Assessment of Interactions between Hydrocolloids and Flavor Compounds by Sensory, Headspace, and Binding Methodologies

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Studies were performed to understand interactions between volatile flavor compounds and hydrocolloids. Sensory analysis of flavored solutions [1-octen-3-ol (mushroom), diallyl disulfide/diallyl sulfide (garlic), and diacetyl (buttery)] showed that both the in-mouth overall and garlic flavors were greatest for water, intermediate for 0.1% xanthan, and lowest for the 0.3% guar gum. This order corresponded to their viscosity at mouth shear rates. No significant differences were found for mushroom and buttery attributes. Equilibrium headspace analysis confirmed that xanthan and guar gum addition lowered flavor release, with the largest decreases found for the diallyl sulfides (50%). The occurrence of molecular interactions between xanthan (0.01%) and 1-octen-3-ol was investigated by exclusion chromatography. Weak reversible hydrogen bonding interactions were found with about one binding site per pentasaccharide repeating unit.

Keywords: *Viscosity; volatility; binding; headspace; sensory*

INTRODUCTION

Hydrocolloids are widely used in the food industry due to their thickening properties at low concentration. An understanding of how they influence the flavor of a product can aid in optimizing product quality. Their effects on flavor release usually show a decrease in release related to increasing viscosity, caused by two main mechanisms. One is diffusion as shown by the Stokes−Einstein equation, where diffusion is inversely proportional to viscosity (Wilke and Chang, 1955). The distance that the molecule must diffuse to be released in the mouth will be less for more spreading materials. The second mechanism comprises molecular interactions of the flavor compounds with the macromolecule. In the evaluation of viscosity effects, sweetness, rather than volatile flavor, has been mainly studied. Several investigators have shown increases in solution viscosity to cause a decrease in the rating of sweetness and other flavor sensations (Morris, 1987; Vaisey et al., 1969). Aroma sensations were different in their time−intensity release and in the rating of dimethyl sulfide (Malkki et al., 1993; Pangborn and Szczesniak, 1974). The decrease in headspace amounts with different thickeners, as measured by analytical means, also depended on the particular flavor compound studied (Roberts et al., 1996; Schirle-Keller et al., 1992).

The aim of the present study was to investigate flavor compound retention by thickeners using different methods for the measurement of interactions between polysaccharides and flavor compounds. Sensory analysis showed which flavor sensations were influenced by hydrocolloid addition. Both viscosity and molecular interactions could be affecting the sensory perception. The static headspace method allowed the determination of global flavor compound retention due to molecular interactions. A chromatographic method was used to directly measure molecular interactions. The most widely used method for ligand−macromolecule interactions (Hummel and Dreyer, 1962) required a column that was able to separate the ligand and the macromolecule by a size exclusion mechanism (Sebille et al., 1987, 1990). The use of several related techniques allows deeper knowledge not only into mechanisms but also of the actual perceptual magnitude of the effects.

MATERIALS AND METHODS

Sensory Analysis. Ten panelists were selected and trained for their ability to describe and distinguish volatile flavor molecules. The flavor mixture gave a medium intensity of each attribute: diacetyl (0.9 ppm), diallyl disulfide (0.4 ppm), and 1-octen-3-ol (7 ppm). The flavor compounds were obtained from Sigma-Aldrich Chemicals (Steinheim, Germany), and their mixture was evaluated in three systems: Volvic mineral water, 0.3% guar gum (Meyprogat 150, Rhône-Poulenc Meyhall, Lyon, France), and 0.1% xanthan gum (Rhodigel Easy, Rhône-Poulenc Meyhall). Final concentrations were selected for a similar apparent viscosity at 10 s^{-1} and to show an intermediate in-mouth viscosity. They were prepared by mixing the flavor in ethanol with cold water and adding the hydrocolloid with additional shear and storage at 20 °C until tasting. Four attributes were evaluated: overall flavor intensity and mushroom, garlic, and butter notes. Tasters scored the attributes 5 s after swallowing. The line rating scale was used with anchors at both ends and in the middle of the scale (1 = not, 5 = medium, and 9 = very strong). Data acquisition was conducted with the FIZZ system (Biosystèmes, Dijon). Hydrocolloids in water were tested at 23 °C and taken with a straw to avoid interaction with direct olfaction. Rinsing was allowed between products (water and crackers were available). The samples were evaluated three times following a balanced presentation order.

