Study of antioxidant properties of green tea extract

Elzbieta SIKORA, Jan OGOROWSKI - Institute of Organic Chemistry and Technology, Cracow University of Technology, Cracow

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Introduction

Oxidation processes are among the chief reasons behind the deterioration of product life and quality during storage and application. It should be emphasized that oxidation is not only responsible for quality deterioration of products but also affect human organism, triggering a number of illnesses and increasing the rate of aging. In order to eliminate or reduce the oxidation rates, the food, pharmaceutical and cosmetic industries use antioxidants. Those agents capture the free radicals and bind them in low reactive, stable systems. On the one hand, the presence of antioxidants in cosmetics ensures the stability of the substance (by protecting the unsaturated ingredients from oxidation), on the other hand, antioxidants as active substances reduce the rate of aging of skin. The most widely used antioxidants in the cosmetic industry are: vitamin C, vitamin E, Q10 coenzyme, gallic acid and its esters, ferulic acid, lipoic acid, butylated hydroxytoluene (BHT) and polyphenol- and flavonoid-rich plant extracts. The plants from which antioxidant-rich extracts are obtained include i.a. soy, grape seeds, lady’s milk, citrus fruits, rosemary, Gingko biloba and green tea.

Tea is a plant of the Theaceae family – Camellia Sinensis (common tea). The tea plant is an evergreen shrub with small white petals and woody seedpods [1]. The green tea originates from China. Green tea was first mentioned in 3rd Century B.C., recalling a Chinese medic who recommended it to his patients for improving concentration and wit. Until 3rd Century B.C. the green tea brew was used solely for medical purposes. Within the next Centuries the popularity of green tea increased, as its taste and therapeutic values came to be appreciated.

The herbal material are tea leaves which, depending on the processing method, give the following types of tea: green, red or black. Green tea undergoes the least processing. It is not subjected to fermentation and, compared to other types, contains the most active compounds, including: flavonoids (rutine, quercetin, kaempferol), catechin tannins, proanthocyanidines, xanthins (caffeine, theobromine, theophylline), theanine (aminoacid), phenolic acids (caffeic, gallic, chlorogenic), microelements (manganese, fluorides, aluminium), B and C vitamins, and essential oils [2, 3].

The antioxidant properties of flavonoids are widely known. The polyphenols in green tea belong to various classes of compounds, including: catechines, flavonols, flavones, their glucosides and phenolic acids [3÷10]. The polyphenol complexes content in dry mass is 20÷30% [2]. Due to high content of antioxidants, green tea functions as dietary supplement and ingredient in care cosmetics, shampoos, sweet waters, masks and anti-aging emulsions [4, 9].

The study investigated the impact of the conditions of green tea leaves extraction on the effectiveness of antioxidant properties. Three types of green tea extracts were obtained: ethanolic, glycolic, hydrous, as well as alcoholic and glycolic macerates. Aside from the effectiveness of active compounds extraction, the solvent selection was also conditional upon its safety in terms of application of liquid green tea extracts as cosmetic raw materials. Next, the rutine content in the obtained products was assayed and the effectiveness of the products antioxidant properties was assessed. The investigation of antioxidant capabilities was conducted on the basis of a colormetric reaction with thiocyanate, tracking the oxidation process of linolic acid [1]. The changes were monitored using UV-vis spectrophotometer.

Experimental study

In order to obtain extracts with maximum active substances content, two extraction methods were applied: continuous in Soxhlet extractor and maceration. Using the continuous method, three types of extracts were obtained: hydrous, ethanolic and glycolic extracts. Using maceration, two types of macerates were obtained: glycolic and ethanolic. The raw material for both extractions originated from the same source – Herba-pol Lublin S.A. After initial mincing in a porcelain crucible, the dried green tea leaves were placed in an extraction thimble and then in Soxhlet extractor. First, the material underwent preliminary processing through extracting the raw material with the mixture of chloroform and petroleum ether (1:1) in order to remove chlorophyll and hydrophobic substances. The preliminary extraction was continued for 2 hours. After separating the eluent and drying the plant material, the proper extraction process was initiated. As solvents were used in the following order: ethanol 96% (POCH Gliwice), propylene glycol (POCH Gliwice), distilled water, in constant mass ratio of solvent to raw material (Tab. 1).

