Release and Perception of Isoamyl Acetate from a Starch-Based Food Matrix

N. Cayot,*,† C. Taisant,† and A. Voilley‡

Laboratoire de Biochimie Alimentaire, Etablissement National d'Enseignement Supérieur Agronomique de Dijon, 21 Boulevard Olivier de Serres, 21800 Quetigny, France, and Laboratoire de Génie de Procédés Agro-alimentaires et Biotechnologiques, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, 1 Esplanade Erasme, 21000 Dijon, France

In the present work, starches of different botanical origins and amylose contents were tested in a concentrated and complex starchy product. The effects of starch/isoamyl acetate interactions were followed by rheology, purge-and-trap GC, and sensory analysis. These methods showed that interactions existed and affected the organoleptic characteristics of the food product. Instrumental and sensory analyses gave different and complementary information on aroma release. It was possible to classify the starches according to their aroma trapping power. The rheology of the products and the interactions with amylose were not the only two factors affecting aroma retention.

Keywords: *Aroma release; starch; aroma compound; sensory analysis; dynamic headspace*

INTRODUCTION

Starch has been shown to exhibit physicochemical interactions with ligands such as iodine, some aroma compounds, and emulsifying agents. [The term ligand is employed here as used by Solms, to define compounds that interact with starch and remain fixed by complexation. Chemically speaking, ligand has a different meaning.] Eliasson and Krog (1985) indicated that the monoglycerides containing C12−C18 saturated fatty acids were very good complexing agents. It is generally thought that these interactions are due to amylose more than to amylopectin. Amylopectin can form only weak complexes or none at all (Godet et al., 1995).

It was mainly Solms et al. (Rutschmann et al., 1989; Rutschmann and Solms, 1990a−f) who studied the physicochemical interactions between potato starch and different ligands. They have shown that, under specific conditions of temperature and time, potato starch can form inclusion complexes with menthone, decanal, 1-naphthol, limonene, glyceryl-1-monostearate, or glyceryl-1-monopalmitate. These starch−ligand complexations were followed through iodine binding capacity. The starch−ligand complexes were also analyzed by X-ray diffraction and gave a typical spectrum of V-amylose, that is to say, a spectrum of complexed amylose.

Indeed amylose is helical in shape, which offers a hydrophobic cavity to the ligands. Rutschmann and Solms (1990c) determined that the shape of the amylose helix was influenced by the ligand. For instance, the number of glucose units per round of the helix varies as a function of the hydrophobicity, the steric hindrance, or the length of the carbon chain of the ligand.

Scatchard plots and Hill coefficients were employed by Rutschmann et al. (1989) to analyze the binding isotherm of menthone on gelatinized potato starch. After analyzing the binding isotherms of different ligands (Rutschmann and Solms, 1990a), they concluded that depending on the ligand, there were one or two sites of interaction. On one hand, there is a complex formation inside the amylose helix, and on the other hand, there could be adsorption external to the helix by hydrogen bonds. The long external branches (at least 20−30 glucose units) of amylopectin might have the same behavior as amylose (Rutschmann and Solms, 1990a).

These results were obtained using simplified model systems, using diluted aqueous solutions of starch. Potato starch was used because it contains no internal lipids and therefore no preexisting complexes (Nuessli et al., 1997). The model systems were binary or ternary systems comprising a low concentration starch solution (2% w/w) and one or two ligands in high concentration (up to 200 mmol/mol of glucose equivalent). In ternary systems (potato starch and two ligands), Rutschmann and Solms (1990e,f) and Godshall and Solms (1992) showed that there could be some competition and even inhibition between the two ligands for binding with starch. The complexation level of the tested compounds (glyceryl monostearate and menthone) varied as a function of concentration and ratio of these two ligands. The determination of the complexation level by means of the iodine binding capacity might then be questioned. Rutschmann and Solms (1990d) indicated that iodimetric titration methods are only applicable when binding and structural characteristics of ternary complexes with the tested ligand and iodine are known.

