Binding of cyclic compounds by starch cryotextures from aqueous solutions

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Binding of cyclohexanol, 2-, 3-, and 4-methylcyclohexanones, and isomeric fenchone and camphor bicycles by the corn starch cryotextures from aqueous solutions in a concentration range of 1—16 mmol L^{-1} was studied using capillary gas chromatography. Cyclohexanone is not sorbed by the cryotexture. All substances except fenchone exhibit linear plots of the amount of substances bound by the cryotexture *vs.* their concentration in the corn starch gel. The empirical constants in the linear dependence of the concentration of cryotexture-bound odorants on their initial concentration in the gel were calculated. The presence and position of methyl substituents in the ring affect the degree of binding of the cyclic compounds. The sorption isotherms of the cycles have different shapes. The apparent binding constants and the number of binding sites were determined for compounds reaching the saturation stage on sorption. Cooperative interactions between the binding sites were found. The most part of compounds are irreversibly bound by the cryotexture pointing to the formation of supramolecular complexes.

Key words: binding from aqueous solutions, cyclohexanol, methylcyclohexanones, fenchone, camphor, corn starch cryotexture, binding constant, capillary gas chromatography.

Binding of low-molecular compounds by biopolymers in various biological systems is very important. Polysaccharides are main components of food products. Their interaction with volatile organic compounds has a significant effect on odor formation of the products. An increasing interest in studying these interactions is caused by the possibility to use polysaccharides as inert supports for food aromatizers.[1—3] Odorants are bound by polysaccharides *via* adsorption, penetration into micropores, complexation, and hydrogen bond formation. It is known that volatiles of different classes of organic substances form inclusion complexes with amylose of starches.[1—10] The experiments were mainly carried out with dry forms of standard or modified polysaccharides and their sols and suspensions.

We pioneered studies on binding of odorants by starch cryotextures, which differ drastically in their structural and sorption characteristics from suspensions and sols of native starch. Binding of different classes of organic compounds by the corn starch cryotexture was studied, and a dependence of the sorption on the type and position of the functional group and the alkyl substituent length was established.[11—17] Odorants can form supramolecular complexes with polysaccharides of amylose-containing cryotextures.[17—20] For aliphatic alcohols, acetates, and ketones, binding was shown to depend to a greater extent on the alkyl substituent length and to a less extent on the type of the functional group and its position. The purpose of this work was to study the influence of the structure and concentration of cyclic compounds on their binding by corn starch cryotextures.

Experimental

Delipidized corn starch produced at the All-Russian Institute of Starch Products of the Russian Academy of Agricultural Sciences containing 25—27% amylose was used as the initial material. The corn starch gel was obtained by heating a 3% suspension of the starch in distilled water to 90 °C with intense stirring. The gel (40 g) was cooled to ~20 °C and placed in polypropylene tubes with caps, and freshly distilled cyclohexanone, 2-, 3-, and 4-methylcyclohexanones, cyclohexanol, or fenchone were added with intense shaking. Camphor was added to the gel as a 58% solution in Et_2O. The concentration of cyclic compounds was varied from 1 to 16 mmol L^{-1}. To form supramolecular structures, gel samples with odorant additives were stored for 3 h at +5 °C. To form cryotextures, the relevant gels were stored for 24 h at −18 °C, and then the samples were unfrozen and stored for 16 h at ~20 °C to establish equilibrium.[12] The cryotexture obtained was separated from the aqueous phase, from which 10-mL samples were taken. A 20% ether solution (10 μL) of the internal standard, *viz.*, heptan-2-one, was added to the aqueous samples. The content of the free part of the ligands in an aqueous phase was determined by capillary gas chromatography. Each experiment was repeated 3—4 times.

In order to study the sorption of ketones by native corn starch granules, corn starch (1.2 g) was placed in a glass 100-mL

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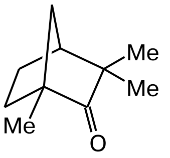
flask, and distilled water was added until the total weight reached 40 g (3% suspension). Individual cyclohexanone, 2-, 3-, and 4-methylcyclohexanones, cyclohexanol, fenchone, or camphor were introduced into the obtained suspension with vigorous shaking in amounts providing their concentration of 6 and 10 mmol L^{-1}. The samples thus prepared were kept for 16 h at ~20 °C with permanent stirring. The suspensions obtained were centrifuged, and the aqueous phase was separated. To determine the remaining nonsorbed substances, 10 mL of the aqueous phase were sampled, the internal standard was added to the sample that was analyzed by capillary gas chromatography. Each experiment was repeated 2—4 times.