Table 1

<table>
<thead>
<tr>
<th>Process</th>
<th>Raw material mass [g]</th>
<th>Extraction time, h</th>
<th>Solvent</th>
<th>Volume, cm³</th>
<th>Preliminary processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext. 1</td>
<td>9.4</td>
<td>5.5</td>
<td>water</td>
<td>226</td>
<td>YES</td>
</tr>
<tr>
<td>Ext. 2</td>
<td>10.5</td>
<td>5.5</td>
<td>propylene glycol</td>
<td>250</td>
<td>YES</td>
</tr>
<tr>
<td>Ext. 3</td>
<td>10.5</td>
<td>5.5</td>
<td>ethanol</td>
<td>250</td>
<td>YES</td>
</tr>
<tr>
<td>Mac. 1</td>
<td>15</td>
<td>505</td>
<td>glycol</td>
<td>250</td>
<td>NO</td>
</tr>
<tr>
<td>Mac. 2</td>
<td>15</td>
<td>505</td>
<td>ethanol</td>
<td>250</td>
<td>NO</td>
</tr>
</tbody>
</table>

The obtained products differed in terms of colour intensity. The alcoholic extract was dark brown. The glycolic extract had a similar colour. The hydrous extract was yellow-brown.

The maceration process was conducted at room temperature (approx. 493 K) for three weeks (Tab. 1). 15 g of minced raw material was placed in dark glass 300 cm³ bottles and flooded with solvent, propylene glycol or 96% ethanol respectively. Thus prepared mixture was placed in dark glass 300 cm³ bottles and flooded with solvent, propylene glycol or 96% ethanol respectively. Thus prepared mixture of raw material and solvent was left in a sealed bottle at room temperature.

Investigation of flavonoids content in terms of rutine

UV spectrophotometry was used to perform quantitative assay of rutine in the extracts. The investigation was conducted on Stełarnet Incorporation EPP 2000°C spectrophotometer. The technical data of the spectrophotometer are provided in Table 2. The quantitative content of rutine in the extracts was assayed on the basis of established calibration curves. For this purpose, alcoholic, glycolic and hydrous standard solutions of rutine were prepared in concentrations of 2·10⁻⁴ [g/cm³], 1.5·10⁻⁴ [g/cm³], 0.2·10⁻⁴ [g/cm³]. By determining the absorbance values, at wavelength λₒ = 270 nm for the standard solutions, the calibration curves were traced.
Assaying antioxidant properties using thiocyanate method

The following procedure was applied each time: in the conical flask of 50 cm³ with ground glass socket the mixture was prepared, containing 10 cm³ of 99.8% ethanol, 10 cm³ of phosphate buffer in 0.1 mol/dm³ and pH=7, as well as 5 cm³ of deionised water. Next, to thus prepared mixture the antioxidant substance (1 cm³ of extract) and linolic acid (in such quantity so that its final concentration in the mixture was 0.02 mol/dm³) were added. The flasks were sealed and left in room temperature. In the intervals of 1, 7 and 14 days the solution samples were collected for analysis in order to establish the degree of oxidation of the linolic acid, basing on colorimetric reaction with ammonium thiocyanate. Using the UV-Vis spectrophotometer the solution absorbance was measured at wavelength $\lambda = 500$ nm. The following procedure was applied each time: from each flask a 0.1 cm³ sample was collected and supplemented with 10 cm³ of 75% ethanol, 0.1 cm³ of 30% ammonium thiocyanate solution. Approx. 3 minutes before the absorbance measurement, 0.1 cm³ of the solution of iron (II) chloride and 3.5% hydrochloric acid was added. On thus prepared samples the absorbance was measured. Conclusions were made on the basis of those measurements, concerning the antioxidant effectiveness of the investigated substances.

Discussion

Impact of extraction conditions on flavonoids content

Figure 1 presents the calibration curve for ethanol solutions of rutine.

![Calibration curve of rutine solution in 96% ethyl alcohol, $\lambda = 270$ nm](image)

Table 3 provides the values of coefficients in equations describing the calibration curves traced for standard solutions of rutine.

<table>
<thead>
<tr>
<th>Coefficient in equation of rutine calibration curve</th>
<th>$a$</th>
<th>$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>for ethanol solutions</td>
<td>1679.8</td>
<td>0.0166</td>
</tr>
<tr>
<td>for glycolic solutions</td>
<td>3170.1</td>
<td>-0.0626</td>
</tr>
<tr>
<td>for hydrous solutions</td>
<td>6830.5</td>
<td>0.0245</td>
</tr>
</tbody>
</table>

Data in Table 4 clearly indicate that the type of solvent plays a major role in the flavonoid extraction process. The obtained results suggest that the best solvent among the examined samples to obtain flavonoid-rich extract is ethanol. The highest rutine concentration was obtained with the use of ethanol ($43.8 \cdot 10^{-4}$ g of rutine in 1 cm³), both in continuous extraction and in maceration. The lowest concentration was obtained when using water as the solvent (only $1.77 \cdot 10^{-4}$ g of rutine in 1 cm³). Furthermore, the data provided in Table 4 can be used to assess the impact of the extraction method on the content of rutine in the extracts. When using ethanol as the eluent, continuous extraction proves to be the more effective method of obtaining rutine. In the case of green tea extraction with propylene glycol, the glycolic macerate exhibits higher rutine content. This may be attributable to the fact that continuous extraction in glycol causes high boiling point which may lead to partial decomposition of isolated flavonoids.

