ABSTRACT

The main waste of the citrus fruits after processing is the citrus peel. The extraction of valuable components from mandarin peels with ethanol-in-water solutions was investigated. The effect of the operational conditions on the total extracted amount, the total polyphenols content and antioxidant activity was studied. The conditions varied were: the ethanol concentration, the particle size and the temperature. Taking into account the thermolability of the polyphenols, vitamin C, etc., the influence of the drying temperature on the properties of extracts after re-dissolution was studied. It was found that the increase in drying temperature leads to decrease in TPPC in the extracts after re-dissolution. The worst results were obtained for drying temperature of 60°C – total polyphenols contents after drying and re-dissolution decreased almost twice (2.22 times).

The comparison in TPPC and AOA of the mandarin peels’ extracts with other citrus peels shows that the source studied is a perspective for possible use in food and cosmetic industries due to the valuable components in it.

Keywords: mandarin peels, extraction, total polyphenols contents, antioxidant activity.

INTRODUCTION

The citrus production is estimated at 80 million tones per year [1, 2], making it an important source for useful to human health components. The main waste of the citrus fruits after processing is the citrus peel. In order to valorize these wastes recently numerous studies were published [1-5]. They are dedicated to a potential use of these peels as a source for natural antioxidants. Citrus processing byproducts are a potential source of natural flavonoids: citrus peels contain a high concentration of phenolic compounds [4-7]. Flavonoids are spread elsewhere in the plant kingdom, but there are several compounds (e.g., flavanones, flavanone glycosides and polymethoxylated flavones) unique to citrus, which are relatively rare in other plants [7]. Epidemiological studies have suggested the beneficial effects of citrus fruits (rich in flavanones) against many degenerative diseases like cardiovascular diseases and some cancers [8]. As it was underlined, citrus by-products are a good source of phenolic compounds, especially the characteristic flavanone glycosides which mainly include naringin, hesperidin, narirutin, and neohesperidin. Their extraction from citrus peels has attracted considerable scientific interest to use them as natural antioxidants mainly in foods to prevent the rancidity and oxidation of lipids [8-10]. One of the main purposes of these investigations is the study of the possibility to replace synthetic addi-
tives as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which might be liver-damaging, carcinogenic with natural low-cost antioxidants extracted from citrus by-products [8].

The aim of this paper is to study the extraction of polyphenolic compounds from mandarin peels, to determine the antioxidant activity of the extracts and to find the effect of the operational conditions on the final extract properties.

EXPERIMENTAL

Materials and methods

Mandarins of Clementine variety bought in local market were used in this study. They were peeled and dried at room temperature and ambient humidity. Thus their humidity decreased from 85 % to about 10 %.

After drying the mandarin peels were grinded and sieved. The following fractions of the particles were used:

- \( d < 1 \text{ mm} \)
- \( 1 < d < 2 \text{ mm} \)
- \( 2 < d < 2.5 \text{ mm} \)
- \( d > 2.5 \text{ mm} \)

For the extractions ethanol-in-water solutions with concentrations 20, 50 and 70 vol % were used. The ethanol was 96 % analytical grade supplied by Valerus (Bulgaria). Solid-to-liquid ratio was 0.1 (2g solid in 20 cm\(^3\) solution). The extraction kinetics was studied on laboratory shaker, the samples withdrawn in the time interval between 3 min and 80 min. It was found that after this time the samples mass almost did not changed. The kinetics was determined gravimetrically in the solid and in the liquid phase. The samples were dried in drying oven Diterm at 50°C until reaching a constant mass, and then were weighted. A laboratory analytic balance Sartorius analytic, with 0.1mg accuracy was used. In Fig. 1 typical kinetic curves for the particles with \( d < 1 \text{ mm} \) and 50 % ethanol-in-water solution are presented. It is obvious, that the plateau of the total extracted amount is reached between the 60th and the 80th min of the extraction process.

Total polyphenols contents

Total polyphenols content (TPC) is representative for rough determination of the antioxidant capacity. As the mean molecular mass of polyphenols mixture is unknown, their global action is expressed as gallic acid equivalent (TPCGAE). A photometric method, based on colour reaction of phenolic compounds with Folin-Ciocalteu reagent is largely used for determination of total polyphenolic content because of its simplicity and reliability. This was the reason to use it in this study.

Folin-Ciocalteau reactive: (2N solution), NaCO \(_3\) (supplied by Sigma) were used. The total polyphenols contents were determined using UV-VIS spectrophotometer (SPEKOL 11) at 765 nm wave length.

