

Influence of Physicochemical Interactions between Amylose and Aroma Compounds on the Retention of Aroma in Food-like Matrices

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In food matrices, where starch is often used as a gelling or texturing agent, the occurrence of amylose–aroma complexes and their effect on the release of aroma compounds are difficult to determine. Indeed, thick or gelled systems are known to reduce the diffusion rate of flavor molecules, resulting in an increase of retention. Moreover, interactions between aroma compounds and matrix components might increase the retention of aroma compounds. The complexing behavior of three aroma compounds with amylose was studied by DSC and X-ray diffraction to determine the relative importance of these two factors. Their interaction properties were different: two of them formed complexes, and the third did not. These aroma compounds were added in food matrices containing different starches that induced different textures. Their retention was studied by static headspace analysis. The retention of aroma compounds appeared to depend on the amylose/amylopectin ratio of starch, both from the formation of complexes and by a viscosity effect.

KEYWORDS: Amylose; aroma compounds; complexes; starch matrices

INTRODUCTION

Starch is widely used in food matrices for its textural properties. The ability of amylose to interact with certain ligands, particularly aroma compounds, has been known for a long time. Some of the formed structures are called complexes and can be described as a “combination of ligand and ligand-induced helicated amylose” (1). It results from nonspecific interactions between the ligand and amylose. These complexes are reversible. They are formed during the gelatinization of starch or during the subsequent cooling (2). Complexes are insoluble in water and this property was for a long time been the sole means of characterization. This property now allows the isolation of amylose–ligand complexes for study by more specific methods, like X-ray diffraction and Differential Scanning Calorimetry (DSC). Precipitation occurs at high concentrations of ligand. Hence, the studies of these complexes could only be made at those concentrations, which are not relevant for food. The formation of such interactions at concentrations used in food has not been confirmed by any method. It was shown that 5 to 10 mmol of aroma compounds per mole of glucose equivalent of starch is necessary to decrease the iodine binding capacity, depending on the aroma compound (3–5), and 50 to 100 mmol of ligand per mole of equivalent glucose is necessary to saturate

amylose helices (4). Different complex structures have been proposed, depending on the properties of the concerned ligand. Not only can the helices have different conformations (6–8), but also the ligand molecules can be located in the helical cavity or at the helix surface (9, 10).

During consumption of a foodstuff, aroma compounds, above a minimal quantity, stimulate the olfactive epithelium, which responds as a function of the intensity of the stimulus, i.e., the quantity of the aroma compound. In simple solutions, the perceived intensity is related to the aroma concentration. But in food matrices, only a fraction of the total aroma is available for perception, partly because of interactions between the matrix and aroma compounds. Perception is better linked with the aroma concentration in the headspace than in the matrix (11). Maier (12) was one of the first to show a decrease in the perception of aroma compounds in food matrices containing starch. Godshall and Solms (13) showed that in matrices containing 1% starch in water the headspace concentration of some volatile compounds was decreased from 0% to 11.5%, compared to the same aroma compounds diluted in water. This behavior may be due to the formation of interactions because at 1%, starch dispersions remain liquid. But at foodstuff concentrations, the texturing agent can also be responsible for an aroma retention increase. Several studies show that at equal viscosity, matrices containing starch retain more aroma compounds than matrices containing other texturing agents (14, 15). This would indicate a synergistic effect between texture and interactions. As a result, each type of starch would induce a different retention rate (16).

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Table 1. Composition of the Starches

type of starch	content (% w/w)		
	water	amylose	lipids
standard corn	10.6	26	0.60
waxy corn	10.0	<1	0.06
stabilized and cross-linked waxy corn	10.2	<1	0.06
amylose-rich corn	9.0	58	0.75

Table 2. Properties of the Aroma Compounds

	isoamyl acetate	ethyl	linalool
	(3-methylbutyl acetate)	hexanoate	
odor	banana, pear	strawberry, pineapple	floral
mol wt (g·mol ⁻¹)	130.18	144.00	134.24
boiling temp at 10 ⁵ Pa (°C)	142	168	198
log <i>P</i> ^a	2.13	2.83	3.54
saturation vapor pressure	733 ^b	346 ^c	27 ^b
<i>P</i> _s (Pa) at 25 °C	714 ^c		51 ^c
solubility in water at 25 °C (g·L ⁻¹)	2.4 ^b	0.51 ^d	2.6 ^b

^a log *P* (log of partition coefficient between octanol and water) values were estimated by the "molecular modeling pro software", chemSW. Copyright James A. Quinn, 1992–1998 (17). ^b Provided by Espinosa-Diaz et al. (18). ^c Calculation with *P* and *T* from the *Handbook of Chemistry and Physics*, 64th ed.; The Chemical Rubber Co.: Cleveland, OH, 1983. ^d Provided by Ramirez Ramirez et al. (19).

