Sorption of aliphatic ketones from aqueous solutions by starch cryotextures

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Capillary gas chromatography was applied to study the sorption of aliphatic ketones (C_6—C_{11}), including metamers, from aqueous solutions by corn starch cryotextures. The amount of ketones sorbed by cryotextures depends linearly on their concentrations in the initial sol. Equations describing the concentration dependence of sorption were proposed. The shape of sorption isotherms reflects the strength of sorption. The binding constants and the number of binding sites were determined for weakly sorbed ketones. The length of alkyl substituent and the position of the functional group are the crucial factors governing the sorption of ketones under conditions of excess binding sites. It was found that the degree of sorption increases with an increase in the carbon chain length from 6 to 9 carbon atoms. The presence of cooperation of binding sites for ketone sorption by cryotextures was demonstrated. The major part of ketones is sorbed irreversibly. This fact points to the formation of supramolecular complexes. Ketones with lower molecular masses are better sorbed by cryotextures than by native starch grains.

Key words: corn starch cryotexture, aliphatic ketones, sorption from aqueous solutions, constants of binding of sorbates, capillary gas chromatography.

Odor-forming compounds can act as ligands in reactions with proteins and polysaccharides. Binding of low-molecular-weight organic compounds by proteins and polysaccharides to yield non-covalent host—guest type complexes can involve adsorption, complex formation, and hydrogen bonding. Currently, there is an increased interest in the studies of retention of odor-forming compounds by carbohydrates, which can be used as matrices for trapping volatile compounds.[1—5] Aldehydes, aliphatic alcohols, fatty acids, and their derivatives are known to form inclusion complexes with starch amylose.[1,6—9]

In previous communications,[10—14] we presented the first data on sorption of odorants by starch cryotextures, which differ sharply in structural and sorption properties from suspensions and sols of native starch. A system consisting of an aqueous solution of odorants and a cryotexture readily separable from the solution is very convenient for quantitative GLC analysis of sorption. Previously, we have studied sorption of essential oil components from rosemary, individual alcohols, acetates, and their mixtures by cryotextures prepared from corn starch sols and have shown that odorants are capable of forming supramolecular complexes with polysaccharides of amylose-containing cryotextures.[15—18] The purpose of this work is to study the influence of the structure and concentration of aliphatic ketones on their sorption by corn starch cryotextures.

Experimental

Delipided corn starch (received from 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 sols were prepared by heating a 3% suspension of starch in distilled water to 90 °C with intense stirring. The sol (40 g) cooled to ~20 °C was placed in polypropylene tubes with caps, and freshly distilled hexan-2-one, 2-, 3-, and heptan-4-ones, 2-, 3-, and octan-4-ones, 6-methylheptan-3-one, 2- and nonan-5-ones, decan-2-one, and undecan-2-one were added with vigorous shaking. The concentration of ketones varied from 1 to 16 mmol L^{-1}. The sol samples with the ketones added were kept for 3 h at +5 °C and for 24 h at −18 °C, unfrozen, and kept for 16 h at ~20 °C to establish equilibrium between the aqueous phase and the sorbent. The resulting cryotexture was separated from the aqueous phase; 10-mL samples of the aqueous phase were taken. Ethereal solutions (2—30 μL) containing 20% of heptan-2-one or octan-2-one as an internal standard were added to aqueous samples. The content of non-sorbed ketones in the aqueous phase was determined by capillary gas chromatography and calculated from the results of two to four parallel runs.

To study sorption of ketones by native starch grains, 1.2-g samples of corn starch were placed in 100-mL glass flasks, and distilled water was added to make a total weight of 40 g (3% suspension). Freshly distilled individual hexan-2-one, heptan-2-one, octan-2-one, nonan-2-one, decan-2-one, and undecan-2-one were introduced with vigorous stirring in the resulting suspension in amounts corresponding to a concentration of 10 mmol L^{-1}. The samples thus prepared were kept for 16 h at ~20 °C with repeated stirring during this period. The resulting suspensions were centrifuged and the aqueous phase was separated. To determine the remaining non-sorbed com-

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pounds, a 10-mL aliquot of the aqueous phase was taken, the internal standard was added, and the sample was analyzed by capillary gas chromatography.