The total polyphenols contents \( \text{C}_{\text{pph}} \) in the peels was calculated using the expression:

\[
\text{C}_{\text{pph}} = \frac{(\text{C}_{\text{ga}} \times \text{Ve})}{\text{M}} \times 100, \%
\]

here: \( \text{C}_{\text{ga}} \) – polyphenols concentration in the liquid extract, found through the calibration curve equation \( \text{A}=\text{f} \left( \text{C}_{\text{ga}}\right), \text{g/l}\); \( \text{Ve} \) – volume of the extracting solution used during the extraction, l; \( \text{M} \) – solid sample mass, g.

Antioxidant activity determination

For determination of the antioxidant capacity of the extracts obtained the DPPH\(^*\) method was used [12]. It is based on the discoloration reaction between nitrogen electron (from DPPH\(^*\)) and hydrogen atom of hydroxyl group (from antioxidant substance). An inconvenience of this method is its light sensitivity. For this reason, the reaction has to be carried out in dark and in non-alkaline medium. In our study this method was applied with the use of spectrophotometer (SPEKOL 11) at 517 nm wave length.
length. We have used IC 50 % parameter as comparative variable. It represents the amount of antioxidant sample, which inhibits 50 % of the initial concentration of DPPH. The blank for comparison is methanol.

Reactives: DPPH (2,2 - Diphenil-1-picrilhydrazil (free radical), 95 %, packed under argon 250 mg C₁₆H₁₂N₅O₆ Alfa Aesar), ethanol, methanol.

The free radicals inhibition by DPPH is calculated using the relationship for the antiradical activity:

\[ I \% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \]  

(2)

Here: Ablank is the light absorbance of the blanc sample, containing all reactives without the plant extract (i.e. 1 ml 50 % ethanol-in-water solution, 4 ml 0.004 % DPPH –in-methanol solution), Amample is the sample light absorbance.

The IC 50 % factor, determining the concentration of the extract leading to 50 % inhibition of free radicals, is calculated using the equation of the calibration line extract concentration, (ml/l), as a function of % inhibition. The calibration line is obtained through preparation of series of extracts, obtained after dilution of the basic extract with 50 % of ethanol-in-water solution. The last one is necessary to make possible the comparison of the plant extracts obtained with standard antioxidant compounds as gallic acid, ascorbic acid, α-tocopherol, etc. After calculation, from the volumes and the extracts’ and DPPH concentrations during IC 50 % analysis, it is possible to find the antioxidant capacity of the dry extract or of the rough material in mg substance / mg DPPH for IC 50 %.

RESULTS AND DISCUSSION

To study the effect of the particle size on the total extracted amount, the extraction kinetics with all particle diameters and 50 % ethanol-in-water solution was followed. The results are presented in Fig. 2. It is evident that the particle diameter does not influence much the total extracted amount. The differences between the kinetic curves are more evident at the beginning of the extraction. At the end of the process (about the 80th min) there is almost no difference in the total extracted mass – the maximal relative error between the data does not exceed 13 %. However, it could be observed a tendency to obtain worse results for the minimal and for the maximal particle diameters.

Total polyphenols contents. To study the process control the extractions were carried out in parallel on the laboratory shaker already mentioned and in the flask using a laboratory magnetic mixer MMS-300 (BOECO, Germany), with variable rotation speed. The total extracted amount and the total polyphenols contents differ in the range of about 15 %, i.e. we can conclude that the process is controlled by internal diffusion in the particles.

The influence of the ethanol-in-water concentration on the TPPC in the extracts obtained was also
investigated. Three concentrations were studied: 20 %, 50 % and 70 % EtOH. It was found that for the higher concentrations (> 50 %) there is no obvious effect on the amount and the qualities of the extracts obtained. The lowest concentration of the ethanol (20 % vol) does not assured satisfactory results and it is not recommended to be used. The results of these experiments are summarized in Table 1.

It can be seen that the best results concerning the total polyphenols contents were obtained with particle diameters < 1 mm and ethanol concentration of 50 %. The results for 70 % EtOH are quite close to those with 50 %. The worst results for TPPC were obtained with 20 % ethanol.

It is well established that the organic compounds exhibiting antioxidant activity as well as the polyphenols are thermolabile. That was the reason for us to study the influence if the drying temperature of the liquid extracts on their properties after re-dissolution in ethanol-in-water solutions. For this purpose three sets of experiments were carried out: each of them on the TPPC in the extract with 50 % EtOH after 80 min of extraction without drying and another sample – after drying at given temperature followed by dissolution of the dry extract in 50 % EtOH. The results of these experiments are presented in Table 2.