Flow Curve Measurements. Flow curves (23 °C) were measured with a Rheometrics RFS II using a cone and plate

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geometry. Powders were mixed in cold water for 15 min. There was a preshearing of 5 min at 100 s^{-1}, and the flow curve was recorded within 5 min from 0.0025 to 500 s^{-1}, with a logarithmic step. Concentrations were screened from 0.1% to 1.4% for guar gum and from 0.04 to 1% for xanthan gum.

Headspace Analysis. Static headspace analysis was conducted in triplicate using 40 mL amber flasks (Supelco, Bellefonte, PA). The samples were composed of 5 mL of an aqueous stock flavor solution and 5 mL of either water, xanthan, or guar gum solutions. Diacetyl and 1-octen-3-ol (Fluka, Buchs, Switzerland) were 91 and 96% pure, respectively. Diallyl disulfide (Acros Organics, Springfield, NJ) was found to be 65% diallyl disulfide, 26% diallyl sulfide, and 9% diallyl trisulfide. Final concentrations were as follows: diacetyl (60 ppm), 1-octen-3-ol (90 ppm), and diallyl disulfide (20 ppm) in water, xanthan (0.01%), xanthan (0.1%), or guar gum (0.3%). A 90 min equilibration time at 30 °C was used after verification that equilibration was complete after 60 min. A gastight syringe was used to sample 1 mL of the vapor phase from the flasks, which was injected onto an HP 5890 GC-FID equipped with a DB-Wax column (J&W Scientific, Folsom, CA) (0.32 mm i.d., 30 m, 0.5 μm) with a hydrogen flow rate of 1.9 mL/min at 130 °C.

For the analysis of 1-octen-3-ol alone, a similar static headspace methodology was used. A closed stainless steel tube (312 mL volume) formed the sampling cell, as described previously for one in glass (Chaintreau et al., 1995). A removable receptor was inserted into the bottom end piece to receive 2 mL of solution. After a 30 min equilibration time at 30 °C, headspace sampling was performed by pressing down the piston at a constant flow of 50 mL/min into a stainless steel tube containing 60−80 mesh Tenax as adsorbent. The loaded traps were desorbed in a Chrompack purge and trap injector connected to an HP 5890 gas chromatograph (250 °C, 10 min, H_2 flow of 20 mL/min). The desorbed volatiles were focused on a cold trap (−80 °C) and then desorbed at 260 °C for 3 min onto the GC column (DB-1701, J&W Scientific), 60 m, 0.25 mm i.d., 1 μm. The column temperature was held at 50 °C for 2 min and programmed at 5 °C/min to 200 °C. The detector was maintained at 270 °C.

Solutions containing different concentrations of xanthan (0.01, 0.5, and 0.1%) or guar gum (0.3%) and different concentrations of 1-octen-3-ol (20, 40, and 60 ppm) were prepared in NaCl solutions (50 mM), which were adjusted to pH 6.6 with NaOH (1 M).

Concentrations of free ligand (L) in the vapor phase were determined using the equation (O'Keefe et al., 1991)

$$L = (R/T)I \tag{1}$$

where R (mol/L) is the measured concentration of ligand in the headspace with macromolecule, T (mol/L) is the measured concentration of ligand in the headspace without macromolecule, and I (mol/L) is the initial concentration of ligand in the solution.

HPLC Analysis. A Waters 6000 pump was equipped with a 20 mL Rheodyne injector. The separation was carried out on a Lichrospher 100 Diol (250 mm × 4.6 mm i.d., 10 mm particle size, Merck, Darmstadt, Germany). Detection was made with a UV spectrophotometric detector (Waters, Milford, MA) at $\lambda = 200$ nm. A multichannel chromatography work station (Almanza and Mielle, INRA-Dijon, France) was used for quantitation. The composition of the eluent was pure water with NaCl (50 mM) adjusted to pH 6.6 with NaOH (1 M), filtered at 0.45 mm, and containing different concentrations of ligand (1-octen-3-ol). At a flow rate of 0.8 mL/min, xanthan eluted after 220 s and 1-octen-3-ol after 540 s. Solutions of xanthan (0.01%) were analyzed in the eluent without ligand and diluted with the same volume of ligand solutions. They were equilibrated for 1 h at 30 °C before injection.

