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Retention of Aroma Compounds in Starch Matrices: Competitions between Aroma Compounds toward Amylose and Amylopectin

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The retention of three aroma compounds—isoamyl acetate, ethyl hexanoate, and linalool—from starch-containing model food matrices was measured by headspace analysis, under equilibrium conditions. We studied systems containing standard or waxy corn starch with one or two aroma compounds. The three studied aroma compounds interact differently: ethyl hexanoate and linalool form complexes with amylose, and isoamyl acetate cannot. However, in systems containing one aroma compound, we observed with both starches a significant retention of the three molecules. These results indicate that amylopectin could play a role in the retention of aroma. In systems containing two aroma compounds in a blend, the retentions measured for isoamyl acetate and for linalool were either equal to or less than those in systems where they were added alone. This phenomenon was attributed to competition between aroma compounds to bind with starch. The retention of aroma compounds blended in starch-based systems gave us additional information which confirmed that interactions occur not only with amylose but also with amylopectin.

KEYWORDS: Starch paste; flavor retention; interactions; complexes; amylose; amylopectin; competition

INTRODUCTION

Flavors are of major importance in food acceptability by consumers. The perception of aroma compounds is directly related to their availability in the gas phase above the food (headspace). The release of aroma molecules from the food matrix to the headspace is governed mostly by interactions between food components and aroma compounds. Starch is one of the food constituents known for its interaction ability. In particular, its linear fraction, amylose, has the ability to form complexes with many low-molecular-weight organic compounds (*1*−*8*). Complexes are the "combination of ligand and ligand-induced helicated amylose", and the ligands can be included in the cavity of amylose helices or in interhelical spaces (*9*). For a long time, only complexes with amylose were studied, probably because they can be isolated by the precipitation of starch by an excess of ligand, followed by lyophilization. More recently, many workers suggested the existence of interactions involving amylopectin molecules (*10*−*12*).

All these studies were conducted on model systems, very different from foodstuffs. Only a few workers have studied more complex systems. Cayot and co-workers work on a matrix very similar to a real food product. They use commercial starches at 7% (mol/mol) concentration, and the process they apply leads to gelled or highly viscous products. Until now, they have studied the release of linalool, isoamyl acetate, limonene, and ethyl vanillin separately (*13*−*15*). In most cases, interaction properties were studied with one aroma compound at a time. Rutschmann and Solms (*16*, *17*) studied the complex formation between starch and decanal, menthone, 1-naphthol, and/or glycerol monostearate under equilibrium conditions in binary or ternary model systems (starch−menthone−glycerol monostearate). All these compounds are able to form inclusion complexes. The degree of complex formation of the ligands in ternary systems varied with concentration, showing apparent competitive, synergistic, and antagonistic characteristics. The authors observed effects that they attributed to the formation of different helical conformations and "mixed complexes".

The aim of the present study was to understand the interactions between starch and aroma compounds in systems that closely mimic real foodstuffs, and to find out if competition occurs between aroma compounds according to their ability to form complexes with amylose. We particularly wanted to know the respective contributions of amylopectin and amylose in the retention of aroma compounds, alone or in blends. For that purpose, starch pastes containing one or two aroma compounds with different interacting properties were prepared. The retention of each aroma compound in the headspace, depending on the nature of the starch and the presence and the concentration of another aroma compound, was followed.

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MATERIALS AND METHODS

Materials. Two different starches were used: a native standard corn starch (26% amylose) and a native waxy corn starch (<1% amylose). These starches were provided by Roquette Frères (Lestrem, France).

Isoamyl acetate (CAS Registry No. 123-92-2; Aldrich, purity >99%), ethyl hexanoate (CAS Registry No. 123-66-0; Aldrich, purity >99%), and linalool (CAS Registry No. 78-70-6; Aldrich, purity 97%) were used as aroma compounds. Their respective physicochemical properties are as follow: molar weight, 130.18, 144.21, and 154.24 g·mol^{-1}; density, 0.876, 0.873, and 0.768; boiling point, 142, 168, and 198 °C; log *P* (hydrophobicity), 2.13, 2.83, and 3.54. Methyl heptanoate (Sigma, purity ~99%) and ethyl caproate (Sigma, purity ~99%) were used as external and internal standards, respectively.