Maier et al. (1987) studied the interactions occurring between aroma compounds, such as vanillin or peppermint, and different starches (potato starch, corn starch, waxy corn starch, tapioca starch, wheat starch) during freeze-drying. They observed that the absorbed amounts generally decreased in the following order: potato starch, waxy corn starch, corn or tapioca starch, wheat starch. However the bound amounts of aroma

* To whom correspondence should be addressed (tel: 03 80 77 27 90; fax: 03 80 77 27 51; e-mail: n.cayot@enesad.fr).

† Laboratoire de Biochimie Alimentaire.

‡ Laboratoire de Génie de Procédés Agro-alimentaires et Biotechnologiques.

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Table 1. Characteristics of the Test Starches and Operating Conditions for Hydrothermic Treatment of Starchy Creams

starch	amylose content (% w/w)	extractable lipids (% w/w)	protein (% w/w)	temp set point (°C)	cooking time (min)	cream water content (% w/w)	cream pH
potato starch, PS	21		0.1	80	0	77.7	6.6
corn starch, CS	26	0.1	0.4	90	3	77.3	6.6
waxy corn starch, WS	1	0.1	0.4	85	0	77.3	6.8
modified waxy corn starch, MWS	1		0.35	98	20	76.3	6.5
standard without starch	0			90	3	92.5	6.6

compounds were better either with high-amylose starches or with high amylopectin starches, as a function of the aroma compound tested.

More work is needed to establish the sensory significance of aroma/starch interactions (Bakker, 1995). In fact, few studies have been done on the sensory effects of these interactions. Hippleheuser (1994) conducted sensory experiments with several types of corn starch in complex food systems and found that the different tested flavor molecules bound differently with the starches.

Thus, the purpose of the study reported here was to conduct sensory analyses simultaneously with instrumental analysis to examine aroma−starch interactions in a complex food product using different types of starch. A dessert cream aromatized with isoamyl acetate was chosen as the model and as a pleasant product for the sensory panel.

MATERIALS AND METHODS

Preparation of the Food Product. The dessert cream was composed of 70 g of starch, 100 g of commercial sucrose, and 100 g of skimmed milk powder rehydrated with Evian water up to 1000 mL. Evian water was chosen as drinking water, neutral from a sensory point of view. It was also used during the sensory tests for the panel to rinse their mouths.

Four starches were tested, all of them provided by Roquette Frères (Lestrem, France): a native potato starch (PS), a native corn starch (CS), a native waxy corn starch (WS), and a modified (cross-linked and stabilized) waxy corn starch (MWS). Their characteristics are given in Table 1. Isoamyl acetate was used as the ligand. This volatile molecule has the following characteristics: mass weight = 130.18 g mol^{-1}; hydrophobicity log P = 2.1 [(log P is the logarithm of the partition coefficient between water and *n*-octanol calculated by the Rekker (1977) method]. A total of 0.333 mL of isoamyl acetate (IFF, Longvic, France) was used to aromatize a quantity of cream corresponding to 1 Liter of reconstituted milk. The amount of the added flavoring substance has been determined to be compatible with both sensory and instrumental analyses.

Starch, sucrose, and reconstituted milk were gently stirred together for 3 min, and the mixture was left to rehydrate for 12 min. Then, the mixture was poured into a reactor vessel, and the aroma compound was added. The reactor vessel (IKA LR 2000 V reactor) was hermetically sealed. The reactor was heated, and the temperature of the product was regulated (±0.1 °C precision) by means of a laboratory thermostat via a double jacket (Lauda C6CS−temperature sensor PT 100). During cooking, the mixture was stirred by an anchor stirring pool with whippers that rotated at 50 rpm. At the beginning of cooking, the temperature was 25 °C. The temperature set point varied from one starch to another. Once the product reached this set point, the temperature was maintained during a period called "cooking time". The cooking time and temperatures for each starch are shown in Table 1. These operating conditions led to well-swollen starch granules, corresponding to the highest viscosity of the starch dispersion.