Determination of the Air−Water Partition Coefficients. A method that does not require the use of internal or external standards was used to determine the air−water partition coefficients (K_{aw}) of the compounds studied (Chaintreau et al., 1995). One, two, or five milliliters of sample was equilibrated with 312 mL of headspace at 30 °C in an enclosed stainless steel sampling cell. After a 30 min equilibration period, the contents of the headspace were pushed onto a Tenax trap, which was subsequently thermally desorbed using an ATD 400 (Perkin-Elmer, Beaconsfield, U.K.).

Determination of the Molecular Interactions. The concentration of free ligand in the eluent was between 10 and 200 ppm. The concentration of bound ligand was determined by internal calibration method. When increasing ligand concentrations were injected with the macromolecule, the trough area decreased and a positive peak appeared. An extrapolation to 0 peak area permitted the calculation of the bound ligand concentration.

The maximum number of binding sites (n) can be calculated using the saturation curve:

$$\nu = nL[((1/K_a) + L)] \tag{2}$$

For a macromolecule having a number of equivalent and independent binding sites (n), the reversible interactions between flavor molecules and macromolecule can be represented thermodynamically by the Scatchard equation (eq 3) or the double-reciprocal equation (eq 4)

$$\nu/L = K_a n - K_a \nu \tag{3}$$

$$1/\nu = 1/n + 1/(nK_aL) \tag{4}$$

where ν is the number of moles of ligand bound per mole of macromolecule, K_a is the intrinsic affinity constant, and L is the concentration of free ligand (Scatchard, 1949).

However, the macromolecule can have some nonequivalent and dependent binding sites. Thus, the binding of flavor compounds on a site facilitates the binding of other molecules on other sites. A cooperation between the sites exists, and the interactions can be represented thermodynamically by the Hill equation (eq 5) or its double-reciprocal form (eq 6)

$$\nu = n/[(1/(K_aL)^h + 1] \tag{5}$$

$$1/\nu = 1/[n(K_aL)^h] + 1/n \tag{6}$$

where h is the Hill coefficient reflecting the cooperation between the sites (Hill, 1910).

RESULTS AND DISCUSSION

Sensory Analysis. Significant differences between products were observed by taking into account differences between tasters and interactions between products and tasters (two-way ANOVA product × tasters).

In most cases, the following results were observed. The taster effect was significant: the tasters used different parts of the scale. Some always gave low scores, others high scores. This result was expected as individuals have different recognition thresholds and the training focused only on taster repeatability and ranking agreement. Interactions between product and tasters were not significant: the tasters ranked the products in the same order.

The products were significantly different regarding two attributes: overall flavor intensity and the garlic note. Table 1 shows that for both attributes the intensities were guar 0.3% < xanthan 0.1% < water. For overall flavor intensity, there was a masking effect of the hydrocolloids. The flavor intensity was higher in water than in hydrocolloid solutions. Furthermore, there was an effect of hydrocolloid type: guar gum masked flavor more than xanthan gum.

Table 1. Sensory Analysis Results Showing Significant Differences at the 5% Level (ANOVA Table, Duncan Test)[a]

Duncan Test	overall flavor			garlic			mushroom			butter		
product	mean	group	SD	mean	group	SD	mean	group	SD	mean	group	SD
water	5.8	A	1.1	5.6	A	1.5	4.0	A	1.0	3.1	A	1.0
0.1% xanthan	4.9	B	1.4	4.1	B	1.9	4.0	A	1.1	3.5	A	1.1
0.3% guar	3.9	C	1.0	3.2	C	1.3	3.5	A	1.5	3.1	A	1.6

ANOVA Table

	overall flavor			garlic			mushroom			butter		
variation	DF	*F* value	*p* value	DF	*F* value	*p* value	DF	*F* value	*p* value	DF	*F* value	*p* value
product (P)	2	18.1	0.0002	2	16.28	0.0004	2	1.11	0.362	2	0.54	0.5958
taster (T)	6	6.88	<0.0001	6	12.42	<0.0001	6	4.6	0.001	6	5.31	0.0004
interaction P × T	12	0.66	0.7764	12	1.26	0.2763	12	0.81	0.64	12	1.39	0.2088
residual error	42			42			42			42		
total	62			62			62			62		

[a] Products with same the letter are not significantly different (DF, degrees of freedom; SD, standard deviation).