Antioxidant properties of green tea extracts

In the thiocyanate method the antioxidant properties of substances are evaluated on the basis of assaying the content of peroxides, created as a result of linolenic acid oxidation, with the use of colorimetric method of creating thiocyanate complexes with Fe$^{3+}$ ions. The Fe$^{2+}$ ions in the solution, originating from iron(II) chloride, are oxidised in the presence of peroxides to Fe$^{3+}$ ions that bind with thiocyanate ions, creating red iron thiocyanates.
The colour intensity of the solution attests to the quantity of Fe^{3+} and thus is the measurement of peroxide concentration. The complex bindings of iron and thiocyanate are unstable and already after 30 minutes the colour begins to fade, which is triggered by the reduction of iron(III) to iron(II). Therefore the absorbance is measured immediately after preparing the sample. The conducted measurement of the colour intensity of [Fe(SCN)]\(^{3-}\) complexes allowed for quantitative assessment of the effectiveness of antioxidant properties of green tea extracts.

Figure 3 presents the sample UV spectra for complexes stabilized with ethanolic and glycolic green tea extract, after 7 days from preparing the mixture of linolic acid with iron(II) chloride. The data on the chart indicate that in the case of extract-stabilized mixtures there are no peaks corresponding to coloured iron thiocyanate complexes present in the mixture without an antioxidant. Those results confirm the anti-radical effect of green-tea extracts.

![UV-Vis spectrum of [Fe(SCN)]\(^{3-}\) complexes stabilized by ethanolic and glycolic green tea extract (after 7 days)](image)

**Figure 3. UV-Vis of [Fe(scn)\(^{6}\)]\(^{3-}\) complexes stabilized by ethanolic and glycolic green tea extract (after 7 days)**

The results of the measurements of colour intensity of [Fe(SCN)]\(^{3-}\) solution complexes after 24 hours from preparing the mixtures with linolic acid were used to determine the antioxidant activity of green tea extracts. Table 5 provides the antioxidant activity values of the examined solutions, calculated with the use of equation 1.

\[
AAC = A_{500} (c) - A_{500} (l)
\]

where:
- AAC – antioxidant activity
- A 500nm (c) – blank test absorbance value
- A 500nm – examined sample absorbance value

**Table 5**

<table>
<thead>
<tr>
<th>Examined sample</th>
<th>Absorbance at (\lambda = 500) nm</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank test ethanolic</td>
<td>0.057</td>
<td>-</td>
</tr>
<tr>
<td>Blank test glycolic</td>
<td>-0.028</td>
<td>-</td>
</tr>
<tr>
<td>Blank test hydrous</td>
<td>0.012</td>
<td>-</td>
</tr>
<tr>
<td>Hydrous extract</td>
<td>-0.046</td>
<td>0.058</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>-0.060</td>
<td>0.117</td>
</tr>
<tr>
<td>Glycolic macerate</td>
<td>-0.135</td>
<td>0.107</td>
</tr>
<tr>
<td>Alcoholic macerate</td>
<td>-0.047</td>
<td>0.104</td>
</tr>
<tr>
<td>Glycolic extract</td>
<td>-0.096</td>
<td>0.068</td>
</tr>
</tbody>
</table>

**Conclusion**

Analyzing the results provided herein we can conclude that the method of extraction and the type of solvent used determine the effectiveness of antioxidant properties of green tea extract. Among the examined extracts the best antioxidant properties were exhibited by the alcoholic extract (Tab. 5). This concurs with the fact that it is the alcoholic extract that contains the highest concentration of rutine (Tab. 4) which, as one of green tea flavonoids, is responsible for its antioxidant properties. The lowest antioxidant effect among the examined extracts was observed for the glycerol extract, in which the rutine concentration per 1 cm\(^2\) of extract was lowest.

**Literature**

8. www.farmakognozja.farmacja.pl

Elżbieta SIKORA - Ph.D., (Eng), graduated and completed her doctoral degree at the Faculty of Chemical Engineering and Technology of the Cracow University of Technology. Currently employed as Assistant Professor on the Organic Technology and Refining Processes Chair. Scientific interests include low-tonnage organic technology, as well as obtaining, investigating the properties of and application of natural raw materials in cosmetic products and household chemicals. e-mail: esikora@pk.edu.pl.

Jan OGNOWSKI - Professor (Ph.D., Eng) is a graduate of the Faculty of Chemistry of the Silesian University of Technology (1996). Currently he is employed as head of the Organic Technology and Refining Processes Chair on the Faculty of Chemical Engineering and Technology, Cracow University of Technology. Specialization: organic technology, biotechnology, catalytic processes.