Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [*Russian original No. 3, May-June, 2011*]

MECHANOCHEMICAL PREPARATION OF WATER-SOLUBLE COMPOSITES BASED ON QUERCETIN

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UDC 547.972

The solubility and antioxidant activity of the bioflavonoid quercetin was increased from its mechanochemically prepared solid composites with metal carbonates and carbohydrate compounds.

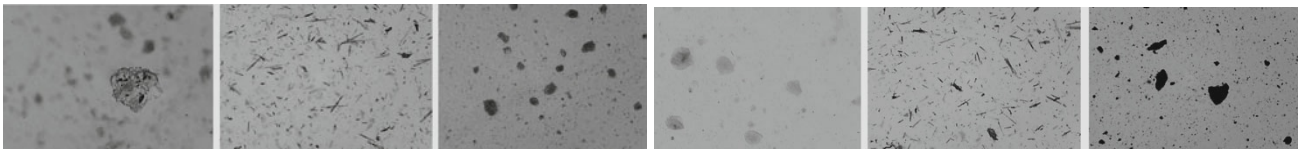
Keywords: quercetin, mechanical treatment, arabinogalactan, basic magnesium carbonate, bioavailability, solubility, activity.

3,5,7,3′,4′-Pentahydroxyflavone (quercetin, **1**) was formerly known as vitamin P. Preparations of quercetin reduce the permeability of capillaries and exhibit anti-inflammatory effects, radioprotector activity, and anti-ulcer action associated with the use of anti-inflammatory agents [1–4]. The development of new drugs and biologically active additives (BAAs) with increased water solubility, bioavailability, and effectiveness is interesting because quercetin is practically insoluble in water. The goal of our work was to prepare water-soluble solid powder composites based on quercetin by forming water-soluble complexes and salt forms of quercetin with $MgCO_3$ and $CaCO_3$ [7–9].

Table 1 presents solubility measurements for **1** from the resulting composites. It can be seen that the solubility of **1** from the composites increased, the exceptions being samples 1 and 13. The dissolution of quercetin from sample 1 took a long time (>>1 h). Thus, its equilibrium solution concentration was not reached under the experimental conditions. The polysaccharide chitosan, which is poorly soluble in water, was used in sample 13. The principal fraction of **1** was found bound to the solid precipitate and not in solution upon forming adducts of chitosan and **1**. This explained the results. The increased solubility in composites 2, 3, 5–10, and 12 was obviously achieved by forming water-soluble intermolecular complexes of quercetin and the carbohydrates through the reported mechanism [5, 6]. The strength of the intermolecular complexation and, as a result, the water solubility of quercetin, increased with the duration of mechanical treatment and the production of a more homogeneous dispersion. The reason for the increased solubility in composites 4 and 11 was apparently the formation of water-soluble salt forms of **1** by the reported mechanism [5, 10, 11]. Thus, both pathways for increasing the solubility were clearly effective.

We studied several physicochemical characteristics such as morphological changes of the powder particles, DSC thermograms, and x-ray diffraction patterns of the produced powdered solid dispersions.

Figure 1 (a, b, c) shows photomicrographs of powdered arabinogalactan (Fig. 1a), quercetin (Fig. 1b), and a water-soluble dispersion of quercetin–arabinogalactan (1:10) composite treated mechanically for 4 h (Fig. 1c). Aggregates of ground particles formed after mechanical treatment. Figure 1d, 1e, and 1f shows photomicrographs of powdered basic magnesium carbonate, quercetin, and water-soluble disperse quercetin–magnesium carbonate (6:4) treated mechanically for 1.5 h. An analysis of these photographs showed that aggregates of ground particles also formed after mechanical treatment.



a *b* *c* *d* *e* *f*

Fig. 1. Photomicrographs of powdered arabinogalactan (a), quercetin (b), quercetin–arabinogalactan sample (1:10) mechanically treated for 4 h (c), magnesium carbonate (d), quercetin (e), quercetin–magnesium carbonate sample (6:4) mechanically treated for 1.5 h (f).