After cooking, the dessert cream was partitioned and conditioned, either into polypropylene boxes for rheology and tasting or directly into purge-and-trap glass vessels for gas chromatographic analyses. The samples were stored, hermetically sealed, at least 24 h at 6 °C before analyses.

Rheology. The rheology of the dessert cream was characterized with a texture analyzer RHEO TA-XT2 (Champlan, France) equipped with a 5 kN sensor. Uniaxial compression measurements at a constant rate (1 mm s^{-1}) were taken with a cylinder 25 mm in diameter. The product was compressed to 30% of its initial height. The cylinder was then returned to its initial position. Each sample was analyzed directly in its box (91 mm diameter, 52 mm deep) as soon as it was taken out of the refrigerator. Three samples were analyzed per product. The cylinder was cleaned after each sample following a standard procedure.

Viscosity measurements were taken to characterize the different products, using a coaxial cylinder viscometer RM180 (Mettler, Schwerzenbach). The measuring system consisted of a cylinder with a 14 mm diameter and a 15−18 mm diameter bowl. The bowl was heated to 60 °C and regulated (±0.1 °C precision) by means of a laboratory thermostat via a double jacket (Julabo MV). Measurements were carried out in triplicate. Flow curves were performed from 10 to 500 s^{-1}. The reported values were the average shear viscosity at 500 s^{-1}.

Purge-and-Trap GC Analysis. The analyses were conducted on a CP-9001 gas chromatograph (Chrompack France, Les Ulis) with a purge-and-trap injector TCT/PTI 4001 (Chrompack France, Les Ulis) and equipped with a flame ionization detector (FID). The conditioned samples were either directly adapted to the purge-and-trap system and helium was used to purge the volatiles at the surface of the product or about 1 g of sample, precisely weighed, was diluted in ultrapure water and strongly stirred and helium was used to purge this solution through a fritted glass. These two means of trapping the volatile compound were named, respectively, sweep mode and bubble mode. The water was retained by a cold trap (−15 °C), and volatile compounds were trapped in a capillary column at −120 °C. This column was then heated to permit the aroma compounds to be introduced onto the chromatographic column. The GC operating conditions were the following: sample volume 10 mL, ambient temperature 19 °C, condensor temperature −15 °C, purge time 5 min, injector temperature 250 °C, detector temperature 270 °C. The volatile compounds were separated by a 25 m × 0.32 mm (i.d.) fused silica capillary column coated with CP wax 57 CB (Chrompack) of film thickness 1.20 μm.

The column oven was temperature programmed from 40 (5 min initial hold) to 200 °C (10 min final hold) at 10 °C min^{-1}. Three replicates per product were analyzed with the sweep mode, and three were analyzed with the bubble mode.

Sensory Testing. The panel consisted of 14−19 members selected from a group of students and laboratory workers in the Laboratoire de Biochimie Alimentaire (ENESAD, France). The two first stages of the Spencer procedure [Spencer (1971) cited by Sauvageot (1982)] were chosen to assess the capability of the panelists to distinguish the different aromatized starchy products. The panel members rated the intensity of the products made with the four different starches containing the same aroma addition level.

Triangle tests were applied to establish whether the samples were different in terms of aromatic intensity. Ranking tests were then performed. Four samples (one of each product) were analyzed per sensory test. The tests were replicated three times (with three different productions). The panelists chewed the food products for a few seconds before swallowing, and then

they attributed the rank 1 to the least aromatic sample and the rank 4 to the most aromatic one (ranks 2 and 3 were of intermediate intensities).

To express the results, the ranks obtained for each sample were added. Then the Friedman (1937) statistical test was used to determine if there were significant differences between the four products. The Newman−Keuls (Newman, 1939; Keuls, 1952) test was used to compare the samples by pairs.

RESULTS AND DISCUSSION

In the present investigation, GC analyses and sensory tests have been used as complementary methods to judge the effects of aroma/starch interactions in a concentrated starchy food product.

