

The first solid state glycosylation of quercetin

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Abstract. This work presents first results of the study possibility of solid state glycosilation of quercetin. The main results were obtained by thermogravimetric and calorimetric analysis. It was shown that the product of glycosylation occurs in the presence of solid alkali as catalyst

1. Introduction

Quercetin-3,3',4',5,7-pentahydroxyflavone, natural flavonoid that is in demand in medicine, preventive nutrition and animal husbandry. Quercetin has a high biological activity - it stabilizes cell membranes, passes antioxidants, anti-inflammatory properties, antihistamine action, antitumor, antithrombotic activity [1]. The main problem of quercetin bioavailability and the problem of isolation from plant raw materials is its small solubility in water. Mechanochemical methods for increasing the solubility and efficiency of extraction of flavonoids [2, 3] are based on the use of solid-phase chemical reactions with the formation of soluble forms of this compound during the joint mechanical treatment of mixtures of a solid reagent and flavonoid. Mechanochemical reactions use reagents that form soluble salts of polyphenols, complex compounds, inclusion compounds in branched soluble polymers. In nature, the main and soluble derivatives of quercetin are its glycosides - compounds with soluble sugars, in particular, rutin - a compound with rhamnose and glucose disaccharide.

The aim of the work is to elucidate the conditions under which the solid state mechanochemical reaction of quercetin and glucose occurs.

2. Experimental part

The main reagent used: quercetin (LTD "DiaM" Russia, > 99.3 %), glucose hydrate > 99.6 %. The other additives has purity higher than 99 %. Mechanochemical treatment of mixtures was carried out in planetary ball mill AGO-2 (Ltd. Novic, Russia) with balls acceleration 200 m/s², intensity treatment 1 W/g, time of treatment 5 minutes.

Thermoanalytical studies were carried out on differential scanning calorimeter DSC-550 (Instrument Specialist Inc.) in air atmosphere. The temperature range is from 20 to 380 °C, the heating rate is 10 deg / min.

X-ray phase analysis was carried out using Bruker D8 advance diffractometer at room temperature. IR-spectroscopic analysis was carried out using IR-Fourier spectrometer Infracum FT-801. Thin layer chromatography was carried out using Sorbfil CTX-1A plates with silicagel 50-120 mikrons and water/MeOH mixture as mobile phase.



3. Results and discussion

Usually mechanical treatment leads to the disappearance of crystalline phases of the initial reagents. The same effect is in our case - the broadening and disappearance of X-ray reflections is apparently associated with size reduction and amorphization of the components [4].

Figure 1 presents the results of gravimetric analysis of individual components (glucose and quercetin), a mixture of glucose and quercetin powders in a 1: 1 ratio by weight, and the same mixture treated under the conditions indicated above.

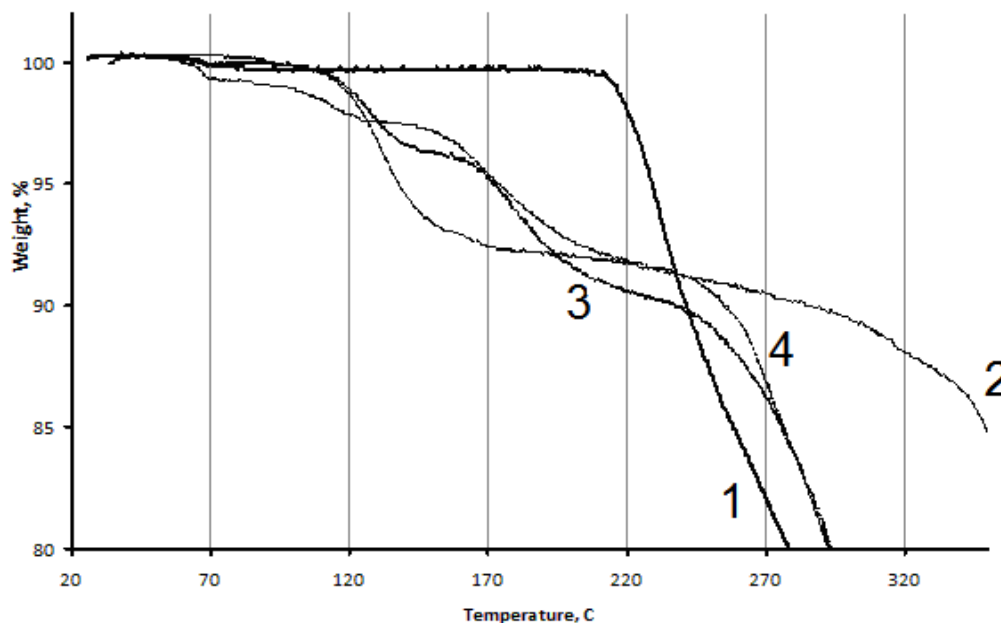


Figure 1. Thermogravimetric profiles of: 1 – glucose, 2 – quercetin, 3 – glucose : quercetin 1 : 1 mixture, 4 – glucose : quercetin mechanochemically treated mixture.

