Polymeric Flavonoids Obtained by Crosslinking Reaction †

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Abstract: Plant polyphenols are becoming more and more popular due to their strong antiaging properties. The best researched and largest group of polyphenols are flavonoids. Flavonoids have high antioxidant and pharmacological activities and these properties are closely related to their structure. Certain structural elements of these compounds condition their properties and improve or degrade the activities. As a result of the polymerization of flavonoids, macromolecular compounds showing more favorable properties, such as, for example, bactericidal and antioxidant activity, can be obtained. The aim of this study is to polymerize selected flavonoids (quercetin and rutin) in reaction with a crosslinking compound. Glycerol diglycidyl ether (GDE) causes the crosslinking of quercetin or rutin monomers and the formation of polymeric structures. The study analyzed the thermal stability of monomeric and polymeric flavonoids and their antioxidant activity. Poly(flavonoids) showed greater resistance to oxidation than their monomeric forms. Moreover, poly(quercetin) and poly(rutin) have a greater ability to reduce transition metal ions. Polymeric forms of quercetin and rutin can potentially be effective stabilizers, e.g., for polymeric materials.

Keywords: quercetin; rutin; polymerization; crosslinking compound

1. Introduction

Quercetin and rutin are popular flavonoids in the diet, arousing great interest due to their beneficial pharmacological properties and strong antioxidant properties. Quercetin is a flavonol occurring in fruit and vegetables. In food products, this flavonoid may appear in the form of glycosides, e.g., quercetin 3-O-rhamnozyl-(1→6)-glucoside (rutin) [1–3]. In the scientific literature, special attention is paid to the prohealth and antioxidant properties of both quercetin and its glycoside—rutin. The properties of flavonoids strictly depend on their chemical structure and may be modified, for example, by polymerization [4–6].

One of the methods of flavonoid polymerization is reaction with a crosslinking compound [7,8]. Sahiner proposed a one-stage microemulsion/crosslinking polymerization of quercetin [7] and rutin [8] using L-α lecithin as a surfactant, cyclohexane as the organic phase and glycerol diglycidyl ether (GDE) as a crosslinking agent. The obtained poly(quercetin) was characterized by higher thermal stability, higher antioxidant activity and better antibacterial properties than the monomeric form [7]. Poly(rutin) also showed higher thermal stability than rutin. In addition, it was characterized by higher fluorescence than the monomer [8].

The aim of the research is to analyze the properties of polymeric forms of quercetin and rutin. Poly(quercetin) and poly(rutin) were polymerized with the crosslinker according to the method described by Sahiner. This manuscript extends and complements Sahiner’s analysis of poly(flavonoids). In the publications [7,8], the author did not analyze the thermal changes of polymeric quercetin and rutin using differential scanning calorimetry and did not study the ability of polymeric flavonoids to reduce multivalent metal ions—iron and copper. The ability of poly(flavonoids) to reduce transition metal ions is...
important as part of the evaluation of these compounds for use as a stabilizing agent which reduces the metals catalyzing the aging process.

2. Experiments
2.1. Polymerization
Flavonoid polymerization was performed according to the method proposed by Sahiner [7,8] with minor modifications.

2.2. Differential Scanning Calorimetry (DSC)
The prepared poly(flavonoids) was subjected to differential scanning calorimetry (DSC). The samples were heated from −80 to 400 °C at a rate of 10 °C/min in an air atmosphere. For comparison, DSC of the reference quercetin and rutin was performed.

2.3. FRAP and CUPRAC Analysis
The FRAP (ferric reducing antioxidant power) and CUPRAC (cupric reducing antioxidant capacity) methods are used to assess the ability of compounds to reduce transition metal ions. The FRAP test is based on the reduction of ferric ion (Fe$^{3+}$ → Fe$^{2+}$) under acidic conditions. The CUPRAC assay is analogous to the FRAP method and consists of the reduction of Cu$^{2+}$ to Cu$^{+}$.

The ferric (FRAP) and cupric (CUPRAC) ions’ reducing capacity was calculated according to the Formula (1):

$$\Delta A = A_{AR} - A_{0}$$

where: $A_0$—absorbance of the reagent test, $A_{AR}$—absorbance after reaction.