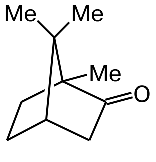
The obtained samples of aqueous solutions were analyzed on a Biokhrom chromatograph with a flame-ionization detector. The temperature of the detector and evaporator was 200 °C. A quartz capillary column (Chrompack, Holland) with the CP-Wax 58CB stationary phase was used (25 m × 0.32 mm, thickness of the phase layer d_f = 0.2 μm). Analysis was carried out in the isothermal regime at 80 and 100 °C. The carrier gas (helium) flow at the inlet of the column was split in a ratio of 1 : 50. The volume of the introduced sample was 2 μL. The amount of nonsorbed substances was calculated from the surface areas of the substance and standard. The amount of sorbed odorants was determined as the difference between the weight of the substance introduced into the gel and the amount of the substance remained in the aqueous phase after separation of the cryotexture. The error of determination, including errors of GC analysis, was 5—7%. The sorption of the substance was calculated in mg per g of the dry starch.

Results and Discussion

Cyclohexane derivatives are components of many food flavor compounds. Therefore, six-membered cyclic ketones and cyclohexanol were chosen as objects of the study. Binding of isomeric bicyclic ketones, *viz.*, fenchone and camphor, which are present in essential oils of kitchen herbs, by the corn starch cryotexture was also studied.



Fenchone



Camphor

As in the case of aliphatic alcohols, acetates, and ketones,[11—17] the amount of sorbed six-membered cyclic compounds increases linearly with an increase in their concentration in the initial gel (Fig. 1). It was found that cyclohexanone was not bound by the corn starch cryotexture in the concentration interval studied.

As can be seen in Fig. 1, the position of the Me group in the ring affects the binding of cyclohexanones: the *para*-isomer shows the lowest degree of binding, and the differences in binding of the *meta*- and *ortho*-isomers are insignificant. The content of the bound *para*-isomer is twofold lower than that of the *meta*- and *ortho*-isomers, and that of the *meta*-isomer exceeds that of the *ortho*-isomer by ~9%. Probably, such a difference in binding of methylcyclohexanones by the cryotexture is caused by differences in conformations of the molecules. It is known that the cyclohexanone molecules exist in the chair conformation and the main difference between them is the quantitative ratio of isomers with the axial and equatorial positions of the Me group.[21] For example, the fraction of the axial isomers in 2-, 3-, and 4-methylcyclohexanones is 5, 15, and 50%, respectively. In our case, 4-methylcyclohexanone, which exhibits a greater number of the axial isomers than the 2- and 3-methyl-substituted compounds, is the most difficult to be bound by the cryotexture.

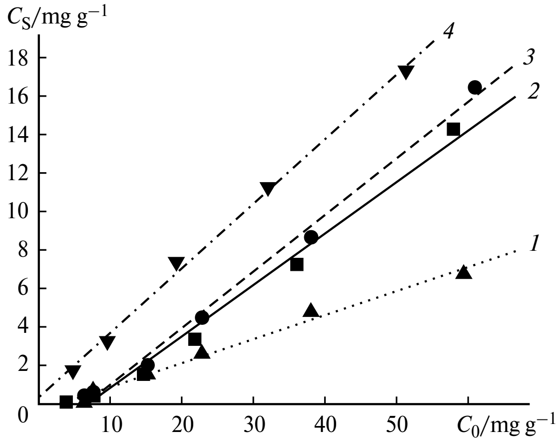


Fig. 1. The amount of six-membered cyclic compounds bound by the corn starch cryotexture (C_S/mg g^{-1} of dry starch) as a function of their initial concentrations (C_0) in the 3% gel: 4-methylcyclohexanone (*1*), 2-methylcyclohexanone (*2*), 3-methylcyclohexanone (*3*), and cyclohexanol (*4*).

Unlike cyclohexanone, cyclohexanol is bound by the cryotexture to a much greater extent than methylcyclohexanones (see Fig. 1). A comparison of binding of cyclohexanol and previously studied *n*-hexanol[14] by the starch cryotexture showed that the presence of the ring increases the binding of the alcohol. The degree of binding of cyclohexanol is twofold higher than that of *n*-hexanol. At the same time, the degrees of binding of methylcyclohexanones and aliphatic isomers of heptanones are comparable.[17] The extent of sorption of these compounds does not exceed 30%, *i.e.*, in ketones with the close molecular weights the structure of the hydrocarbon framework has no substantial effect on the sorption.