Preparation of the Starch-Based Food. Standard corn starch or waxy corn starch was hydrated with Evian water (7%), and this blend was gently stirred for 12 min before being cooked in an IKA LR 2000 V reactor. Just before cooking, one or two aroma compounds were added. The temperature of the product inside the cooking device was regulated (±0.1 °C) by means of a laboratory thermostat via the double jacket (Lauda C6CS, temperature sensor PT 100). The oil contained in the double jacket was heated to 120 °C to ensure fast heating of the starch dispersion. The temperature of the product was 25 °C at the beginning of the cooking and was raised asymptotically to 85 °C over 20 min. Cooking then followed for 15 min. The temperature continued to increase due to the inertia of the regulating system. The maximal temperature of the starch paste was 95 °C. Throughout the cooking time, the starch paste was stirred by a stirring tool with wipers, at a rate of 50 rpm.

These operating conditions led, as judged by microscopy, to well-swollen but not totally disrupted starch granules, corresponding to the highest viscosity of the starch dispersion. The product obtained was sampled into hermetic glass vessels for static headspace analysis and stored at 6 °C for 24 h before analysis.

Solutions of aroma compounds in water were used as references. One or two aroma compounds were weighted in 500 mL of Evian water, and these solutions were stirred at ambient temperature, sheltered from light, for 1 h. They were then sampled into glass vessels for static headspace analysis.

Extraction of Aroma Compounds from the Products. As the aroma compounds could be partly lost during cooking, quantification was made in the cooled products by extracting the aroma compounds with a Likens-Nickerson apparatus and quantifying them by GC. After 1 day at 6 °C, 10 g of the starch paste or gel was dispersed in 100 mL of pure water, saturated with NaCl (360 g·L^{-1}). The addition of NaCl improves the extraction output by a "salting-out" effect. Methyl heptanoate was used as the external standard; it was added to the solution at a concentration of 2.5 mg·L^{-1}. This solution was extracted using 25 mL of methylene chloride for 30 min after the boiling point was reached. Ethyl caproate was added to the extracts as the chromatography standard (at a concentration of 0.1 g·L^{-1}). The extraction outputs determined by chromatography were very close to 100%. For those lower than 100%, the obtained concentrations were corrected by the proportion of recovered extraction standard. These extractions were done in triplicate.

Determination of Aroma Compounds Concentration in the Headspace (Starch Pastes and Water). One hour before the analysis, the samples in headspace glass vessels were placed at 25 °C. One milliliter of the headspace was then collected with a gas syringe and directly analyzed by chromatography. Four analyses were done for each product.

Chromatography Conditions. The obtained extracts or gas samples were analyzed with a gas-phase chromatograph (HP 6890) fitted with a split/splitless injector (230 °C) and a flame ionization detector (250 °C; H_2, 30 mL·min^{-1}; air, 300 mL·min^{-1}). It was equipped with a high-resolution gas chromatography column, DB-Wax (J&W Scientific) of 15 m × 0.25 mm i.d. The film thickness was 0.15 μm. Nitrogen was used as the vector gas at a rate of 1 mL·min^{-1}. (makeup rate, 24 mL·min^{-1}). Extracts were injected automatically, and headspace samples were injected manually. Using calibration curves, the weight of aroma compound contained in the injected quantity of headspace or extract was calculated. The corresponding concentrations were expressed in mass fraction (the mass of air was calculated from the injected volume by the perfect gas equation and the apparent molar mass of air, estimated to be 28.8 g·mol^{-1}).