Absorbance measurements in the FRAP and CUPRAC tests were performed using a UV-spectrophotometer (Evolution 220, Thermo Fisher Scientific, Waltham, MA, USA). A detailed description of the determinations is presented by the authors in the following publications [9,10].

3. Results and Discussion
The research began with the thermal analysis of monomeric and polymeric flavonoid forms. Figure 1 shows DSC thermograms, and Table 1 summarizes the results of DSC analysis of the tested flavonoids and poly(flavonoids). Polymeric quercetin and rutin obtained by polymerization with a crosslinking compound, were characterized by a lower melting temperature $T_m$ than the melting temperature of reference polyphenols. The addition of a GDE crosslinker can lower the $T_m$ of the poly(flavonoids). Interesting results were obtained for the changes in the oxidation temperature $T_o$. Poly(quercetin) has a higher final oxidation temperature $T_o$ (by 39.2 °C) and a higher enthalpy of oxidation $\Delta H_o$ (about six times) than monomeric quercetin. Thus, the polymeric form of quercetin showed greater resistance to oxidation. The polymeric form of quercetin glycoside-poly(rutin) was characterized by a higher enthalpy of oxidation (about 15 times), but the oxidation temperature $T_o$ was lower than temperature of rutin by 58.8 °C.
Figure 1. Differential scanning calorimetry (DSC) thermograms of quercetin and poly(quercetin) (A) and rutin and poly(rutin) (B).

Table 1. DSC analysis of quercetin and poly(quercetin) (A) and rutin and poly(rutin) (B).

| Sample        | $\Delta H_m$ [J/g] | $T_m$ [°C] | $\Delta H_o$ [J/g] | $T_o$ [°C] |
|---------------|--------------------|------------|---------------------|------------|
| Quercetin     | 193.0              | 111.0      | 99.9                | 312.4 (endset) |
| Poly(quercetin)| 119.2              | 48.6       | 626.5               | 351.6 (endset) |
| Rutin         | 115.7              | 160.3      | 31.3                | 313.3 (endset) |
| Poly(rutin)   | 118.3              | 52.4       | 460.4               | 258.5 (endset) |

$\Delta H_m$—enthalpy of melting, $T_m$—melting temperature, $\Delta H_o$—enthalpy of oxidation, $T_o$—oxidation temperature (final-endset).

The next step of the research was to determine the ability of flavonoids and poly(flavonoids) to reduce transition metal ions. Polyvalent metal ions can catalyze the aging processes of, e.g., polymeric materials. Therefore, the ability to reduce and chelate such ions is a very important property that a stabilizer should fulfill. Figure 2 shows the ability of quercetin and poly(quercetin) (A) as well as rutin and poly(rutin) (B) to reduce iron (FRAP measurement) and cupric (CUPRAC assay) ions. The solutions of the reference quercetin and rutin flavonoids, at a concentration of 1 mg/mL, showed a much lower ability to reduce iron and copper ions than solutions of poly(flavonoids). The poly(flavonoids) of quercetin and rutin, obtained as a result of the reaction with glycerol diglycdyl ether (GDE), were characterized by a better ability to reduce transition metal ions. The polymerization of these compounds resulted in a positive effect on the improvement of this property.
Figure 2. Ability of quercetin and poly(quercetin) (A) as well as rutin and poly(rutin) (B) to reduce iron and cupric ions measured by ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) methods.

4. Conclusions

Flavonoid polymerization with a crosslinking compound influenced their thermal and stabilizing properties. Poly(quercetin) was characterized by a higher oxidation temperature than its monomeric form. Poly(rutin) had a much higher enthalpy of oxidation than rutin. Moreover, both poly(flavonoids) showed a significantly higher ability to reduce the multivalent metal ions that catalyze the aging processes. Due to their good resistance to oxidation and great ability to reduce metal ions, poly(quercetin) and poly(rutin) can potentially be good stabilizers, e.g., for polymeric materials and environmentally friendly materials.

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Abbreviations

The following abbreviations are used in this manuscript:

- DSC: Differential Scanning Calorimetry
- CUPRAC: Cupric Reducing Antioxidant Capacity
- FRAP: Ferric Reducing Antioxidant Power
- GDE: glycerol diglycidyl ether
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