The degree of binding of bulky bicyclic compounds is particularly sensitive to the position of the Me substituents in the ring. The fenchone molecule with two Me groups located in the immediate vicinity of the keto group is sorbed much more poorly than the camphor molecule with the Me groups distant from the carbonyl groups. It is of interest that during GLC fenchone is retained on

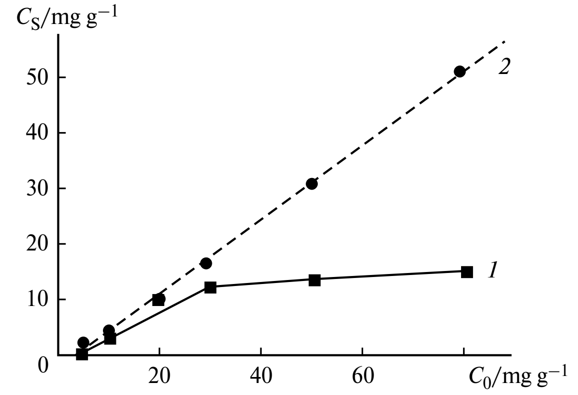


Fig. 2. The amount of fenchone (*1*) and camphor (*2*) bound by the corn starch cryotexture (C_S/mg g^{-1} of dry starch) as a function of their initial concentration (C_0) in the 3% gel.

the nonpolar stationary phase more weakly than camphor, their retention indices being 1070 and 1175, respectively. The plots of the amounts of the bound bicycles (C_S) *vs.* concentration of the bicyclic compounds in the initial gels (C_0) are presented in Fig. 2. For camphor this dependence is linear (R = 0.999), *i.e.*, similar to that observed for six-membered cyclic and previously studied aliphatic compounds.[12–17] The nonlinear character of the dependence of the amount of the sorbed compound on its concentration in the initial sol was first found for fenchone (see Fig. 2, curve *1*). When C_0 increases from 30 to 80 mg g^{-1}, the amount of sorbed fenchone remains virtually unchanged, while that of sorbed camphor increases. The degree of binding of camphor by the cryotexture is twofold higher than that of six-membered cyclic ketones.

The sorption isotherms were calculated to examine the influence of the structure of odorants on binding by the cryotexture. Figure 3 presents the sorption isotherms plotted in the coordinates of the Scatchard equation[4,22]

$$V/L = nK - KV, \qquad (1)$$

where V is the degree of filling of the polymer (mmol per g-equiv. of glucose), L is the equilibrium concentration of the unbound odorant in the aqueous phase (mmol L^{-1}), and n is the number of binding sites (per 1000 glucose units), and K is the apparent binding constant.

The sorption isotherms of 2- and 3-methylcyclohexanones (see Fig. 3, *a*, curves *2* and *3*) and camphor (see Fig. 3, *b*, curve *6*) are concave. Similar curves have previously been obtained for octan-4-one, nonan-2-one, *n*-octanol, and *n*-octyl acetate.[15,17] Probably, polylayer sorption occurs along with monolayer sorption in these cases. The shapes of the sorption isotherms of 4-methylcyclohexanone, cyclohexanol (see Fig. 3, *a*, curves *1* and *4*), and fenchone (see Fig. 3, *b*, curve *5*) indicate that the polymer is saturated with the odorant. When the binding sites in the macromolecule are identical (*i.e.*, of the same type) and independent, binding is defined as noncooperative and the plot of V/L *vs.* V is linear. It is found that for 4-methylcyclohexanone, cyclohexanol, and fenchone this plot is nonlinear, which points to interactions between the binding sites.[22] Similar plots of V/L *vs.* V were obtained for the sorption of *n*-hexanol, hexan-2-one, octan-2-one, and hexyl acetate by the cryotexture.[15,17] The nonlinear plot of V/L *vs.* V implies that the polymer contains binding sites of different types and binding of one odorant molecule changes the affinity of the polymer toward the next sorbate molecule.[22] This binding is defined as cooperative, and the semiempirical Hill approach[4,22] is used for its description

$$\ln L = -(1/h)\ln[(n/V) - 1] + \ln K, \qquad (2)$$

where h is the Hill coefficient. The K values obtained from Eq. (2) are apparent binding constants, *i.e.*, they