The resulting samples of aqueous solutions were analyzed by gas chromatography on a Hewlett—Packard 5730 A chromatograph with a flame ionization detector; the detector and evaporator temperature was 250 °C, a quartz capillary column with the SE-30 stationary phase was used (50 m × 0.32 mm, thickness of the phase layer d_f = 0.25 μm). The analysis was carried out under isothermal conditions at 120 °C. The carrier-gas (helium) flow at the inlet of the column was split in 1 : 50 ratio. The sample volume was 2 μL. The amounts of ketones not sorbed by the cryotexture or the native starch were found from the peak areas of the analyte and the standard. The amounts of sorbed substances were found as the difference between the quantity introduced in the sol and that remaining in the aqueous phase after separation of the cryotexture. The degree of binding upon sorption was found as the ratio of the concentration of the sorbed ketone to the ketone concentration introduced in the sol.

The error of the determination of the amount of non-sorbed compound including the error GC analysis was 5—7%. The amount sorbed was expressed in mg per g of dry starch taken for the preparation of the initial sol for cryotexture or the suspension of native starch.

The reversibility of sorption by the cryotexture was investigated for heptan-2-one and decan-2-one present in the sol in an initial concentration of 10 mmol L^{-1}. After separation of the aqueous phase, distilled water was added to give a total weight of 40 g. The cryotexture—water system was kept for 16 h at –20 °C and the amount of desorbed ketone in the aqueous phase was determined by gas chromatography.

When describing the process of odorant binding, the concentration of an odorant in a cryotexture was expressed in mg g^{-1}, while in constructing the binding isotherms, the concentration of an odorant in the equilibrated solution was expressed in mmol L^{-1}, and that in starch was defined as mmol g-equiv.$^{-1}$ of glucose.

Results and Discussion

The study was carried out with aliphatic C_6—C_{11} methyl ketones and their isomers with different positions of the functional group.

Figure 1 shows the amount of ketones sorbed by the cryotexture from aqueous solutions as a function of their concentration in the sol. The dependence of the ketone adsorbed by starch C_s on the initial ketone concentration in the sol C_0 is reflected by the following linear equation:

$$C_s = A + BC_0. \qquad (1)$$

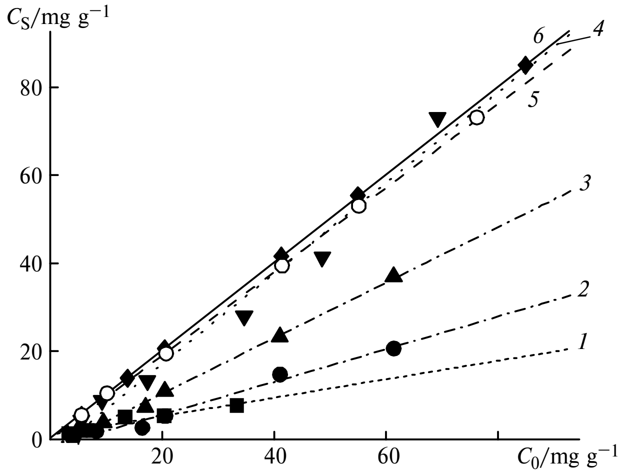

Fig. 1. Amount of ketones sorbed by corn starch cryotexture (C_S/mg g^{-1} of dry starch) *vs.* their initial concentration (C_0/mg g^{-1} of dry starch) in a 3% sol: hexan-2-one (*1*), heptan-2-one (*2*), octan-2-one (*3*), nonan-2-one (*4*), decan-2-one (*5*), undecan-2-one (*6*).