Rheology was used to characterize the product and also to observe if the assumptions of Nuessli et al. (1995) that a modification of rheology induced by ligands was true in the present case. Nuessli et al. (1995) worked with an aqueous dispersions of potato starch (2% w/w) and 0−200 mmol of aroma compound (decanal or fenchone)/mol of glucose. Through dynamic rheological measurements, they observed gelation-like changes that might be caused by physical aggregation of the insoluble amylose−ligand complexes. The dessert creams in the present study were concentrated dispersions of various starches (7% w/w) with isoamyl acetate added up to 5 mmol/mol of glucose. These concentrations were chosen to obtain a product compatible with sensory testing (texture and aroma level). The unflavored products were the standards for the corresponding flavored creams.

Rheology. The graphic representations obtained by uniaxial compression of unflavored creams are reported in Figure 1a−d. The positive peak represents the resistance of the product to deformation. Several positive peaks corresponds to fractures of the gel. The negative peak represents the adhesiveness of the product when the cylinder was returned to its initial position. These force−time curves are different for each starch. PS and CS creams showed fractures and adhesiveness. MWS showed adhesion but no fracture. WS had little resistance to deformation and showed neither adhesiveness nor fracture.

The graphic representations obtained with flavored creams resembled those obtained with unflavored creams. There are only slight differences in the curve amplitude. After preliminary studies, we chose to take into account only two of the parameters of the force time curves: the maximum strength (F_{max}) and the surface of the positive peak (positive area).

Sensory tests revealed that the panel was unable to distinguish between flavored and unflavored creams in terms of texture. It is uncertain whether the panel was insufficiently trained or the differences between textural characteristics were too small.

Even if rheological measurements can characterize only the mechanical component of texture, it could be convenient to correlate rheological measurements to texture terms. The maximum strength and the positive area can be linked to the hardness of the product.

The mean values of our measurements are reported in Table 2. The RSDs for triplicates range between 3 and 9% for the maximum strength and between 1 and 6% for the positive area.

First, as shown by Inaba et al. (1994), the uniaxial compression test can be useful in characterizing different types of starch gels. The different products were well differentiated, and the positive area values of these products increased in the order WS, MWS, CS, and PS. The maximum strength increased in the order WS, CS, MWS, and PS.

Second, it can be noted from Table 2 that the resistance of the cream to uniaxial deformation, expressed through F_{max} and positive area, was enhanced by the presence of isoamyl acetate. This is a tendency with differences between aromatized and nonaromatized creams being significant only for the creams prepared with CS.

It may be that the limits of sensitivity for these operating conditions were reached or that the differences were insignificant. Different aroma concentrations should be tested.

The difference in rheological characteristics between aromatized and nonaromatized creams might be directly due to the presence of ligand molecules in the helix of amylose and between the macromolecules. In fact, as gel stiffness is closely related to the association of amylopectin chains (Inaba et al., 1994), the formation of insoluble amylose/aroma complexes might influence the constitution of the gel network and then the textural behavior of food products.

Moreover, as written by Godet et al. (1995), heat-formed complexes actually show specific properties such as a decrease in amylose solubility and an increase in gelatinization temperature. So the aroma compound added to the cream at the very beginning of cooking might also have influenced the hydrothermal modification of starch granules (this is yet to be demonstrated). This is all the more important as the state of swelling and disintegration of the starch granules determines the accessibility of amylose for complexation (Nuessli et al., 1997).

Nuessli et al. (1995) reported that gels induced by complex formation with decanal (molecular weight = 156.26) in a starch dispersion were softer than those obtained with lecithin. Under other operating conditions, Condé-Petit and Escher (1992) observed a gelification induced by complex formation with glyceryl monostearate but not with lecithin. They supposed that this phenomenon depended on the molecular weight of the ligand and on the intensity of the binding forces. It is not totally clear whether the gelation-like phenomenon is dependent only on the complexation of the ligand. In the present case, an increase in the hardness was observed in CS creams aromatized with a small molecular weight compound (isoamyl acetate molecular weight = 130.18), similar to the decanal-induced gel. But isoamyl acetate is less polar than decanal and can better stabilize interactions with starch. In fact, Rutschmann and Solms (1990b) considered that a combination of dipolar and hydrophobic characteristics is necessary to stabilize the inclusion complexes.