The present study was undertaken to determine if interactions between amylose and aroma compounds really have an effect on aroma retention in real foodstuffs. We tried to determine the respective roles of texture and interactions. The ability of three aroma compounds to interact with amylose was studied first and their retention in four starch pastes was measured.

MATERIALS AND METHODS

Materials. Potato amylose (Merck) was used to determine the interaction between aroma compounds and amylose. Four corn starches provided by Roquette Frères (Lestrem, France) were used to prepare the pastes. Their composition is given in **Table 1**. Isoamyl acetate (Aldrich, purity >99%), ethyl hexanoate (Aldrich, purity >99%), and linalool (Aldrich, purity 97%) were used as aroma compounds. Their physicochemical properties are reported in **Table 2**.

Methyl heptanoate (Sigma, purity ~99%) and ethyl caproate (Sigma, purity ~99%) were used as extraction and chromatography standards.

Amylose Preparations. Amylose (1% w/w) was dispersed in pure water in a glass tube with a screw cap. Isoamyl acetate (20% v/v) or ethyl hexanoate or linalool was added. Oxidation of aroma compounds was avoided by bubbling U Nitrogen in those dispersions for 10 min. Then the dispersions were placed in an oil bath at 155 °C for 30 min, followed by a bath at 80 °C for 10 min to favor crystallization. The dispersions were next cooled to room temperature and stored for 48 h before being centrifuged at 625 g for 30 min. The precipitates were removed and stored over saturated NaCl solution, at a water activity (*A*_w) of 0.75, and then they were studied by X-ray diffraction.

X-ray Diffraction. X-ray diffraction data were collected with a diffractometer that operated in a transmission mode and was powered by an Inel (CPS 120, France) generator operated at 40 kV and 30 mA. A copper X-ray tube was used along with a quartz monochromator, which provided a Cu Kα₁ beam and was monochromatized with a bent crystal. The full diffraction curves were obtained with samples conditioned at 75% relative humidity. The samples (100 mg) were sealed between two aluminum foils to prevent any significant change in the water content during the measurement. The resulting diffraction diagrams were normalized between 3 and 30° (2θ).

DSC Measurements. An automated heat flux differential scanning calorimeter SETARAM DSC 121 (France) was used for thermal analysis of 100-mg samples. The samples were composed of 20 mg of

precipitated amylose and 80 mg of water. The reference sample was 100 mg of water. Samples and references were sealed in stainless steel high-pressure cells. Scans were run at 3 °C/min between 5 and 160 °C.

Preparation of Starch-Based Matrices. Starch was hydrated with mineral water (Evian, France). The mixture was gently stirred for 12 min before being cooked in an IKA LR 2000 V reactor. Just before cooking, an aroma compound was added to a concentration in the final products of 0.4 mmol of aroma per kg of starch paste, taking into account the loss of flavor during cooking. This amount corresponds to 1.3 mmol per mol of glucose equivalent.

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 previously 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 raised asymptotically to 85 °C. This temperature was reached after 20 min. The product was then maintained at 85 °C for 15 min. During all the cooking time, the starch paste was stirred by an anchor, at a rate of 50 rpm.

For partition coefficient measurements, 7% starch pastes were heated as previously described. These operating conditions led, as it was judged by microscopy, to well-swollen but nonruptured starch granules, corresponding to the highest viscosity of the starch dispersion. The obtained product was sampled into glass vessels for static headspace analysis, or in polypropylene boxes for other measurements. The samples were stored, hermetically sealed, at 6 °C for 24 h before analysis.

Characterization of the Suspension Structure by Viscometry.