The coefficients of the linear equation are listed in Table 1. It can be seen from Fig. 1 that the dependence of the amount of sorbed ketones on the number of C atoms becomes noticeable at elevated (>10 mg g^{-1}) starting concentrations of the sorbates. The increase in the chain length from six to nine carbon atoms results in a greater amount of the material sorbed, whereas the increase in the chain length from 9 to 11 carbon atoms has no influence on sorption. A similar effect of sharp increase in the amount sorbed on passing from heptan-2-one to nonan-2-one has been noted in a study of binding of odorants by albumin;[19] in that case, sorption was accompanied by conformational changes in the protein. In a study of sorption of nonan-2-one by β-lactoglobulin, it was found that an increase in the ketone concentration changes the number of binding centers and induces a conformational change in the protein.[20] This means that during binding, the character of interaction of the odorant with the sorbent changes.[20] In the sorption of odorants by proteins and starch cryotextures, the amount of the bound material is found to be proportional to its initial concentration.[21,22]

Unlike the reversible sorption of ketones by proteins,[2,20] sorption of the major part of ketones by starch

Table 1. Coefficients of linear equation (1) describing the dependence of the amount of ketone adsorbed by starch on the initial ketone concentration

Ketone	*A*	*B*	*R*	*SD*
Hexan-2-one	1.11	0.21	0.963	0.80
Heptan-2-one	−1.76	0.37	0.991	1.17
Heptan-3-one	−2.04	0.37	0.991	1.18
Heptan-4-one	0.50	0.19	0.975	1.01
Octan-2-one	−2.19	0.63	0.998	0.83
Octan-3-one	−1.19	0.32	0.983	0.96
Octan-4-one	−3.33	0.83	0.998	1.25
6-Methyl-heptan-3-one	−1.81	0.29	0.991	0.93
Nonan-2-one	−3.41	1.03	0.987	4.61
Nonan-5-one	−2.37	0.89	0.999	0.71
Decan-2-one	−0.007	0.95	0.999	0.66
Undecan-2-one	−0.02	1.0	1.0	0.01

Note. R is the correlation coefficient; *SD* is the standard deviation.

cryotextures is irreversible. In a study of sorption reversibility, it was found that only a part of ketones is desorbed by water: ~3% for heptan-2-one and 10% by decan-2-one.

Sorption of ketones depends substantially on the position of the carbonyl group. Data on sorption of heptanone, octanone, and nonanone metamers present in the sol in identical initial concentrations, equal to 10 mmol L^{-1}, are presented below.

Ketone	Sorption (%)
Heptan-2-one	30.0
Heptan-3-one	30.0
Heptan-4-one	22.0
6-Methyl-Heptan-3-one	24.0
Octan-2-one	57.0
Octan-3-one	30.5
Octan-4-one	75.0
Nonan-2-one	87.5
Nonan-5-one	85.0

Symmetric heptan-4-one is bound by the cryotexture to the lowest degree. The migration of the functional group from the second to the third C atom in heptanone does not affect sorption. The shift of the C=O group from position 2 to position 3 results in a decrease in the amount sorbed. Sorption of octan-3-one by the cryotexture is almost twice as low as that of octan-2-one. Of octanone isomers, octan-4-one is sorbed most efficiently by cryotexture (75% of the ketone is sorbed). It was found that chain branching remote from the functional group has little influence on sorption. As shown above, octan-3-one is sorbed only 7% more efficiently than 6-methyl-heptan-3-one, which has a branched alkyl chain. Thus, the shift of the functional group changes sharply the character of sorption. The degree of sorption of octan-3-one is comparable with that of heptan-2-one and heptan-3-ones (Fig. 2), while the sorbed amount of octan-4-one is somewhat lower than that of nonanones. The position of the oxo group in the beginning or in the center of the nonanone molecule does not affect sorption of the isomer, as indicated by the fact that the sorbed amounts of nonan-2-one and nonan-5-one are equal.