Gas Chromatography. GC with purge-and-trap injection was used to obtain instrumental information for comparison with sensory analyses. Through the sweep mode, the evaluation of the fraction of aroma released in the headspace was carried out for comparison with the orthonasal aroma detected by sniffing during sensory testing. Through the bubble mode, the fractions of the aroma compound that might be present either in the aqueous phase or bound to starch were recovered. This is to be related to the retronasal aroma released during eating when saliva dilutes the sample and chewing deletes the gel structure. The results obtained with the two different modes of trapping aroma compounds could partly be explained by the gelled

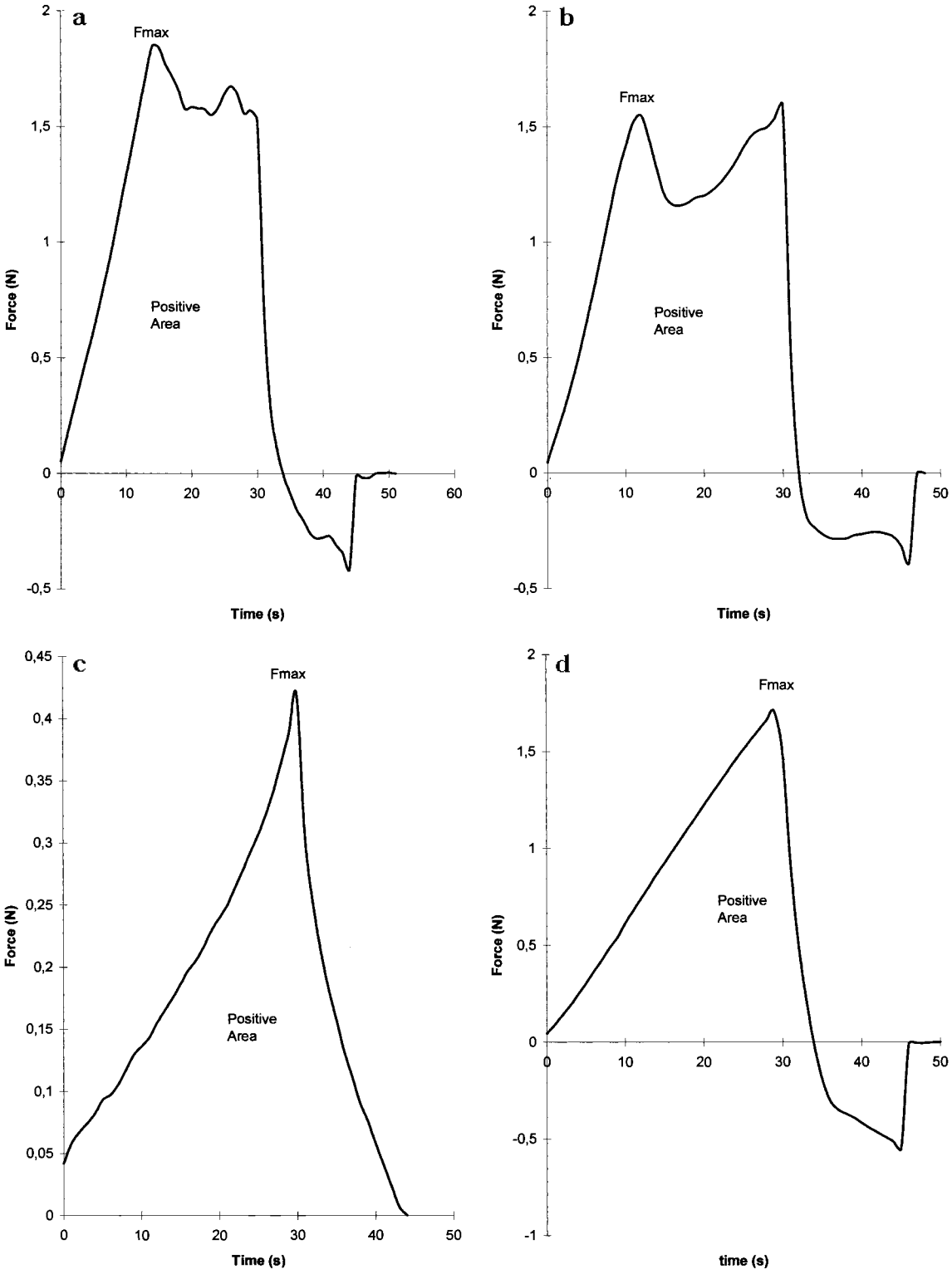

Figure 1. Force−time curves obtained by uniaxial compression of unflavored creams by a 25 mm diameter cylinder to 30% of the sample height: 1a, cream made with potato starch (PS); 1b, cream made with corn starch (CS); 1c, cream made with waxy corn starch (WS); 1d, cream made with modified waxy corn starch (MWS).

structure of the food matrix and by the ability of the volatile molecules to reach the vapor phase.

The mean values of purge-and-trap GC measurements are reported in Table 3. The RSDs for triplicates ranged between 6 and 12% for the sweep mode and increased to 20% for the bubble mode. The results obtained with the bubble mode are dependent upon the capacity of the cream to be dispersed in water before analysis.

Considering the results obtained by the sweep mode, the quantities of isoamyl acetate in the headspace of the four different creams were significantly different. CS and WS creams released the greatest quantities of isoamyl acetate into the headspace, followed by the PS cream and finally the MWS cream.

It is probable that the release of the aroma compound was in relation to the viscosity of the medium. The shear viscosity measured at a shear strain rate of 500