Immediately after processing, the products were maintained at 65 °C in a water bath until viscosity measurement. Viscosity measurements were performed on a coaxial cylinder viscometer RM180 (Mettler, Schwerzenbach). The measuring system consisted of an inner cylinder with a 14 mm diameter and a 15–18 mm diameter bowl. The bowl was heated to 65 °C and regulated at ±0.1 °C by means of a laboratory thermostat via a double jacket (Julabo MV). Measurements were carried out in triplicate. Flow curves, loading and unloading, were performed from 10 to 500 s⁻¹ for standard corn starch matrices and from 80 to 500 s⁻¹ for other matrices. The loading curves were modeled with the power law between those limits: $\tau = K\dot{\gamma}^n$, with τ = stress (Pa), $\dot{\gamma}$ = shear strain rate (s⁻¹), *K* = consistency index (Pa·s^{*n*}), and *n* = flow index. For a given shear rate ($\dot{\gamma}$), the viscosity, η , is given by the following: $\eta = K/\dot{\gamma}^{(1-n)}$. So, η is proportional to the consistency index and increases when the behavior index decreases.

Rheological Analysis of the Gels. The rheology of the products was characterized 24 h after their preparation, with a texture analyzer RHEO TA-XT2 (Champlan, France) equipped with a 5 kN sensor and placed in a refrigerated enclosure (10 °C). Fracture experiments were conducted on amylose-containing matrices and penetrometry measurements on waxy starch matrices.

Fracture Experiments. The products were removed from the moulds they had been sampled in (40 mm diameter, 38 mm deep) and were immediately compressed up with a 100 mm diameter cylinder, at a constant speed (1 mm·s⁻¹), until macroscopic fracture. The plunger was then stopped and returned to its initial position, before being cleaned between each measurement with ethanol and distilled water. Measurements were made in triplicate.

The force needed to fracture the gels was measured as a function of the displacement. The noticed parameters were as follows: σ_f , the maximum stress (also considered as fracture stress), in N·m⁻²; ϵ_f , the displacement at which the maximum stress was reached, expressed relative to the initial height of the sample (no dimension); *E*, the elasticity modulus, which corresponded to the values of σ/ϵ when $\epsilon \rightarrow 0$ (expressed in N·m⁻²); and *W*, the energy required to induce the fracture, $W = \int \sigma d\epsilon$, measured in N·m⁻².

Penetrometry Tests. Penetrometry experiments were conducted in waxy starch matrices, sampled within a polypropylene box (55 mm diameter × 38 mm deep). They were compressed by a 25 mm diameter cylinder, at a constant speed (1 mm·s⁻¹), until the matrices deformation was 50% of their initial height. The plunger was then cleaned with ethanol and distilled water. Measurements were made in triplicate. The force needed to deform the material was measured as a function of the

displacement. The noticed parameters were as follows: F_{\max} , the maximum applied force in N; s_p , the elasticity modulus, which corresponded to the values of σ/ϵ when $\epsilon \rightarrow 0$ (expressed in $\text{N}\cdot\text{m}^{-1}$); and W_p , the energy required to deform the matrix of 50%, $W = \int \sigma \, d\epsilon$, measured in $\text{N}\cdot\text{m}^{-2}$.

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, followed by quantification by CG. After 1 day at 6 °C, 10 g of the gelled product was dispersed in 100 mL of pure water, saturated with NaCl ($360 \text{ g}\cdot\text{L}^{-1}$). The addition of NaCl improves the extraction output, by a "salting-out" effect. Methyl heptanoate, used as the extraction standard, was added to the solution at a concentration of $2.5 \text{ mg}\cdot\text{L}^{-1}$. This solution was extracted by dichloromethane, during 30 min after the boiling point was reached. These extractions were made in triplicate, and we added ethyl caproate to the extracts as the chromatography standard. The extraction outputs determined by chromatography were very close to 100%. When it was not 100%, we corrected the obtained value by the proportion of recovered extraction standard.

Determination of the Concentration of Aroma Compounds in the Headspace. 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 with a flame ionization detector (250 °C; H_2 , $30 \text{ mL}\cdot\text{min}^{-1}$; air, $300 \text{ mL}\cdot\text{min}^{-1}$) equipped with a high-resolution gas chromatography column DB-wax (J&W Scientific) of $15 \text{ m} \times 0.25 \text{ mm}$ (i.d.). Film thickness was $0.15 \mu\text{m}$. Nitrogen was used as the vector gas, at a rate of $1 \text{ mL}\cdot\text{min}^{-1}$ (makeup rate: $24 \text{ mL}\cdot\text{min}^{-1}$). Extracts were injected automatically and headspace samples were injected manually. The temperature of the oven increased from 100 °C to 180 °C at a $5 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ rate.

With the assistance of calibration curves, we calculated from the obtained areas the weight of aroma compound contained in the injected quantity of headspace or extract. The corresponding concentrations were expressed in molar fraction.

Calculation of Partition Coefficients and Retention Coefficients.