The results of glucose analysis correspond to those known [5]. The glucose is the most thermally stable component of the mixture. A slight decrease in weight at temperatures of 70 is associated with the removal of adsorbed water, dehydration with the formation of anhydrous glucose is observed at 210 °C. Further decomposition of anhydrous glucose occurs at temperatures above 310 °C.

Removal of water from quercetin is observed at 60 °C, the dehydration stage begins at 100 °C. Melting of quercetin occurs at 330 °C, which also corresponds to the literature data [6].

The mechanical mixture of powders of quercetin and glucose possesses stability corresponding to the stability of quercetin. The mixture of mechanically treated powders decomposes faster - at 100-190 °C. Thus, the preliminary mechanical activation of the powder mixtures accelerates the interaction of the components.

The glycosylation reactions of quercetin in the liquid phase provide acceptable rates of yield in aprotic solvents and presence of catalysts, hydrochloric acid is usually used. Recently, it has been found that alkali can act as a catalyst in some glycosylation reactions [5]. The both types of catalyst were used in our work.

The effect of additives was studied in a mechanically treated mixtures by adding up to 5% by weight concentrated hydrochloric acid (with HCl concentration of 30%) and the addition of solid adipic acid in an ration of 5-20% by weight, but no catalytic effect on glycosylation reaction was observed.

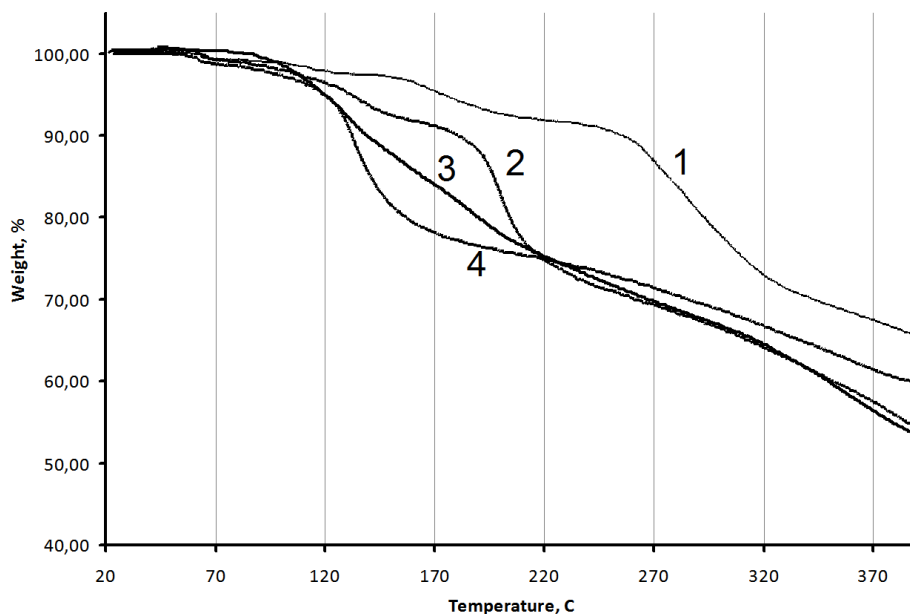


Figure 2. Thermogravimetric profiles of mechanochemically treated samples (AGO-2 5 min) of mixtures glucose : quercetin 1:1 : 1 – without additives, 2 - 5% NaOH, 3 – 10 % NaOH, 4 – 20% NaOH.

The solid alkali (Na_2CO_3 , NaOH) was used as an alkaline catalyst. The significant changes were observed in samples treated in the presence of the strongest alkali - sodium hydroxide. Thermoanalytical data of mechanochemically treated samples depended on various amounts of sodium hydroxide are presented in figure 2. It can be seen that the reaction between the components proceeds in the range 120-200 °C is substantially accelerated by the introduction of increasing from 5 to 20% amounts of sodium hydroxide.