From the results in Table 2 it is evident that the increase in drying temperature leads to decrease in TPPC in the extracts after re-dissolution. The worst results were obtained for drying temperature of 60°C – total polyphenols contents after drying and re-dissolution decreased almost twice (2.22 times).

The effect of the extraction time and the ethanol concentration on the total polyphenols contents in the extracts was also studied. The results are summarized in Table 3.

It is evident that the best results for TPPC are ob-

### Table 1. Effect of the particle size and the ethanol concentration on TPPC.

<table>
<thead>
<tr>
<th>Particle size, mm</th>
<th>EtOH, %</th>
<th>A</th>
<th>Cpph(%)</th>
<th>C_{GA} (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d &lt; 1mm.</td>
<td>20</td>
<td>0,275</td>
<td>3,987</td>
<td>0,1864</td>
</tr>
<tr>
<td>d &lt; 1mm</td>
<td>50</td>
<td>0,901</td>
<td>4,323</td>
<td>0,643</td>
</tr>
<tr>
<td>d &lt; 1mm</td>
<td>70</td>
<td>0,864</td>
<td>3,038</td>
<td>0,616</td>
</tr>
<tr>
<td>1 mm &lt; d &lt; 2 mm.</td>
<td>50</td>
<td>0,5599</td>
<td>5,283</td>
<td>0,5599</td>
</tr>
<tr>
<td>2 mm &lt; d &lt; 2.5 mm</td>
<td>50</td>
<td>0,5187</td>
<td>2,731</td>
<td>0,5187</td>
</tr>
<tr>
<td>d &gt; 2.5 mm</td>
<td>50</td>
<td>0,3699</td>
<td>2,433</td>
<td>0,3699</td>
</tr>
</tbody>
</table>

### Table 2. Effect of the drying temperature on the TPPC in the dry extracts.

<table>
<thead>
<tr>
<th>Particle size, mm</th>
<th>EtOH, %</th>
<th>A</th>
<th>Cpph(%)</th>
<th>C_{GA} (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d &lt; 1mm.</td>
<td>50</td>
<td>1,014</td>
<td>2,422</td>
<td>0,7255</td>
</tr>
<tr>
<td>d &lt; 1mm. (re-dissolution, drying at 40°C)</td>
<td>50</td>
<td>1,122</td>
<td>2,685</td>
<td>0,8042</td>
</tr>
<tr>
<td>d &lt; 1mm.</td>
<td>50</td>
<td>0,632</td>
<td>3,991</td>
<td>0,4468</td>
</tr>
<tr>
<td>d &lt; 1mm. (re-dissolution, drying at 50°C)</td>
<td>50</td>
<td>0,448</td>
<td>2,825</td>
<td>0,3126</td>
</tr>
<tr>
<td>d &lt; 1mm.</td>
<td>50</td>
<td>1,117</td>
<td>3,389</td>
<td>0,8006</td>
</tr>
<tr>
<td>d &lt; 1mm. (re-dissolution, drying at 60°C)</td>
<td>50</td>
<td>0,512</td>
<td>1,522</td>
<td>0,3593</td>
</tr>
</tbody>
</table>
Table 3. Effect of the extraction time and the ethanol concentration on the total polyphenols contents.

<table>
<thead>
<tr>
<th>Particle size, mm</th>
<th>EtOH, %</th>
<th>A</th>
<th>C_{GA} (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d &lt; 1 mm (extraction at 60°C for 60 min)</td>
<td>20</td>
<td>0.247</td>
<td>0.166</td>
</tr>
<tr>
<td>d &lt; 1 mm (extraction at 60°C for 60 min)</td>
<td>50</td>
<td>0.986</td>
<td>0.705</td>
</tr>
<tr>
<td>d &lt; 1 mm (extraction at 60°C for 120 min)</td>
<td>80</td>
<td>1.111</td>
<td>0.7962</td>
</tr>
</tbody>
</table>

tained for the case of extraction at 60°C for 120 min and 80 % ethanol. Nevertheless these results are quite close to those for 50 % ethanol at 60°C for 60 min (in 12 % limit).

**Antioxidant capacity (radical scavenging effect).**

The DPPH⁺ assay was used to determine the radical scavenging effect of the extracts obtained. For the purpose samples with different contents of the extract were prepared and the relationship I % = f(C, ml/l) was built. After that the radical scavenging effect I% was calculated as described (eq.(1)). Then the quantities IC50 % for the extract and IC50 %, calculated as equivalents of vitamin C (IC50 %AA) were found.