Partition coefficients K_i^∞ are the ratios of Y_i , concentration of the aroma compound i in the headspace, and K_i , concentration of the aroma compound i in the product (these concentrations are expressed here in molar fraction):

$$K_i^\infty = \frac{Y_i}{X_i}$$

Solutions of aroma compounds in water were used as references. The three aroma compounds were weighted in three separated 500 mL of mineral water, and these solutions were stirred at ambient temperature, shelter from light, during 12 h. Then they were sampled into glass vessels for static headspace analysis. Retention was calculated from partition coefficients calculated in starch pastes and in water, from the following equation:

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

RESULTS AND DISCUSSION

X-ray Analysis. When it is not forming a complex, amylose is in the form of double helices (B type). Complexes of amylose are also called V amylose, which is a single left-handed helix. It is known from the literature that depending on the complexing molecule, different types of V amylose exist. The best known and described complex is V_h amylose, which is obtained with linear alcohols and monoacyl lipids (9). It was therefore of interest to know if amylose formed complexes with the aroma

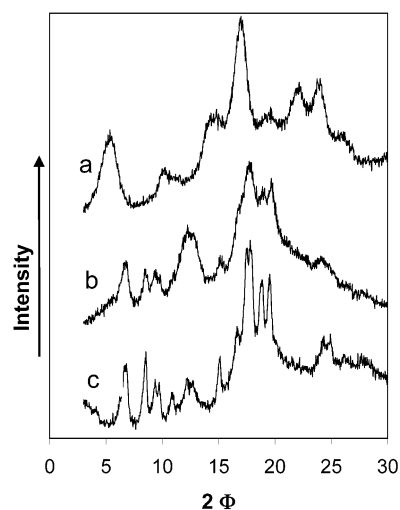


Figure 1. X-ray diffractograms obtained for crystallized amylose with isoamyl acetate (a), linalool (b), and ethyl hexanoate (c).

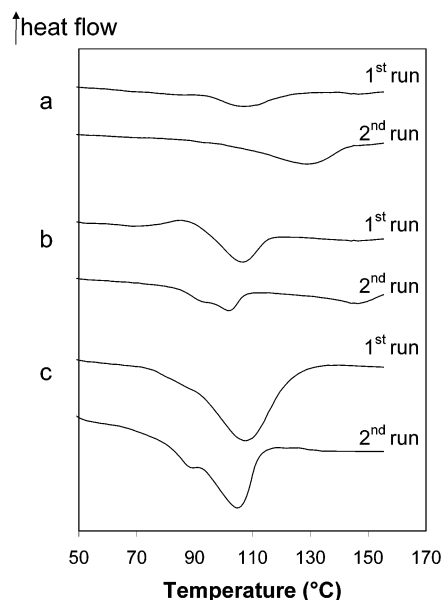


Figure 2. Thermographs obtained for amylose with isoamyl acetate (a), linalool (b), and ethyl hexanoate (c).

used in this study, and what was the type of these complexes. We determined the type of wide-angle X-ray diffraction diagram obtained with each aroma compound. Figure 1 shows a wide-angle X-ray diffraction diagram of amylose–isoamyl acetate. The reflections at $2\Theta = 5.3^\circ$, 14.6° , 17° , 22° , and 24° are characteristics of the B type retrograded amylose. This result indicates that amylose did not form complexes with isoamyl acetate.

X-ray diffraction diagrams of amylose precipitated with ethyl hexanoate and linalool are shown in Figure 1b,c. The reflections at $2\Theta = 6.7^\circ$, 12.5° , and 17.8° are comparable to those obtained for amylose complexed with 2-propanol (9, 10). With linalool the crystallinity seems to increase. The reflections are quite sharp, although the formation of the precipitate was very fast, which is usually an indication for a less organized system.

DSC Analysis. Thermographs obtained with isoamyl acetate, linalool, and ethyl hexanoate are given in Figure 2. The thermograph obtained with isoamyl acetate shows endothermic