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0009-3130/11/4703-0373

TABLE 1. Composite Compositions and Solubility of Quercetin in Aqueous Solutions

Number	Composite and mass ratio	Mechanical treatment time, h	Quercetin concentration in water, g/L	Solubility increase by n times
1	Quercetin–arabinogalactan 1:10	0	0.003	0.16*
2	Quercetin–arabinogalactan 1:10	1	0.0495	2.75
3	Quercetin–arabinogalactan 1:10	4	0.2086	11.59
4	Quercetin–magnesium carbonate 6:4	1.5	1.21	67.22
5	Quercetin–arabinogalactan 1:20	0	0.1125	6.25
6	Quercetin–arabinogalactan 1:20	1	0.5762	32.01
7	Quercetin–arabinogalactan 1:20	4	1.2774	70.96
8	Quercetin–sorbitol 1:10	0	0.0718	3.98
9	Quercetin–sorbitol 1:10	1	0.0705	3.91
10	Quercetin–sorbitol 1:10	4	0.3625	20.13
11	Quercetin–acetylsalicylic acid–magnesium carbonate 2.2:11:6.8	2	0.1512	8.4
12	Quercetin–citrus pectin 1:10	2	0.1014	5.63
13	Quercetin–chitosan 1:10	2	0.0175	0.97

*Quercetin solubility in water at +20°C was 0.019 g/L.

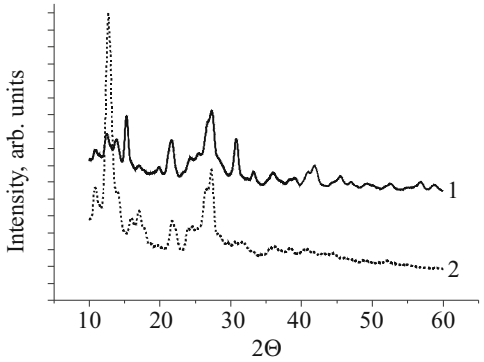

Fig. 2. Diffraction patterns of quercetin and quercetin–basic magnesium carbonate. Quercetin–$MgCO_3$ (4:6) (1) and quercetin (2).

We think that formation of the particle aggregates assisted faster interaction of the components during hydration and an increased rate. This correlated with published data [5, 10, 11].

The endothermic peak related to melting of quercetin shifted to lower temperatures in DSC thermograms of solid dispersions of quercetin with calcium and magnesium carbonates. This effect suggested that the crystal structure of quercetin was significantly disordered as a result of the mechanical treatment.

A peak corresponding to melting of quercetin could not be reliably identified in samples of quercetin–carbohydrate excipients because its relative content in the mixture was insignificant.

The relative intensity of reflections for crystalline quercetin decreased significantly in x-ray diffraction patterns of the produced composites (Fig. 2). This also confirmed the hypothesis about the disordering of the quercetin crystal structure.

The phase change of quercetin as a result of the mechanical treatment of composites based on it apparently helped to accelerate the dissolution of quercetin. Partial formation of amorphous quercetin in the case of basic magnesium carbonate accelerated the dissolution of quercetin as the magnesium salt. Dispersion of quercetin in the matrix of the water-soluble filler probably occurred in addition to disordering of the crystal structure with arabinogalactan and sorbitol. This also helped to accelerate the release of quercetin into solution and the formation of intermolecular complexes during hydration.

Different induction periods were observed on kinetic curves of oxygen absorption obtained from AIBN-induced cumene oxidation in the presence of quercetin and samples 2-13. Their values depended on the degree of binding of quercetin phenols with the excipients.

The following equation was used to calculate the content of oxidation inhibitors (C, mol/kg) of the studied extracts:

$$C = W_i \cdot \tau / P,$$

where W_i is the initiation rate ($6.8 \cdot 10^{-8}$ mol/L·s); τ, the induction period determined graphically (s); P, the weight of analyzed sample (kg/L) (considering a reaction volume of 10 mL).

TABLE 2. Antioxidant Activity

Sample*	Antioxidant concentration, mol/kg			K_7, L/mol·s		Quercetin, %		N**
	total	type I	type II	type I	type II	added	determined	
1	11.33	3.84	7.49	5.86	2.44	100	100	1
3	1.29	0.48	0.081	10.15	8.56	9.1	11.4	1.25
4	9.48	3.48	6.00	3.94	1.85	60.0	83.7	1.40
7	0.39	0.39	No data	14.8	No data	4.8	3.4	0.70
11	1.30	0.48	0.81	10.15	8.58	9.1	11.5	1.26

*Sample numbers correspond to those in Table 1.
**Change of antioxidant activity by n times.

The inhibition rate constant (k_7, L/mol·s) was determined from the equation

$$k_7 = 2.303k_3/\text{tg}\,\alpha,$$

where k_3 is the rate constant of chain propagation (1.75 L/mol·s) and tg α was determined by plotting the kinetic curve for oxygen absorption by the cumene reaction mixture in coordinates

$$X = -\log(1-t/\tau);\ Y = [V_{O2}]/[RH]_0,$$

where $[V_{O2}]$ is the concentration of absorbed oxygen (M); $[RH]_0$, the cumene concentration (7.14 M); and *t*, the time (s).

