Gas chromatographic determination of sorption of aroma substances by corn starch cryosponges

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The sorption of *Rosmarinus Officinals L.* essential oil components by the corn starch cryosponge was studied. The hydrophobic interactions of monoterpene hydrocarbons with polysaccharides of the starch result in their quantitative sorption. The sorption of aroma compounds containing oxygen atoms is twofold lower. The templating effect of mono- and disaccharides (glucose, sucrose, maltose, and some essential oil components) was found.

Key words: capillary gas chromatography, sorption, essential oil components, corn starch cryosponge.

In recent years, templating and imprinting of synthetic polymers are of interest for many researchers. G. Wulff[1] studied templating, *i.e.*, sorption of molecules to form cavities in organic polymers ("stamping" cavities), and imprinting (sorption of molecules in the cavity after removal of the template). The main results of the development of molecular templating and experimental data are presented in the previously published works.[2—6] To obtain highly specific binding sites with a definite shape containing functional groups in the predetermined orientation, a suitable template has to be chosen. Removal of these templates gave polymers with the formed cavities, whose structure and environment of the functional groups were predetermined by the chemical nature of the template. The functional groups in these cavities retain a definite mutual orientation. The fixation of the polymer conformation offered new possibilities of its sorption activity. It was shown that when the template was fixed due to noncovalent bonds, more than 90% of it can be easily removed. The empty cavities formed can be subsequently reoccupied by 80—90% by new molecules in organic solvents. However, when the reoccupation is performed in an aqueous medium, the binding effect decreases to 10—15% due to the relaxation and swelling of the polymer, resulting in a change in the shape of the cavities and losses in their sorption activity.[3—6]

For food biopolymers, unlike synthetic polymers, information on templating and imprinting processes is scarce.[2,7—11] It has been shown previously[12] that freezing of polysaccharide sols gives water-insoluble cryotexturates with retained food properties. Polysaccharide molecules in the cryotexturates are additionally bound to form the rigid texture of the sponge.

Sorption properties of polysaccharide cryotexturates obtained from food biopolymers have not yet been studied. The purpose of this work is to establish conditions for preparing the corn starch cryosponge; to study the sorption by the cryosponge of a mixture of essential rosemary oil components from 0.05% aqueous solutions; to determine the effect of the saccharide-templates on the sorption of aroma compounds by the cryosponge; and to evaluate the nature of noncovalent interactions of the aroma compounds with the cryosponge.

Experimental

Preparation of corn starch sol and cryotexturates. The corn starch sol (the content of amylose in the corn starch was 25—27%) was obtained by heating a 3% slurry of starch (300 mL) in distilled water to 90 °C with vigorous stirring. The sol cooled (for 3 h) to ~20 °C was divided into 40-g aliquots, which were placed in polypropylene tubes with caps. The rosemary oil *Rosmarinus Officinals L.* (Robertet) (20 mg) and carbohydrates (400 mg) (glucose, sucrose, or maltose), which gave concentrations of oil and saccharide in the solution of ~0.05 and 1.0%, respectively, were used as additives to the sol. The mixture of the sol with the additives was vigorously shaken. The samples of the original sol and the sol with the oil or saccharide additives were stored for 3 h at 5 °C and for 16 h at −18 °C. After slow freezing out (16 h) at ~20 °C, the cryosponge formed was separated. The negative reaction with iodine showed that the dissolved starch was completely absent in the aqueous phase. Unsorbed oil components in the aqueous phase were determined by capillary gas chromatography (GC). The sorption properties of the cryosponge were studied in special experiments. Each experiment was twice repeated.

Characterization of samples. Sample I. The rosemary oil (20 mg) was added to a 3% aqueous solution of the starch slurry (40 g) with stirring, and the mixture was stored for 3 h.

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The starch was separated by centrifuging, and the aqueous phase was analyzed by GC.

Samples II—IV. Cryosponges were obtained from the sol with an additive of the rosemary oil (20 mg). After freezing out at −20 °C, the aqueous phase was separated from the cryosponge for the GC determination of the unsorbed oil components.

Sample V. Cryosponges were obtained from the starch sol. After freezing out, the rosemary oil (20 mg) was added to the aqueous phase above the cryogel, the mixture was thoroughly stirred and stored at 20 °C for 16 h, and the aqueous phase was separated from the cryosponge for the gas chromatographic determination of the unsorbed oil components.