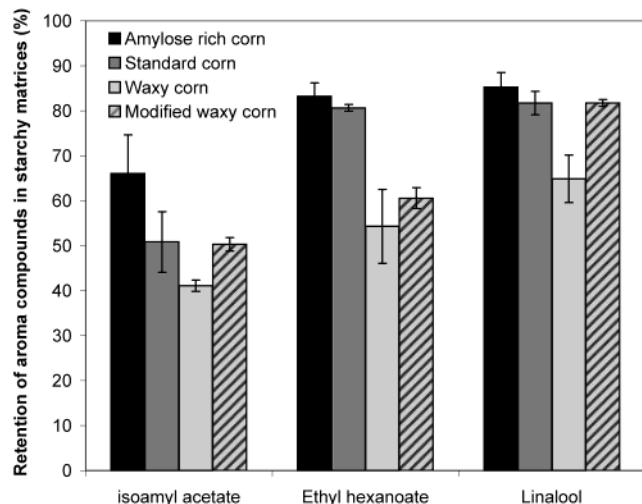


Figure 3. Retention of aroma compounds in four different starchy matrices.

peaks at 106 and 129 °C for the first and the second run, respectively. This shift to higher temperature on the second run indicates that these endothermic peaks do not concern complexes. They correspond to the fusion of retrograded amylose that generally occurs between 120 and 140 °C. On the first run, the amylose chains have a less perfect crystalline arrangement, and thus they melt at a lower temperature. With ethyl hexanoate and linalool, the thermographs show endothermic peaks at very close temperatures at the first and the second run (107 and 102 °C respectively for ethyl hexanoate, and 107.5 and 105 °C for linalool). This indicates the fusion of amylose complexes, confirmed by X-ray diffraction results. Melting temperature during the second run is slightly lower for both amylose complexed with ethyl hexanoate and linalool. It could be expected to be higher because the water content in the pan during the second run is probably lower than at the time of the complexes formation.

Our results confirm behaviors previously reported for the same compounds, by using different methods. Langourieux and Crouzet (20) noticed by dynamic headspace analysis that ethyl hexanoate interacted with amylose and that isoamyl acetate was not retained by amylose. Cayot et al. (21) and Arvisenet and Cayot (22) studied interactions between starch and aroma compounds by rheological measurements and hypothesized that interactions between isoamyl acetate and starch could exist, but were different from inclusion with amylose.

Previous studies showed some influence of the chemical structure of aroma compounds on their complexation ability with amylose (evaluated by precipitation or iodine binding): most alcohols have a great ability to form complexes (23) and for other compounds such as ketones, aldehydes, or esters, the complexing behavior depends primarily on their solubility. Our results seem to confirm this tendency: the alcohol and one of the two tested esters have the ability to form complexes. The different behaviors of isoamyl acetate and ethyl hexanoate probably are caused by their differences in solubility and/or polarity.

Retention of Aroma Compounds by Four Starch Matrices.

The retention of the aroma compounds by four different starch-based food matrices is given in Figure 3. In the four matrices, linalool and ethyl hexanoate are more retained than isoamyl acetate. But for the same aroma compound, the retention depends a lot on the starch used. These results are in accordance with literature, which shows that both the nature of the aroma

Table 3. Consistency Index and Behavior Index Calculated by the Oswald Model Law from Flow Curves at 62 °C of Four Different Starch Matrices without Aroma Compound

	behavior index	consistency index
amylose-rich corn	0.86 ± 0.07	0.04 ± 0.02
standard corn	0.30 ± 0.03	16.93 ± 2.73
unmodified waxy corn	0.57 ± 0.03	4.05 ± 0.80
modified waxy corn	0.42 ± 0.01	12.60 ± 0.95

Table 4. Parameters Measured by Uniaxial Compression until Fracture on Containing Amylose Starch Matrices after 24 h at 6 °C

	standard corn	amylose-rich corn
σ_F ($10^3 \text{ N}\cdot\text{m}^{-2}$)	6.4 ± 0.4	4.5 ± 0.4
ϵ_F (mm)	13.2 ± 0.4	9.1 ± 0.3
E ($\text{N}\cdot\text{m}^{-3}$)	120 ± 9	158 ± 5
W ($\text{N}\cdot\text{m}^{-1}$)	25.8 ± 0.3	14.6 ± 0.9

compounds and the type of starch play a role in the retention of aroma compounds by starch-based matrices.

Retention of Aroma Compounds in Unmodified Starch-Based Matrices: Influence of the Formation of Complexes. Considering only unmodified starches, the retention of linalool and ethyl hexanoate is significantly higher in matrices with amylose-containing starches. These two aroma compounds are precisely those that were shown to be able to form complexes with amylose. Therefore, these results demonstrate the existence of complexes between aroma compounds and amylose in the matrices, and prove that such complexes can be formed at concentrations relevant for foodstuff. High amylose corn starch hybrids are known for their relatively greater content of fatty acid, compared with normal or waxy starches, and lipids are known for their aroma retention ability. Here, such interactions could have a role in the retention of aroma compounds, but the formation of complexes seems to be predominant in the retention, because isoamyl acetate does not exhibit the same behavior as the other two.