In Fig. 3 the IC50 % time evolution is presented. It is evident that the radical scavenging activity increases with time and reaches a plateau after 20th min of the extraction. For the same operational conditions the plateau for the total extracted amount is reached about 60th min, and the TPPC plateau – after 120 th min.

The effect of the particle size and the ethanol concentration on the antioxidant activity was investigated. The results are summarized in Table 4.

From the results presented, it is evident that the highest values for AOA are obtained for the particle sizes in the range 2 mm < d < 2.5 mm with 50 % ethanol-in-water solutions. There is no evident relationship between the TPPC and the radical scavenging effect of the extracts studied. The same conclusion can be derived from the results in Fig. 3. Similar conclusions were made by other authors investigated citrus by-products [11].

In Fig. 4 a comparison between the results obtained in this study and our other results with citrus peels is presented. As a base for this comparison the TPPC and the antioxidant activity of the extracts is chosen.

It can be seen that all the citrus peels exhibit quite high antioxidant activity, which together with

![Fig. 3. IC50% evolution, particle size d < 0.5 mm, solid-to-liquid ratio 1 : 10, 20 % EtOH, 20°C.](image)
Table 4. Effect of the particle size and the ethanol concentration on the AOA of mandarin peels extracts.

<table>
<thead>
<tr>
<th>Particle size, mm</th>
<th>% EtOH</th>
<th>IC 50% (ml/l)</th>
<th>IC 50% AA (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d &lt; 1 mm</td>
<td>20</td>
<td>237.58</td>
<td>0.065</td>
</tr>
<tr>
<td>d &lt; 1 mm</td>
<td>50</td>
<td>72.63</td>
<td>0.211</td>
</tr>
<tr>
<td>d &lt; 1 mm</td>
<td>70</td>
<td>82.1</td>
<td>0.187</td>
</tr>
<tr>
<td>1 mm &lt; d &lt; 2 mm</td>
<td>50</td>
<td>152.88</td>
<td>0.1</td>
</tr>
<tr>
<td>2 mm &lt; d &lt; 2.5 mm</td>
<td>50</td>
<td>50.84</td>
<td>0.302</td>
</tr>
</tbody>
</table>

polyphenols contained makes them a valuable source for natural antioxidants and the citrus flavonoids in the cosmetic and food industries.

CONCLUSIONS

The extraction of valuable components from mandarin peels with ethanol-in-water solutions was investigated. The effect of the operational conditions on the total extracted amount, TPPC and antioxidant activity was studied. The conditions varied were: the ethanol concentration, particle size and the temperature. The best results concerning the total polyphenols contents were obtained with particle diameters < 1 mm and ethanol concentration of 50 %. The results for 70 % EtOH are quite close to those with 50 %. The worst results for TPPC were obtained with 20 % ethanol. The highest values for AOA are obtained for the particle sizes in the range 2 mm < d < 2.5 mm with 50 % ethanol-in-water solutions. There is no evident relationship between the TPPC and the radical scavenging effect of the extracts studied. Taking into account the thermolability of the polyphenols, vitamin C, etc., the influence of the drying temperature on the properties of extracts after re-dissolution was studied. It was found that the increase in drying temperature leads to decrease in TPPC in the extracts after re-dissolution. The worst results were obtained for drying temperature of 60°C – total polyphenols contents after drying and re-dissolution decreased almost twice (2.22 times).

The comparison in TPPC and AOA of the mandarin peels’ extracts with other citrus peels shows that the source studied is a perspective for possible use in food and cosmetic industries due to valuable components in it.
Acknowledgements

This work was partially supported by the Research division of UCTM-Sofia, Grant No 11015.

REFERENCES

2. Khizar Hayat, Sarfraz Hussain, Shabbar Abbas, Umar Farooq, Baomiao Ding, Shuqin Xia, Chengsheng Jia, Xiaoming Zhang, Wenshui Xia, Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro, Separation and Purification Technology, 70, 2009, 63–70.
5. B.B. Li, B. Smith, Md. M. Hossain, Extraction of phenolics from citrus peels II. Enzyme-assisted extraction method, Separation and Purification Technology, 48, 2006, 189-196.
8. Muhammad Kamran KHAN, These, Polyphénols d’Agrumes (flavanones): extraction de glycosides de la peau d’orange, synthèse de métabolites chez l’homme (glucuronides) et étude physico-chimique de leur interaction avec la sérum albumine, Académie d’Aix-Marseille, Université d’Avignon et des Pays de Vaucluse, 2010, 18.