The content of **1** in samples 1–13 was determined by converting to the concentration of antioxidants found in starting quercetin (11.33 mol/kg for 100% quercetin). Table 2 presents the results for the antioxidant activity of samples exhibiting the greatest activity.

The produced samples had various levels of antioxidant activity. Sample 4, which was treated mechanically for 1.5 h, had the greatest activity. Composite 7 gave a poorer result than pure quercetin. In addition, this result contradicted that for composite 3 with the same amount of treatment. The probable cause for the reduced antioxidant activity of composite 7 was the higher solution concentration of arabinogalactan. This could change specifically the antioxidant activity under the applied conditions. In general, with the exception of sample 7, the antioxidant activity increased upon increasing the solubility of quercetin.

Thus, we produced solid composites of quercetin and excipients such as metal carbonates and carbohydrates that had increased (up to 70.9 times) water solubility for **1**. The antioxidant activity of **1** also increased in most of the studied samples. As expected, the studied quercetin composites should have increased bioavailability owing to the higher concentrations of active ingredient released into the aqueous medium and were a promising basis for creating drugs and BAAs.

EXPERIMENTAL

Quercetin (Chemapol, Czech. Rep., 200740181) was purified beforehand by column chromatography over L 60/100 grade silica gel (Czech. Rep.). The purity was determined by HPLC on a Milikhrom-2 chromatograph using a column (4 mm diameter, 100 mm height) packed with C-18 sorbent. The eluent was CH_3CN and aqueous acetic acid (2%) (30:70 ratio) with detection at 254–290 nm and elution at 100 μL/min.

Arabinogalactan was supplied by Prof. V. A. Babkin (Irkutsk Institute of Chemistry, SB, RAS), isolated from Siberian larch by the literature method [10, 11], certified according to TU 9363-015-39094141-03, registered as raw material for production of BAAs under the trade name Fibrolar, and purified additionally by reprecipitation from EtOH. The purity was >99.5%; moisture, 0.01%; content of phenolic impurities (flavonoids), 0.15%.

Pharmacopoeial acetylsalicylic acid drug substance (LSR-003514/10-270410) and basic magnesium carbonate (FSP 42-3989-08) were used. Chitosan was 97% deacetylated (TU 9289-058-04689375-2001).

Composites of quercetin and excipients were prepared by mechanical treatment of powder mixtures in a BM-1 rotary ball mill (drum volume 300 mL, 15 steel balls with d = 15 mm, loading 20 g, acceleration of grinding bodies 1 g, drum rotation rate 150 rpm). Samples were finely ground light-yellow to greenish-yellow powders.

Samples were studied using photomicrography on a Mikmed-1 laboratory microscope with a digital ocular for transferring the images to a computer. Thermal analysis was performed on a DSC-550 instrument (Instrument Specialists Inc.)

with temperature programmed at 20–400°C and heating rate 10°C/min. X-ray phase analysis used powder patterns of samples from a DRON-3 diffractometer (Cu cathode, $\lambda = 1.54$ Å) at room temperature. The angle measurement uncertainty was 0.005°.

Quercetin solubility was measured by dissolving a weighed sample of the mass required to reach a calculated quercetin concentration of 10 g/L in doubly distilled water (10 mL) at +20°C, stirring on a magnetic stirrer for 30 min, and filtering to produce a transparent solution. An aliquot (1 mL) was taken from the solution and diluted with distilled water in a volumetric flask (25 mL). The quercetin concentration was measured by HPLC relative to alcohol solutions of standards.

Antioxidant activity was determined by kinetic analysis based on a model chain reaction of induced oxidation of cumene. The kinetic parameters of water-soluble antioxidants were measured by a modified method with added DMSO. The initiator was azo-*bis*-diisobutyronitrile (AIBN). Antioxidant activity was measured from the oxygen absorption rate on an automated gas-measuring apparatus. Results were processed using the ANTIOXIDANT program under the following conditions: thermostat temperature 60°C, initiation rate $W_i = 6.8 \cdot 10^{-8}$ mol/L·s. The method enabled the content and activity of antioxidants in a multi-component mixture to be estimated quantitatively without preliminary isolation and separation and could be used to determine total lipid- and water-soluble natural antioxidants of unknown structure and composition [12].

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