Table 2. Results of Texture Analyses Obtained for Various Dessert Creams by Means of Uniaxial Compression with a 25 mm Diameter Cylinder

starch	F_{max} (N)	positive area (N s)	statistical significance
PS			
unflavored	1.96 ± 0.17	42.49 ± 1.70	NS
flavored	1.96 ± 0.18	40.59 ± 2.32	
CS			
unflavored	1.48 ± 0.07	34.82 ± 0.24	S
flavored	1.62 ± 0.06	36.99 ± 0.22	
WS			
unflavored	0.44 ± 0.02	8.04 ± 0.26	NS
flavored	0.48 ± 0.03	8.25 ± 0.23	
MWS			
unflavored	1.67 ± 0.08	29.10 ± 0.96	NS
flavored	1.75 ± 0.05	29.96 ± 1.33	

Table 3. Purge-and-Trap GC Results: Peak Areas (Arbitrary Units) of Isoamyl Acetate Obtained from Four Different Dessert Creams

	isoamyl acetate peak area		aroma recovery (bubble mode − sweep mode)/ bubble mode (%)
starch	sweep mode (/1 g dry matter)	bubble mode (/1 g dry matter)	
PS	563 243	915 413	38.5
CS	776 954	1 782 909	56.4
WS	797 329	1 179 739	32.4
MWS	164 743	192 669	14.5
standard (without starch)	2 273 632	52 660 809	95.7

Table 4. Viscosity Measurements of Four Different Flavored Creams Obtained with a Coaxial Viscometer

starch	shear viscosity measured at a shear strain rate of 500 s^{-1} (Pa s)
PS	0.60 ± 0.10
CS	0.49 ± 0.09
WS	0.29 ± 0.03
MWS	0.84 ± 0.12

s^{-1} gave the following results: the least viscous cream is WS, followed by CS and PS, and finally MWS cream (Table 4). The order in terms of viscosity is nearly the same as the order in terms of aroma recovery through the sweep mode.

The aroma fraction recovered from the aqueous dispersion of the creams by the bubble mode should correspond to the nonlinked aroma molecules, and the lack of diffusivity due to the gel structure should be minimized. At the present, we are testing samples with different starch contents. The recovered aroma fraction was lowest for the MWS cream, high for the CS cream, and intermediate for WS and PS creams.