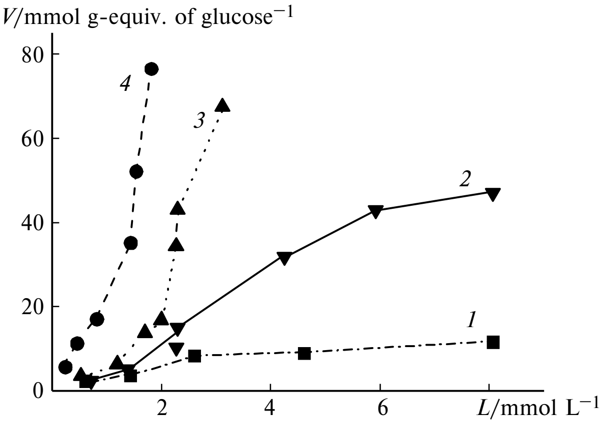

Fig. 2. Isotherms of sorption of hexan-2-one (*1*), octan-2-one (*2*), octan-4-one (*3*), nonan-2-one (*4*) by starch cryotexture.

To elucidate the relationship between sorption and structure of ketones, we calculated the dependences of the concentrations of sorbed substances (*V*, mmol per g-equiv. of glucose, which is equal to 167) on the equilibrium concentration of the non-bonded odorant in the aqueous phase (*L*/mmol L^{-1}). As an example, Fig. 2 shows the sorption isotherms for octan-4-one and nonan-2-one (curves *3* and *4*), whose degrees of binding are more than 70%, and for hexan-2-one and octan-2-one, which are sorbed less efficiently (curves *1* and *2*). The pattern of the sorption isotherms for the readily sorbed compounds attests to a complex mechanism of the binding of these substances by the cryotexture. A similar shape of sorption isotherms has been observed for nonan-5-one and decan-2-one and for individual *n*-octanol and *n*-octyl acetate whose degrees of sorption exceed 70%.[14] Since the shapes of adsorption isotherms for two octanone metamers differing in the position of the functional group (2 or 3) are different, it can be concluded that the position of the C=O group influences the mechanism of sorption. In accordance with the interpretation of various shapes of sorption isotherms,[23] in the case of readily sorbed compounds, the monolayer packing of the sorbed material in the cryotexture bulk might be accompanied by the formation of multilayers.

The sorption isotherms for hexan-2-one, octan-2-one (see Fig. 2, curves *1* and *2*), heptan-4-one, and octan-3-one by the cryotexture show that the sorbents are saturated with ketones even at relatively low ketone concentrations. The binding of low-molecular-weight ligands with macromolecules is accompanied by the interaction of adsorption sites with ligands if they are bound to one macromolecule.[23] The binding sites in a macromolecule may be sites of the same type. In this case, binding is called non-cooperative and the variation of the concentrations ratio of the bound ligand to the free ligand (V/L) *vs.* free ligand concentration (V) is linear (Skatchard dependence). A nonlinear dependence of V/L on V implies that the macromolecule has binding sites of more than one type and, in addition, the binding of one ligand molecule changes the affinity of the macromolecule to the next ligand molecule. If the subsequent ligand molecules are bound more strongly than the first ones, the Skatchard plot is a curve convex toward the abscissa axis. This situation corresponds to the case where an increase in the number of bound molecules of the ligand results in interaction between the sorbed molecules. The presence of convex curves is evidence for the fact that the sites of binding also interact with one another.[23] These curves for the variation of V/L as a function of V describe the behavior of hexan-2-one, octan-2-one as well as hexan-1-ol and *n*-hexyl acetate. The point of intersection of the curves with the abscissa axis shows the number of binding sites *n*. As an example, Fig. 3 shows the plots for V/L *vs.* V

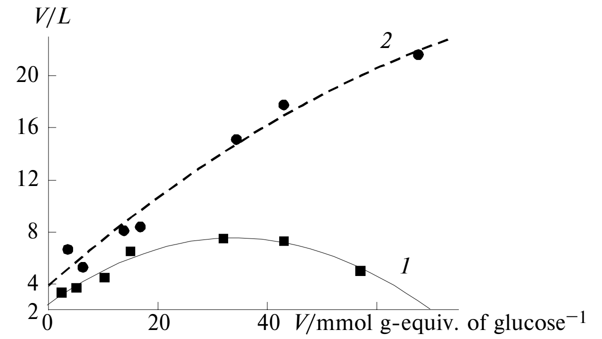

Fig. 3. Skatcherd curves for sorption of octan-2-one (*1*) and octan-4-one (*2*) by starch cryotexture.