Aroma retention is about 20 to 30% less in waxy corn starch matrices than in amylose-containing matrices. Nevertheless, the aroma compound mostly retained in waxy starch matrices is linalool, followed by ethyl hexanoate and isoamyl acetate, i.e., the two mostly retained aroma compounds are the same in both amylose-containing matrices and matrices without amylose. This result suggests the possibility of interactions between linalool or ethyl hexanoate and amylopectin. Indeed, the external branches of amylopectin have the same structure as amylose, and they could be able to interact in the same way with small ligands. This would be all the more possible if linalool and ethyl hexanoate interact at helices surface, because in this case, the chain lengths are not so important.

Isoamyl acetate retention is at least 40%, even though it does not form complexes with amylose. But it could interact with amylopectin by a mechanism other than complexes. An influence of the texture of matrices could also explain the interaction.

Influence of the Texture of Matrices on Retention of Aroma Compounds in Unmodified Starch-Based Matrices. Texture is known to influence aroma retention. That is why the texture of starch matrices was characterized by flow curves at 62 °C (Table 3) and uniaxial compression after 24 h for amylose-containing matrices (Table 4), or the penetrometry test after 24 h for matrices without amylose (Table 5). The viscosity of the matrices depends on the starch they contain and increases as following: amylose-rich corn starch < waxy corn starch <

Table 5. Parameters Measured by Penetrometry on Starch Matrices without Amylose, after 24 h at 6 °C

	waxy corn, not modified	modified waxy corn
F_{\max} (10^3 N)	115 ± 4	380 ± 14
S_p (10^3 N·m ⁻¹)	7.2 ± 0.3	87.0 ± 7.0
W_p (N·m)	0.75 ± 0.05	3.26 ± 0.18

modified waxy corn starch < standard corn starch (**Table 3**). After 24 h at 6 °C, amylose-containing matrices gelled, and waxy corn starch matrices did not. The gel obtained with amylose-rich starch was firmer than that containing standard corn starch: it was more fracture resistant (**Table 4**). Concerning non-gelled matrices, modified waxy starch was more deformation resistant.

The retention is the same in rich amylose corn starch matrices and in standard corn starch matrices, which differ in amylose contents. This cannot be explained by an equivalent amount of complexes: too low concentrations were used for it to be possible, particularly if ethyl hexanoate and linalool are located on the amylose surface. Amylose-rich starch matrices are less viscous than standard corn starch ones. When no interactions exist between the matrix and the aroma compounds, the less viscous the matrix is, the less the aroma compounds are retained. In our study, interactions are also involved in the retention. The texture of the matrices would increase the retention in standard corn starch compared to amylose-rich starch, and the interactions would increase the retention in amylose-rich starch compared to standard corn starch.

The texture of matrices could also be responsible for this retention. In unmodified starches, the more the matrix is gelled, the more isoamyl acetate is retained.

Retention of Aroma Compounds in the Modified Starch Matrix. For all aroma compounds, the retention is significantly higher in modified waxy corn starch matrices than in unmodified waxy starch matrices (**Figure 3**). This could be caused by an interaction between aroma compounds and the chains added by chemical modifications, or to the effect of the modifications on the texture. This can be particularly interesting, because chemical modifications are generally used to modify textural properties of starch-based products. They could also be used to influence the retention of aroma compounds.

CONCLUSION

The characterization of complexes of amylose–aroma compounds by X-ray diffraction and DSC had to be done in model systems with high aroma concentration because the low sensibility of those methods would not have allowed us to identify complexes in food products containing low concentrations of aroma compounds. Hence, we used the results obtained by these methods to understand the retention of aroma compounds in matrices containing different types of starch. It would be advisable to determine a suitable method to identify complexes and measure aroma retention in the same matrices and for the same concentrations.

With this work, we have shown that interactions between amylose and aroma compounds can be formed at foodstuffs concentration because a significant effect on the retention of aroma compounds was reported under those conditions. We confirmed that the formation of such interactions is widely dependent on the nature of aroma molecules. It would be of great interest to find a way to prove if amylopectin is able to form complexes with aroma compounds.

The texture of the starchy matrices also has an important influence on aroma retention. More work is necessary to evaluate the effect of structure and texture characteristics of the food matrix on the aroma retention and to compare it to the effect of the formation of complexes between amylose and aroma compounds.

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