Table 1. Aroma Content in the Different Matrices after Thermal Treatment

	concn of aroma compounds in the matrices (mmol/mol of glucose equivalent, mean values)		
	isoamyl acetate	ethyl hexanoate	linalool
standard corn starch	1.2	–	–
(matrices presented	–	0.7	–
in **Figure 1**)	–	–	1.1
	0.6	0.4	–
	0.6	–	0.6
	–	0.4	0.5
standard corn starch	–	0	1.1
(matrices presented	–	1.9	0.9
in **Figure 2**)	–	3.0	0.9
standard corn starch	–	0.4	0
(matrices presented	–	0.4	1.8
in **Figure 3**)	–	0.3	3.3
	–	0.5	6.1
waxy corn starch	1.1	–	–
(matrices presented	–	0.6	–
in **Figure 4**)	–	–	0.9
	0.6	0.3	–
	0.7	–	0.3
	–	0.3	0.3

Calculation of Partition Coefficients and Retention Coefficients. Partition coefficients K_i^∞ are the ratios of Y_i, the concentration of the aroma compound *i* in the headspace, and X_i, the concentration of the aroma compound *i* in the product (these concentrations were expressed in molar fraction):

$$K_i^\infty = Y_i/X_i$$

Retention was calculated from partition coefficients between air and starch pastes, and between air and water, from the following equation:

$$R = 1 - (K_i^\infty)_{\text{starch paste}}/(K_i^\infty)_{\text{water}}$$

RESULTS AND DISCUSSION

Determination of the Quantity of Aroma Compounds in the Starch Pastes. Because of the loss of aroma during cooking, the exact concentrations of the aroma compounds were measured in the final products. The flavoring rates (the ratio of the quantity of the aroma compound introduced in the system before cooking to the quantity of the aroma compound measured after cooking) were different for each aroma compound, and the one measured for linalool varied with the type of starch. With standard corn starch, the flavoring rates were 38% for isoamyl acetate, 25% for ethyl hexanoate, and 70% for linalool. With waxy corn starch, the flavoring rates were 37% for isoamyl acetate, 24% for ethyl hexanoate, and 57% for linalool. These flavoring rates could be a first indication of the affinity of the aroma compounds for the starch pastes.

As our reasoning was based on molecular interactions between aroma compounds and starch, we managed to obtain similar molar concentrations for the aroma compounds (**Table 1**). Several experiments were necessary to adjust the quantities of aroma compound added before cooking. When aroma compounds were added in blends, their respective quantities were approximately divided by 2, so that there were enough interaction sites available to accept the total amount of aroma

Table 2. Partition Coefficients Calculated for Each Aroma Compound, in Binary or Ternary Systems, in 7% Starch Pastes, or in Water[a]

Studied aroma compound	Other aroma compound in the matrix	K_i^∞ in water (mean value ± standard deviation)	K_i^∞ in 7% standard corn starch gels (mean value ± standard deviation)	K_i^∞ in 7% waxy corn starch pastes (mean value ± standard deviation)
Linalool	none	0.18 (± 0.05)^b	0.03 (± 0.00)^a	0.06 (± 0.01)^a
	isoamyl acetate	0.09 (± 0.01)^a	0.03 (± 0.01)^a	0.07 (± 0.00)^a
	ethyl hexanoate	0.16 (± 0.04)^{ab}	0.03 (± 0.01)^a	0.06 (± 0.02)^a
Ethyl hexanoate	none	11.63 (± 2.31)^a	2.20 (± 0.09)^a	5.01 (± 1.05)^b
	isoamyl acetate	9.31 (± 0.65)^a	1.90 (± 0.02)^b	5.91 (± 1.50)^b
	linalool	9.91 (± 1.15)^a	1.51 (± 0.18)^c	2.68 (± 0.17)^a
Isoamyl acetate	none	12.17 (± 0.60)^b	6.56 (± 0.95)^a	7.15 (± 0.19)^{ab}
	ethyl hexanoate	10.63 (± 0.31)^a	8.96 (± 0.92)^b	7.45 (± 0.70)^b
	linalool	11.23 (± 0.51)^{ab}	10.47 (± 1.42)^b	6.41 (± 0.22)^a

[a] The statistical significance is reported with the letters a, b, or c. Different letters means that there is a significant difference. The values are to be compared inside the cells defined by the bold line.