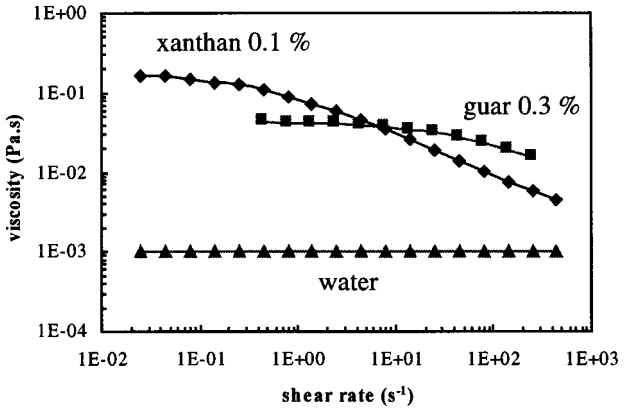

Figure 1. Flow curves of solutions measured with a Rheometrics RFSII cone and plate viscometer at 23 °C.

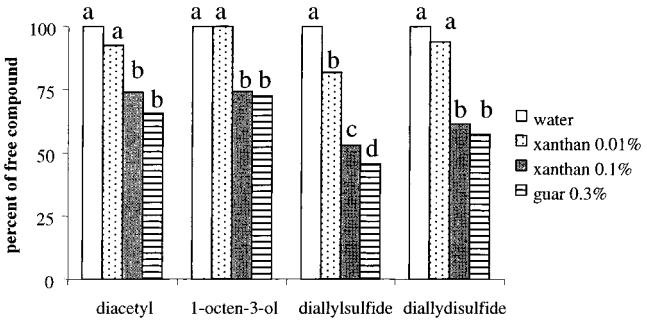

Figure 2. Amount of free compound as shown by relative headspace analysis of compounds analyzed together in the presence of different hydrocolloid solutions. Different letters mean that the results are significant at the 5% level (*t* test).

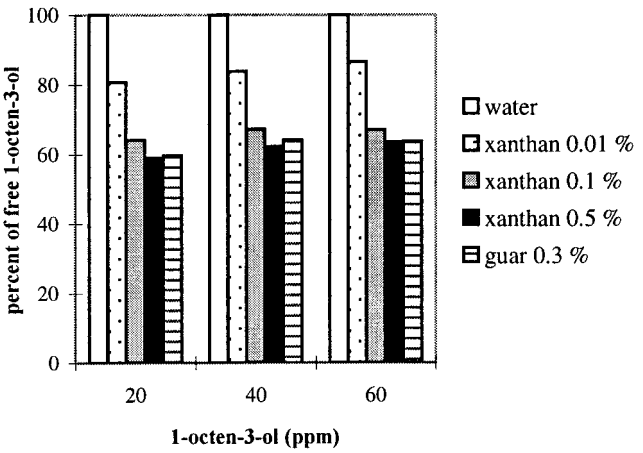

Figure 3. Amount of free compound as shown by relative headspace analysis of 1-octen-3-ol in the presence of different hydrocolloid solutions.

The flow curves (Figure 1) show that the differences between the products can also be interpreted in terms of rheological behavior. Water, a Newtonian fluid, had the lowest viscosity. Guar and xanthan had the same apparent viscosity at 10 s^{-1}, but xanthan was more shear thinning. The overall flavor intensity showed that xanthan had a reduced intensity from water and guar had the lowest. The results can be explained in terms of their viscosities at mouth shear rates. The mouth shear rate for 0.5% xanthan was found to be between 10 and 100 s^{-1} (Cutler et al., 1983). This less viscous 0.1% xanthan solution would have a shear rate in this range or higher. In fact, at shear rates above 10 s^{-1} (mouth shear rates), the viscosity order from lowest to highest corresponded with overall flavor intensity: higher viscosity solutions had lower intensities.