The result obtained for the standard without starch showed the great influence of starch on aroma retention. It seems that the best aroma traps, when the gel structure is preserved, are in ascending order and for isoamyl acetate: CS < PS < WS < MWS. In relation to rheological measurements, it appears that the gel structure of the creams was not the unique explanation for the lack of released aroma. For example, the proportion of aroma recovery (between the sweep and bubble modes) was higher for CS than for PS, even though the PS creams were harder than CS creams. Thus, it may be concluded that PS induces more physicochemical interactions than CS with isoamyl acetate.

Physicochemical interactions with aroma molecules are supposed to be due to amylose. In the results presented here, however, WS cream retained the aroma compound better than CS, and moreover, MWS cream showed maximum aroma retention. The fact that PS cream shows a better aroma retention than CS cream is understandable because in contrast to other starches PS has no lipid internal to the amylose helix and may thus form stronger complexes with ligands.

Table 5. Sensory Analyses Results of Ranking Test on Aromatic Intensity of Four Different Creams[a]

	starch			
	PS	CS	WS	MWS
ranks sum	162[a]	150[a]	106[b]	67[b]

[a] Same letter in the table indicates that the ranks sums are not significantly different.

Sensory Testing. Sensory tests were performed to assess the effect of aroma/starch interactions on aroma expression and were based on the retronasal aromatic intensity perceived during tasting. As shown by the rank sums reported in Table 5, the highest score, corresponding to the most intense flavor, was obtained for PS cream, followed by CS, WS, and finally MWS, which exhibited the least aromatic intensity.

The Friedman statistical test indicated that the creams are globally perceived as different ($F = 54.41 > Fr = 7.815$). It was then possible to classify the creams into two groups: the first is constituted by PS and CS creams that were not different, and the second is constituted by WS and MWS creams. So, by retronasal assay, for the same aroma content, PS and CS creams had more aroma than WS and MWS creams.

As underlined by Ingham et al. (1995), dynamical headspace analysis can provide nearly the same information as that given by sensory testing. Here we attempted to classify four different starches in relation to the observed aroma release. The classifications obtained by these two methods were the same, but with dynamic headspace PS and WS are very similar, even while with retronasal release PS is very similar to CS.

In fact, at 37 °C, in the mouth several phenomena are taking place: a destruction of the gel structure, a dilution in an aqueous medium that might displace the chemical equilibrium, and a hydrolysis of starch with salivary α-amylases. Matheis (1993) indicated that a mastication of at least 20 s is necessary for the amylose to release the aroma compound. As we did not impose such a delay on our panel, we can conclude that the panel only had a sensation corresponding to nonlinked aroma. So the results might have closely resembled those of gas chromatography by the bubble mode. The fact that there exist some differences might be explained in Hussein et al. [1983, cited by Roberts and Acree, 1995]. They indicated that saliva might also influence the retronasal aroma through its high polarity, through its neutral pH, or by emulsification with the protein it contains.

CONCLUSIONS

The effects of physicochemical aroma−starch interactions have been tested through rheology, dynamic headspace, and sensory analyses. A modification of rheological characteristics was observed following the aromatization of the CS creams, but more precision is needed to obtain clear conclusions and explanations. Rheology seems to be indispensable in this type of study because it makes it possible to consider aroma release as a function of diffusion and of interactions in a starchy

jelled food. Dynamic headspace analysis allowed us to distinguish among the abilities of the different starches to trap isoamyl acetate. The release of the aroma compound was not correlated only to the hardness of the medium. Finally, the performed sensory analyses showed that GC did not account for all the slight differences in the interaction phenomena. It must not be forgotten that these experiments have been conducted in a complex matrix and that volatile compounds can also be bound to proteins, for instance, by hydrophobic interactions (Bakker, 1995).

Among the four tested starches, MWS appeared as the starch that might be the best trap for isoamyl acetate. Then came WS. The classification between PS and CS was not so clear. The amylose content was not the major factor in controlling aroma release. The results have to be completed by a quantification of starch/aroma complexes.

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