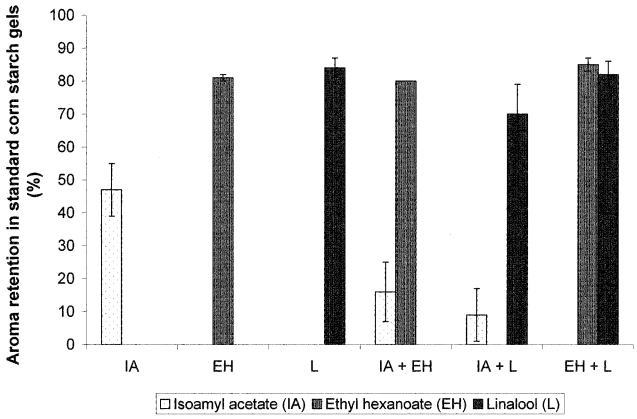

Figure 1. Retention of aroma compounds alone or blended in standard corn starch gels. The aroma compounds were added to a total concentration of 1.5 mmol/mol of glucose equivalent.

compounds added. We expressed our results in the form of partition coefficients and retention values, which are not dependent on the concentrations.

Aroma Retention in Standard Corn Starch Gels. Partition coefficients measured between air and 7% standard corn starch pastes are given in **Table 2**. In systems containing two aroma compounds in a blend, in two cases out of three, the partition coefficient of each aroma compound was modified with respect to the starch suspension containing one aroma compound alone (**Table 2**). This result could be due, on one hand, to the behavior of aroma compounds blended in water, and on the other hand to a competitive or antagonistic effect between aroma compounds and starch. To understand this situation, we measured the partition coefficients of the aroma compounds between air and water. These coefficients were different, depending on whether the aroma compounds were alone or blended (**Table 2**). To take into consideration at once the retention of aroma compounds in starch and in water, the results were given as retention values (**Figure 1**). In other words, retention represents only the retention of aroma compounds by starch. For these calculations, the $(K_i^\infty)_{\text{starch paste}}$ values were used with the corresponding $(K_i^\infty)_{\text{water}}$ values.

In systems composed of standard corn starch, water, and only one aroma compound, linalool and ethyl hexanoate were retained more than isoamyl acetate (**Figure 1**). These results were discussed previously (*15*).They were shown to be due to the formation of complexes between amylose and linalool and between amylose and ethyl hexanoate, whereas isoamyl acetate did not form such complexes.

In systems containing two aroma compounds in a blend, the retention of isoamyl acetate was drastically decreased (**Figure 1**). This can be explained by the ability of linalool and ethyl hexanoate to form complexes with amylose. When there was no ligand or when isoamyl acetate was present, amylose was under the form of double helices (type B). In contrast, it was shown that the presence of linalool or ethyl hexanoate resulted in an arrangement of amylose chains in the $V_{2\text{-propanol}}$-type, i.e., in a simple helical conformation (*15*). So, in the present experiment, amylose was in the form of double helices when isoamyl acetate was added alone and, it can be assumed, in the form of simple helices when the other aroma compounds were present. These different conformational states of amylose might explain the different retentions of isoamyl acetate.

In contrast, the retention of linalool and ethyl hexanoate was not or was only very slightly modified by the presence of another aroma compound (**Figure 1**). It was not surprising that isoamyl acetate had little effect on the retention of linalool and ethyl hexanoate in a medium containing amylose. Conversely, competition between linalool and ethyl hexanoate was expected since both of them were able to interact with amylose to form $V_{2\text{-propanol}}$ structures. We observed that the retention of ethyl hexanoate was very slightly improved in the presence of linalool. In fact, Rutschmann and Solms (*17*) mentioned a possible cooperative effect: the formation of a complex with a first molecule favored the formation of a complex with a second one.