Headspace Analysis. Figure 2 shows the equilibrium headspace results for the three compounds measured together in the different carbohydrate matrixes. The sample of diallyl disulfide had a significant impurity: diallyl sulfide. Because this flavor compound is also garlic-like and could have contributed to the garlic sensory perception, its headspace results are also reported. At a xanthan concentration of 0.01%, only the diallyl sulfide showed a significant binding. At a xanthan concentration of 0.1%, all compounds were significantly bound. A slightly higher binding was found for guar (0.3%) than xanthan (0.1%), although this was only significant for diallyl sulfide. Other studies with diacetyl have shown that hydrocolloid addition causes a decrease in headspace concentration when using dynamic headspace sampling (Rankin and Bodyfelt, 1996) but not at equilibrium (Schirle-Keller et al., 1992).

The study was repeated for one compound in isolation to see if the same results would be obtained. Figure 3 compares the relative percent in the headspace for gums, repeated at several different concentrations of 1-octen-3-ol. This is a situation without competition for the binding sites between the molecules. The binding levels are slightly higher than in the mixture, indicating that competition may be occurring in more complex flavorings. For example, in the xanthan (0.01%) solution, diallyl sulfide may have taken the binding site that 1-octen-3-ol occupied when alone.

Table 2. Air−Water Partition Coefficients of Aroma Compounds Measured at 30 °C

diacetyl	1.1×10^{-3}	diallyl sulfide	2.1×10^{-2}
1-octen-3-ol	3.1×10^{-3}	diallyl trisulfide	2.4×10^{-2}
diallyl disulfide	1.6×10^{-2}		

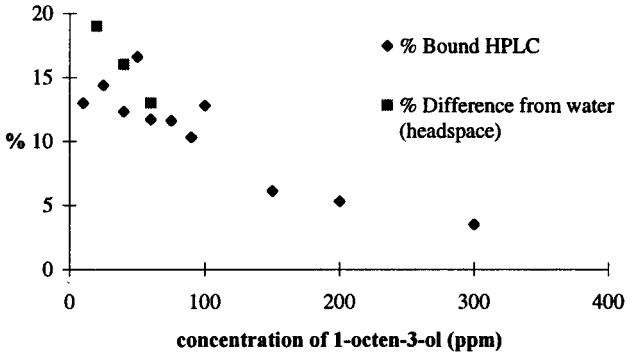

Figure 4. Comparison of percent change in headspace with percent bound as measured by HPLC for 1-octen-3-ol and xanthan (0.01%).

Sensory Analytical Correlations. The static headspace and sensory analysis results are quite similar, even if the former was at equilibrium and the latter is more dynamic. The garlic perception was caused by diallyl disulfide and diallyl sulfide and showed the same order of perception/headspace concentration. The order from greatest to least was water, 0.1% xanthan, and 0.3% guar.

While the garlic perception of diallyl disulfide was diminished by the presence of hydrocolloids, the buttery and mushroom perceptions of diacetyl and 1-octen-3-ol were not. The analytical results show that at equilibrium, the headspace concentrations of these molecules decreased by ∼30%, as compared to ∼50% for the garlic-smelling compounds. A 30% decrease may not have been perceptible. The Steven's law constant (Stevens, 1960) that relates compound concentration to intensity could vary for the different compounds, causing some differences to be better perceived than others.

As demonstrated previously (Roberts et al., 1996), different flavor compounds were affected differently by thickener addition. Compounds with higher volatility in water were more retained by the matrix than those with low volatility in water. Table 2 shows the air−water partition coefficients for the compounds in this study. Indeed, the compound with the highest air−water partition coefficient (diallyl sulfide) was affected most by the gums, followed by diallyl disulfide (with the second highest air−water partition coefficient). Finally, 1-octen-3-ol and diacetyl, which have the lowest air−water partition coefficients, were affected least.

Similarly, gelled thickeners showed the effects of food structure (Guinard and Marty, 1995): flavor release decreased as gel strength increased for carageenan and gelatin but not for starch gels. The use of compounds with high water volatility (ethyl butyrate, limonene, and benzaldehyde) allowed differences to be seen. The ability of thickeners to change flavor depends, in part, on the characteristics of the flavor compound.

Binding Analysis. Although reductions in the headspace during equilibrium indicate that binding is present, a direct measurement of the binding was desired. However, the binding analysis was performed only for 1-octen-3-ol and xanthan due to purity and coelution limitations of the other combinations. Both methods of binding analysis, HPLC and equilibrium headspace analysis, gave similar results. Figure 4 shows this similarity.