Sample VI. The cryosponge of sample II was washed with distilled water (5×100 mL) to remove the sorbed oil components, and the mass of the sponge was brought to 40 g by distilled water. The rosemary oil (20 mg) was added, the mixture was stored at 20 °C for 16 h, and the aqueous phase was separated from the cryosponge for the GC determination of the unsorbed oil components.

Sample VII. The cryosponge obtained from the sol with the glucose additive (400 mg) was separated and washed with distilled water (5×100 mL) to a negative reaction for glucose (the silver mirror reaction). The mass of the sponge was brought to 40 g by distilled water, and the rosemary oil (20 mg) was added with vigorous shaking. The sample was stored at 20 °C for 16 h to establish equilibrium. The unsorbed oil components were determined by GC.

Sample VIII. A cryosponge was obtained from the sol with the sucrose additive (400 mg). For subsequent procedures, see sample VII.

Sample IX. A cryosponge was obtained from the sol with the maltose additive (400 mg). For subsequent procedures, see sample VII.

Reference sample. The rosemary oil (5 mg, 0.05%) was added to distilled water (10 mL), and the mixture was vigorously stirred. The oil components in the sample were determined by GC after addition of an internal standard (*n*-dodecane, 500 μg) and extraction of organic compounds with diethyl ether (2 mL).

Gas chromatographic analysis. To determine the unsorbed oil components, the internal standard (*n*-dodecane, 500 μg) was added to the aqueous phase (10 mL) obtained after separation of the cryosponge in samples II—IX, and the organic compounds were extracted with diethyl ether (2 mL). The analyzed probe was 1 μL of the ether extract.

Ether extracts of samples I—IX and the reference sample were analyzed by GC on a Hewlett—Packard 5730A chromatograph using a fused silica capillary SPB-1 column (60 m × 0.32 mm, d_f = 0.25 μm) in the temperature-programmed regime (from 60 to 250 °C) with a rate of 8 deg $\min^{-1}$. The temperature of the injector and detector was 250 °C, and the flow rate of the carrier gas (helium) was 1.5 mL $\min^{-1}$. The retention time and peak areas were recorded on a Hewlett—Packard 3390A integrator. Each sample was analyzed 3—4 times. The concentrations of unsorbed oil components in the solution were calculated from the peak areas of the compound and standard. The averaged data of the analyses of two parallel samples were used for the calculation of the sorption percentage of the compounds, which was determined from the difference between the concentrations of the compounds added (reference sample) and the concentration of samples I—IX found in the solution.

Results and Discussion

Noncovalent hydrogen bonds and electrostatic interactions are the most important types of binding in polysaccharides. Hydrophobic interactions, which are typical of proteins, are not usually considered. In our case, the reactions of the OH groups of the corn starch with the functional groups of low-molecular aroma compounds mainly occur.

Initially we supposed that the low oil concentration (0.05%) in the corn starch sol resulted in the almost quantitative sorption of all compounds containing the —OH, —O—, or —CO groups and in lower sorption of terpene hydrocarbons. However, unexpected results were obtained for the sorption of the essential oil components. As can be seen in Table 1, the corn starch (3%) in the aqueous slurry (sample I) sorbs the hydrocarbons (the first five compounds) by 68—98% and γ-terpinene by 45%. The γ-terpinene molecule contains double bonds. The compounds with the functional groups are sorbed by 10—42%.

The cryosponges obtained from the sols containing 0.05% oil (samples II—IV) sorb more the compounds with the hydroxyl, carbonyl, and ether functional groups (on the average, 51—58%). The terpene hydrocarbons are sorbed by 75—87%, including γ-terpinene (82%) (see Table 1). The cryosponge sorbs the essential oil components better than the starch slurry, which suggests hydrophobic interactions between the terpene hydrocarbons and the starch cryosponge. The low sorption percentage for the compounds with the functional groups in the case of the starch slurry may indicate the competitive sorption with water molecules, which also form hydrogen bonds with the OH groups of the starch polymer.

Table 1. Sorption of the rosemary oil components by the corn starch cryosponge

Compounds	Sorption (%)				
	I	II	III	IV	II—IV*
α-Pinene	94	88	82	85	85
Camphene	80	89	83	83	85
β-Pinene	90	87	86	87	87
β-Myrcene	87	86	86	87	86
p-Cymene	68	77	75	74	75
1,8-Cineole	19	58	56	53	56
γ-Terpinene	45	80	83	82	82
Linalool	10	52	51	52	51
Camphor	15	52	49	52	51
Terpinen-4-ol	42	58	50	51	53
α-Terpineol	32	62	57	55	58
Bornyl acetate	28	86	86	82	85

Note. I is the sample of a slurry of the corn starch in distilled water; II, III, and IV are cryosponge samples obtained from the corn starch sol containing 0.05% oil. * Average value (%).