A competition phenomenon might occur at higher aroma concentrations when the free interhelical spaces of amylose

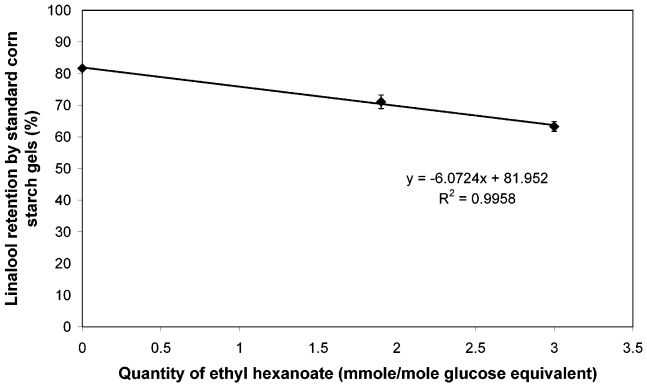

Figure 2. Retention of linalool in standard corn starch gels as a function of the concentration of ethyl hexanoate.

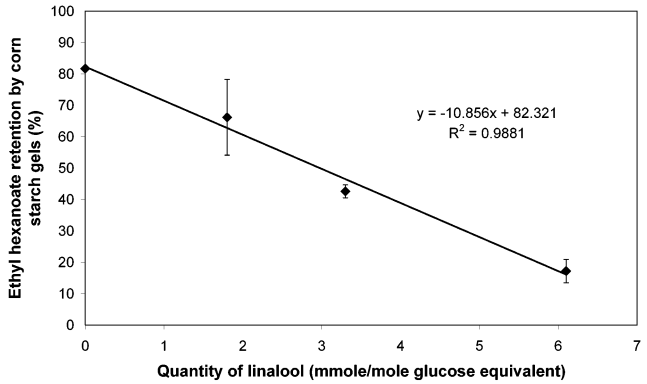

Figure 3. Retention of ethyl hexanoate in standard corn starch gels as a function of the concentration of linalool.

become less important. To confirm this assumption, different concentrations were tested: the concentration of linalool was kept constant (between 0.9 and 1.1 mmol/mol of glucose equivalent), and its retention was measured when the concentration of ethyl hexanoate was varied from 0 to 3 mmol/mol of glucose equivalent (**Figure 2**). The same experiments were done at a constant concentration of ethyl hexanoate (between 0.3 and 0.5 mmol/mol of glucose equivalent) when the concentration of linalool was varied from 0 to 6 mmol/mol of glucose equivalent (**Figure 3**). As expected at these huge concentrations in aroma compounds, a competitive effect was observed. The higher the concentration of one aroma was, the less the second one was retained. For both aroma compounds, we observed a linear decrease of the retention with increasing concentration of the second aroma compound, but it was clear that the addition of linalool had far more influence on the retention of ethyl hexanoate than the opposite.

Aroma Retention in Waxy Corn Starch Pastes. To find out if the retention of the aroma compounds was due to amylose only or to amylose and amylopectin, we made measurements with waxy corn starch, which contained less than 1% of amylose (**Figure 4**).

The retention of linalool and ethyl hexanoate was lower in waxy corn starch pastes than in standard corn starch gels. This was expected because of the higher affinity of these compounds for amylose than for amylopectin, because of the ability to form complexes with the amylose chains (*15*). However, for waxy starch pastes, the retention of these aroma compounds was at least 55%, which could indicate that they interacted with external unbranched chains of amylopectin. It could be assumed that in standard corn starch gels, interactions occurred mainly with amylose because the small concentration of the aroma compound