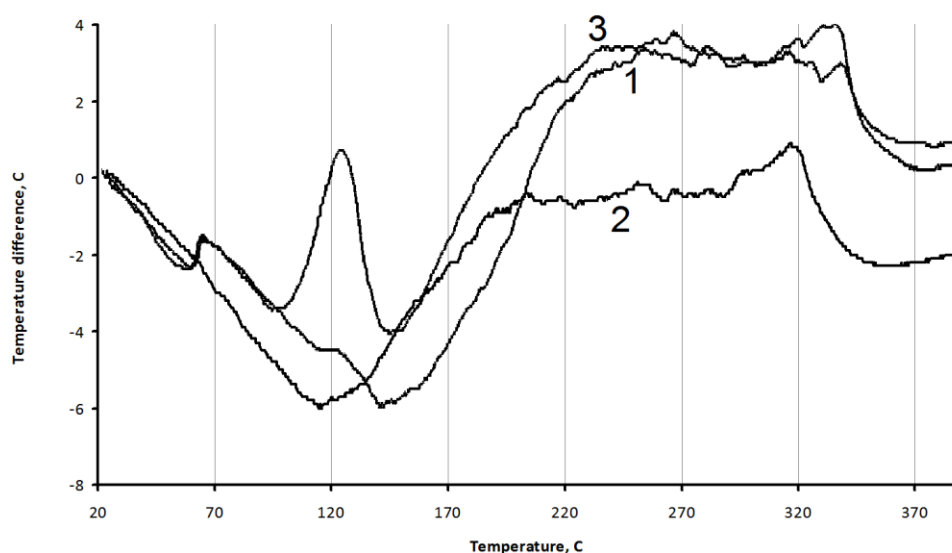


Figure 3. Differential thermal analysis of mechanochemically treated samples (AGO-2 5 min) of mixtures glucose : quercetin 1:1 : 1 - 5% NaOH, 2 – 10 % NaOH, 3 – 20% NaOH.

Figure 3 presents the results of differential thermal analysis of samples of the glucose-quercetin mixture treated mechanochemically in the presence of 5, 10 and 20% of sodium hydroxide. Curve of the sample with 10 % NaOH in the given temperature interval corresponds to the results of [6] for one of the samples of quercetin. The minimum of the endoeffect corresponding to the release of water, with an increase in the amount of alkali up to 10%, is shifted to 110 °C. With an addition amount of 20% against the background of a broad peak of the endoeffect, a relatively narrow peak of the exoeffect appears at 130 °C. This exoeffect could be due to some reaction proceeds or recrystallization of components. The recrystallization process was refuted by X-ray analysis. There is no decomposition of components at so low temperature so we assume that this exoeffect corresponds to glycosylation reaction.

The next exoeffect, apparently associated with the decomposition of the formed product, is already observed at a temperature of about 370 °C.

To confirm the nature of the endoeffect 124 °C, the following additional experiments were carried out. Qualitative analysis by thin-layer chromatography showed that there are the compounds with retention times between reference samples - quercetin and rutin in the samples obtained in the presence of 20% alkali. These compounds can be interpreted as the glycosides of quercetin with 1 glucose molecule.

It is known that glycosylation of sugars in only one position requires special precautions for four of the five hydroxyl groups of sugar capable of glycosylation [7]. Under our conditions, when the protection of the hydroxyl groups of the sugar was not carried out, it is also possible to form diglycosides, in which 2 glucose molecules are attached to two possible glycosylation sites of quercetin. This is indirectly indicated by the appearance of new substances with retention times close to the time of retention of rutin-glycoside quercetin with disaccharide ramnogalactose.

The IR spectra of samples obtained with 20% alkali and heated at 124 °C for 5 minutes, in the region characteristic for the manifestation of glycosidic bonds 950-1010 cm⁻¹ [8], new signals appeared that were absent in the samples obtained without alkali.

4. Conclusions

The possibility of solid-phase mechanochemical glycosylation of quercetin is shown for the first time, the reaction proceeds in the presence of alkaline catalysts.

The formation of glycosides in absence catalysts is not observed, quercetin decomposes.

The formation of water-soluble glycosides with a different degree of glycosylation is observed after mechanochemical treatment of powder followed by heating of mixtures when stronger bases are used as catalyst.

Acknowledgment

The work was supported by grant of Russian scientific foundation N. 16-13-10200

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