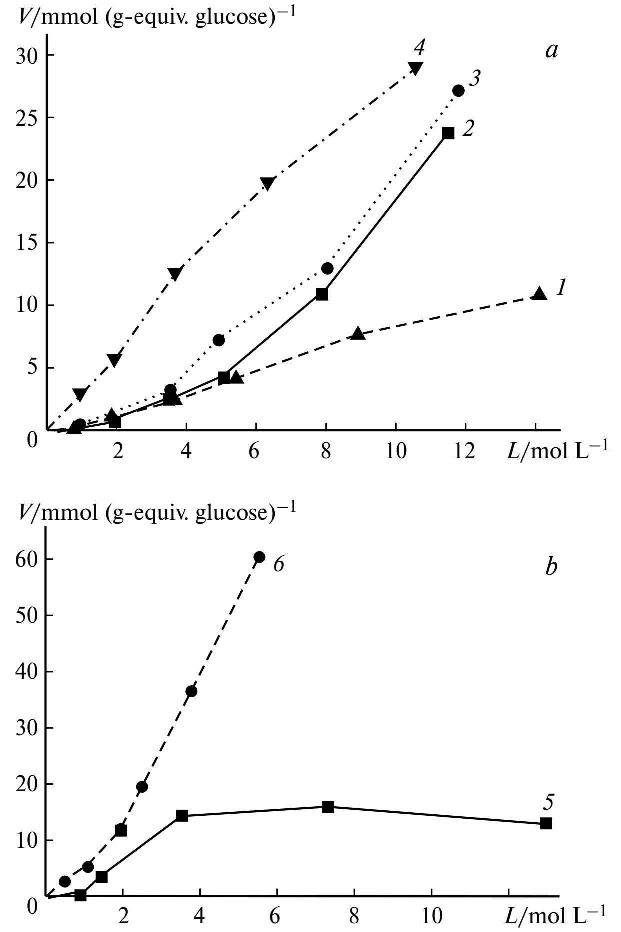


Fig. 3. Sorption isotherms of six-membered cyclic compounds (*a*) and bicyclic compounds (*b*) on the corn starch cryotexture: 4-methylcyclohexanone (*1*), 2-methylcyclohexanone (*2*), 3-methylcyclohexanone (*3*), cyclohexanol (*4*), fenchone (*5*), and camphor (*6*); V is the degree of filling of the polymer, and L is the equilibrium concentration of the odorant in the aqueous phase.

Table 1. Quantitative binding characteristics of odorants by the corn starch cryotexture from aqueous solutions

Compound	n	h	K/mol^{-1}
4-Methylcyclohexanone	15	2.1	120.8
Hexan-2-one[17]	12	1.8	217.0
Cyclohexanol	50	1.3	115.1
n-Hexanol[17]	16	2.4	165.2
Fenchone	17	2.7	480.7

Note. The following designations were used: n is the number of binding sites (per 1000 glucose units), h is the Hill coefficient, and K is the binding constant.

depend on the concentrations of the components because the number of binding sites can change with concentration changing.

The number of binding sites n was determined from the plot of V/L *vs.* V for the compounds reaching the saturation stage on sorption. Using n in Eq. (2), we calculated the Hill coefficients and apparent binding constants (Table 1). The Hill coefficients provide an information on the degree of cooperativity.[22] If $h > 1$, binding is of cooperative character, and the higher Hill coefficient, the higher the degree of cooperativity of the process.[22] The highest cooperativity of the process is observed for binding of fenchone by the cryotexture ($h = 2.7$). This compound is also characterized by the highest binding constant. The hexan-2-one and 4-methylcyclohexanone exhibit close n and h values but differ in binding constants. For example, for aliphatic ketone K is higher than that for the cyclic compound by 1.8 times. The greater distinctions are observed for alcohols. Less sorbed *n*-hexanol exhibits a higher cooperativity of interactions in binding by the cryotexturate. The Hill coefficient for the aliphatic alcohol nearly doubles that for cyclohexanol. At the same time, the number of binding sites of cyclohexanol triples that for *n*-hexanol, and that probably results in a higher degree of binding of the cyclic compound.

Aliphatic ketones are sorbed mainly irreversibly: depending on the length of the hydrocarbon chain, from 3 to 10% of the adsorbed substances undergo desorption.[17] At the same time, only 25—30% of cyclic ketones and camphor are desorbed by diethyl ether. Sharp distinctions in desorption capability are observed for aliphatic and cyclic hexanols. The extent of desorption of *n*-hexanol from the cryotexture achieves[14] 80%, and it does not exceed 12% in the case of cyclohexanol. Thus, binding of cyclic compounds by the starch cryotexture is mainly irreversible.

The study of binding of cyclic compounds by native corn starch granules showed that in the region of concentrations studied cyclohexanones, unlike aliphatic ketones,[17] were not sorbed by native starch. The initial concentrations (C_0) and the amounts of cyclohexane, *n*-hexanol, and camphor sorbed by the cryotexture and native starch (C_S) are presented below.

Sorbate	C_0	C_S	
		Cryotexture	Granules
Cyclohexanol	16	6.0	5.8
n-Hexanol	16	—	1.2
Camphor	29	16.5	10.7

The sorption activity of cyclic alcohol toward native starch is higher than that of aliphatic alcohol. Camphor is bound by native starch granules somewhat weakly than by the cryotexture, although the degree of its sorption on native starch reaches 40%. Unlike camphor, fenchone is not bound by native corn starch in the concentration region studied.

Thus, the results outlined above showed that the binding of cyclic compounds by the corn starch cryotexture depends on the presence and position of substituents in the ring and the type of the functional group. Distinctions in conformations of the molecules also affect the degree of binding. The process of binding of cyclic compounds is mainly irreversible and includes the formation of supramolecular complexes of the compounds studied with polysaccharides of the starch cryotextures.

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