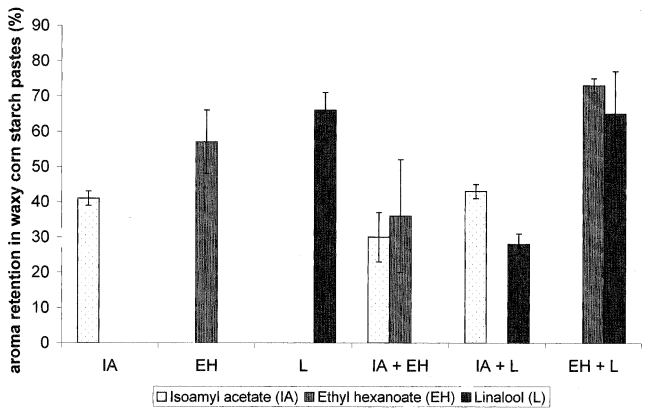

Figure 4. Retention of aroma compounds alone or blended in waxy corn starch pastes. The aroma compounds were added to a total concentration of 1.5 mmol/mol of glucose equivalent.

might not favor additional binding with amylopectin. When there was almost no amylose, i.e., in waxy starch pastes, linalool and ethyl hexanoate could bind with amylopectin. Isoamyl acetate was equally retained by normal and waxy corn starch pastes. This could be explained by a higher affinity of this compound for amylopectin than for amylose. Noncharacterized interactions between isoamyl acetate and amylopectin were hypothesized by Langourieux and Crouzet (*12*). Since standard corn starch contained almost 74% of amylopectin, amylopectin binding sites were available for isoamyl acetate in both corn starches pastes, and so the retentions were equal in those two matrices.

In the waxy corn starch pastes containing two aroma compounds in a blend, the presence of isoamyl acetate clearly decreased the retention of linalool and seemed to decrease also the retention of ethyl hexanoate. The opposite was verified for ethyl hexanoate: the presence of this compound decreased the retention of isoamyl acetate. These two aroma compounds seemed to be in competition for amylopectin, and this result would confirm the assumptions made about interactions between ethyl hexanoate and amylopectin. The formation of interactions between amylopectin and aroma compounds has not yet been demonstrated, but several studies have shown the possibility of such interactions with lipids and surfactants (*18*−*20*) or with alcohols (*21*). According to Eliasson and Ljunger (*22*), some type of inclusion complexes could occur between lipids and the outer branches of amylopectin. The reduced retention of ethyl hexanoate in the presence of isoamyl acetate might be due to an interaction between isoamyl acetate and amylopectin, different from formation of complexes. Morrison (*21*) thought that interaction between lipids and amylopectin could not be in the form of a complex. As far as we know, no type of association between amylopectin and ligands was isolated and characterized. If it interactsin a different way, isoamyl acetate probably did not have the same fixation sites as ethyl hexanoate and linalool on the amylopectin molecule. Different sites close to each other could be imagined for the two compounds, and steric hindrance effects could lower the retention of ethyl hexanoate when isoamyl acetate was present.

As observed for standard corn starch gels, the presence of linalool increased slightly the retention of ethyl hexanoate. For the tested concentrations, it seemed that a cooperative effect took place. Once again, the retention of linalool was not modified by the presence of ethyl hexanoate.

It was noticeable that linalool had no influence on the retention of isoamyl acetate in waxy corn starch pastes.

CONCLUSION

This paper focused on competitions between aroma compounds for the retention by starch at concentrations relevant for food products. Our reasoning was based upon whether these compounds were able to form complexes with amylose. We showed that isoamyl acetate, which is unable to form complexes with amylose, was less retained in starch matrices when compounds that are able to interact with amylose were added, because the formation of complexes modified the structure of amylose and thus the structure of the matrix. The two aroma compounds able to form $V_{2\text{-propanol}}$ complexes with amylose were in competition with each other at high concentrations, but not at food concentrations. This type of behavior is still to be studied for aroma compounds that form other types of interactions (V_h or others).

Linalool and ethyl hexanoate seemed to show the behavior previously described for decanal and 1-naphthol by Rutschmann and Solms (*6*). These compounds form complexes with amylose, and when there is no amylose available, they interact with amylopectin. As in previous studies, our results seem to indicate that isoamyl acetate interacts with amylopectin, although it does not form complexes with amylose (*12*). This interaction may be of a different nature. The characterization of interactions between amylopectin and ligands is not possible because the methods are still to be found, but it would be very interesting because of the large use of amylopectin and waxy starches in food and cosmetic applications.

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