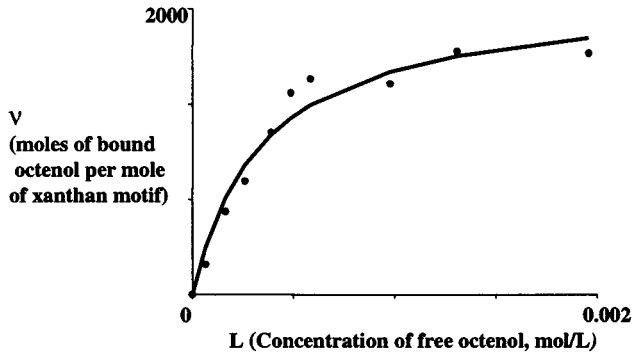

Figure 5. Saturation curve showing the maximum number of binding sites.

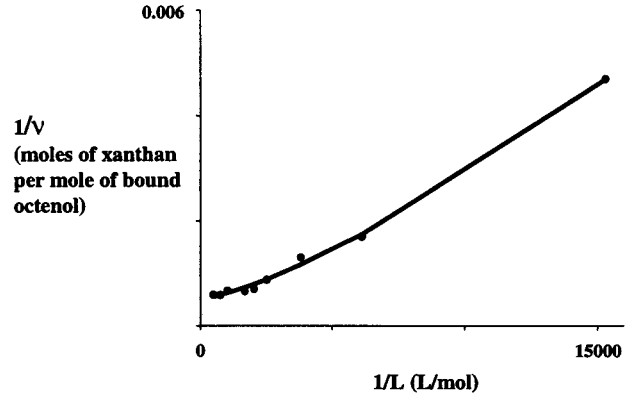

Figure 6. Double-reciprocal Hill plot for xanthan (0.01%) and 1-octen-3-ol obtained by HPLC with the Hummel and Dreyer method.

Table 3. Binding Parameters of 1-Octen-3-ol with Xanthan

parameter	saturation curve	Hill plot
R^2	0.963	0.967
n	2100	2000
K_a (L/mol)	2900	3100
h		1.3

The method used an eluent containing the ligand to be studied dissolved at a given concentration. A small amount of macromolecule was injected into the column; a positive peak appeared corresponding to the ligand−macromolecule complex, and a negative peak emerged at the ligand retention volume. The negative peak area depended directly on the amount of bound ligand.

Figures 5 and 6 show the saturation curve and double-reciprocal Hill plot, respectively. The number of binding sites was determined by the intersection with the y-axis and the association constant by the slope. The line was not completely linear, so the value of h, the Hill coefficient, was determined. This value indicates the cooperativity.

Both the saturation and Hill curves gave very similar values for the number of binding sites and the association constants (Table 3). The Hill constant indicates some cooperativity, although this value may actually not be different from 1. The free energy of binding was ∼−20 kJ/mol, which indicates weak hydrogen bonding.

Xanthan is made up of a pentasaccharide repeating unit with β-1−4 linked glucose as the backbone. Every other glucose has a trisaccharide of mannose, glucuronic

acid, and mannose attached. The mannose units are specifically and variably acetylated and pyruvylated. With ~2700 repeating units per xanthan molecule, this means ~1 binding site per unit. The hydrogen bonding could be occurring between the hydroxy group of octenol and the oxygen from a carbonyl on xanthan. In comparison with previous studies on inclusion complexes of limonene and menthone with potato starch (Rutschmann and Solms, 1991), the xanthan/1-octen-3-ol system had a greater number of binding sites per sugar molecule and a greater association constant but a lower Hill constant for copperativity.

Conclusion. Flavor compound interactions with hydrocolloids have been shown, with direct evidence of binding. Sensorily significant differences from water were found for the garlic flavor but not for the other attributes. Equilibrium headspace analysis showed confirmation in the order of flavor compound retention (0.3% guar > 0.1% xanthan > water). In addition, the diallyl sulfides (garlic flavor) were bound more than the other molecules. Competition for binding sites was observed by comparing the equilibrium headspace results in competitve and noncompetitive environments. These results can be useful in understanding the molecular and sensory-relevant interactions between hydrocolloids and flavor compounds.

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