel. The main flavanones present in obtained extracts were HES and NRT. DID was also identified but not quantified due to low concentration. By Plackett-Burman experimental design it was determined that the number of extraction stages influences the yield of extraction, type of solvent influences the amount of extracted HES and particle size of material influences the amount of extracted NRT.

5. References

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Povzetek

Flavanoni so pomembna skupina flavonoidov, ki so karakteristični za citruse. V naših raziskavah smo s konvencionalno ekstrakcijo izvedli izolacijo flavanonov iz lupin mandarin, pri čemer smo kot topilo uporabili vodo, aceton, etanol ter vodne raztopine acetona in etanola. Dobljene ekstrakte smo analizirali in določili sestavo in vsebnost flavanonov ter določili njihovo delovanje na stabilni DPPH radikal. V nadaljevanju smo s pomočjo Plackett-Burman-ovega eksperimentalnega načrta določili tudi vpliv ekstrakcijskih parametrov (velikost delcev, temperatura in čas ekstrakcije, razmerje material-topilo, število stopenj ekstrakcije ter vrsta topila) na učinkovitost ekstrakcije.

Rezultati so pokazali, da je najbolj učinkovito topilo za izolacijo flavonoidov iz lupin mandarin 70% vodna raztopina acetona. Glavna flavanona, prisotna v ekstraktih, sta bila hesperidin in narirutin. Na izkoristek ekstrakcije vpliva število stopenj ekstrakcije, na učinkovitost ekstrakcije hesperidina vrsta topila, medtem ko ima velikost delcev materiala pomemben vpliv na učinkovitost ekstrakcije narirutina.