The pure cryosponge obtained from the starch sol without additives (Table 2, sample V) sorbs from an aqueous solution of essential oil a smaller amount of *p*-cymene and 1,8-cineole than the cryosponge obtained from the sol containing 0.05% oil (sample IV).

When the starch cryosponge was preliminarily templated by the oil components and washed out from the template molecules (see Table, sample VI), the repeated sorption of the same compounds is considerably greater than that for the untreated cryosponge V. If the sorption of each of the compounds in sample V is assumed to be 100%, in cryosponge VI, for example, the sorption is increased to 171% for *p*-cymene, to 139% for 1,8-cineole, to 118% for γ-terpinene, to 119% for camphor, and to 113% for bornyl acetate. A comparison of the sorption properties of the starch slurry (sample I) and the cryosponge templated by the oil components (sample VI) shows that the sorption of all components is increased by 1.5—6.0 times for alcohols and by 3—4 times in the case of ethers, esters, and ketones (samples I and VI).

Thus, cryostructuring of the corn starch sol increases the sorption activity of the cryosponge obtained due to the additional noncovalent interactions resulting in conformational changes and fixation of the polysaccharide structure. In our case, the cryosponge is probably formed by the formation of a three-dimensional polymer network stabilized by noncovalent interactions as has been previously shown for the chemically cross-linked polymers.[13]

The increase in the sorption of the aroma compounds by the oil-treated cryosponge (see Table 2, sample VI) is related to an increase in the surface of the starch polymer due to noncovalent interactions of the compounds with the polymer and the formation of cavities around the sorbed molecules. In the case under question, these cavities are fixed, because they are formed at the stage of formation of the cryosponge. This makes it possible to study sorption in aqueous solutions.

Table 2. Sorption of the rosemary oil components by the corn starch cryosponge treated with the oil, glucose, sucrose, or maltose

Compound	Sorption (%)				
	V	VI	VII	VIII	IX
α-Pinene	82	94	97	96	97
Camphene	83	99	98	99	99
β-Pinene	87	98	99	97	100
β-Myrcene	89	97	97	97	96
p-Cymene	55	94	97	96	97
1,8-Cineole	48	67	70	72	73
γ-Terpinene	80	95	89	84	97
Linalool	52	59	70	71	71
Camphor	52	62	67	69	68
Terpinen-4-ol	58	61	73	75	76
α-Terpineol	62	64	86	72	79
Bornyl acetate	84	95	80	81	88

Note. Cryosponge samples obtained from the corn starch sols: V, without template additives; VI, templated by the rosemary oil; VII, templated by 1% glucose; VIII, templated by 1% maltose; and IX, templated by 1% sucrose.

We also studied the effect of glucose, sucrose, and maltose as template-molecules on the sorption properties of the corn starch cryosponge. Mono- and disaccharides stabilize starch sols due to the formation of inclusion complexes.[14—16] It can be assumed that the orientation of the polysaccharide fragments of the starch and the formation of the cavities in the polymer occur due to the hydrogen bonds of the OH groups of amylose and, perhaps, the starch amylopectin. In this case, after removal of the templates by water, the sorption activity of the cryosponge should increase as compared to the untreated sponge. In fact, after the removal of glucose, the glucose-templated sponge VII (see Table 2) sorbs more linalool (70%), terpinen-4-ol (73%), and α-terpineol (86%) than the nontemplated (sample V) and oil-templated (sample VI) sponges.

A similar effect was established in the case of sucrose and maltose, whose molecular weights are twofold higher than that of glucose. Sucrose and maltose should form half as many cavities as glucose with sizes twice as large as those of glucose. The sorption properties of the cryosponge templated by 1% glucose, sucrose, or maltose were equivalent (see Table 2). This sponge sorbs all the substances 11—12% better than that untreated with mono- and disaccharides. Such high sorption of aroma compounds in an aqueous solution under equilibrium conditions was obtained for the first time.

Thus, the results obtained allow one to conclude that the corn starch cryosponge sorbs low-molecular-weight aroma compounds from aqueous solutions due to noncovalent hydrophobic interactions and hydrogen bonds. The templating effect of low-molecular-weight food compounds such as glucose, sucrose, and maltose results in an increase in the sorption of essential oil components.

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