Table 2. Quantitative characteristics of odorants sorption by corn starch cryotextures from aqueous solutions

Compound	*n*	*h*	*K*/mol^{-1}
Hexan-2-one	12	1.82	217.0
Octan-2-one	75	1.68	190.6
n-Hexanol	16	2.43	165.2
n-Hexyl acetate	34	2.03	1053.1

Note. *n* is the number of binding sites (per 1000 glucose units); *h* is the Hill coefficient; *K* is the binding constant.

Table 3. Sorption of 2-methyl ketones by an aqueous suspension of native corn starch grains (I) and its cryotextures (II)

Ketone	Degree of sorption (%)	
	I	II
Hexan-2-one	19	23
Heptan-2-one	13	30
Octan-2-one	24	57
Nonan-2-one	73	85
Decan-2-one	92	94
Undecan-2-one	97	100

Note. The initial concentration of the ketone in the suspension and in the sol was 10 mmol L^{-1}.

for 2- and octan-4-one. It can be seen from Fig. 3 that the *n* value can be calculated only for octan-2-one. The *n* values found can be used to determine the Hill coefficient (*h*) and the apparent binding constants[23]

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

where *K* is the apparent constant of binding. The *K* values depend on the concentration of components because the number of binding sites can change upon the variation of the concentration.

Table 2 contains the quantitative characteristics found for two ketones, and also for *n*-hexanol and *n*-hexyl acetate, whose binding has been studied in our previous works.[14] The smallest and similar numbers of binding sites were found for *n*-hexanol and hexan-2-one close in molecular mass. The greatest *n* value equal to 75 corresponds to octan-2-one.

The *h* value can be used to judge what interactions are cooperative. It can be seen from Table 2 that *n*-hexanol is bound by the cryotexture more weakly than ketones; it is characterized by the highest coefficient *h*, while the binding constant for *n*-hexanol is lower than those for ketones or hexyl acetate. The last-mentioned compound is fixed much more firmly than ketones: its binding constant is 5 times higher than the *K* values for ketones or for the alcohol.

In order to evaluate the influence of the polysaccharide structure on the binding of volatile organic compounds, we compared sorption of methyl ketones on the pellets of native corn starch and on its cryotexture prepared from a sol containing these compounds in an initial concentration of 10 mmol L^{-1}. As follows from the data listed in Table 3, ketones with lower molecular masses are better sorbed by the cryotexture than by native starch. Indeed, the degree of sorption of heptan-2-one and octan-2-one on the cryotexture is 1.5—2 times greater than that on native starch. As the molecular mass increases, the difference between the degrees of sorption of ketones on the two types of starch becomes less pronounced. Decan-2-one and undecan-2-one are sorbed by native starch and the cryotexture to virtually equal degrees. The close quantitative characteristics do not rule out the possibility of different mechanisms of sorption by starch grains and by its cryotextures.

The results of this study demonstrate that the length of the alkyl substituent is the main factor affecting the degree of sorption of *n*-alkyl ketones by the amylose-containing cryotexture. This indicates that the crucial role in the binding of these compounds by the starch cryotexture is played by hydrophobic interactions. The position of the functional group influences sorption in the case of metamers, *i.e.*, on migration of the functional group in the center of the carbon chain. In the case of irreversible sorption, supramolecular complexes of ketones with polysaccharides of starch cryotextures arise during cryotrexturing.

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