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The Influence of Gel Strength on Aroma Release from Pectin Gels in a Model Mouth and in Vivo, Monitored with Proton-Transfer-Reaction Mass Spectrometry

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The course of events from taking a food into the mouth to the perception of the food's flavor involves many steps, from dilution with saliva, mastication, and transportation of the compounds to the olfactory epithelium to transformation into signals that go to the brain. In addition, there are also the effects of the food's structure and properties. In this study, a proton-transfer-reaction mass spectrometer (PTR-MS) was used to investigate how four pectin-containing systems with different structures and strengths affected the release of aroma compounds in a model mouth and in the nose of an assessor. Both the model mouth and the in-nose measurements showed that the strength and structure of pectin-containing systems are important with regard to the quantity of aroma compounds that are released. Mastication and saliva were also shown to have a large influence on how much of the aroma compound is released from the mouth to the nose.

KEYWORDS: Flavor; mastication; model mouth; pectin gels; proton-transfer-reaction mass spectrometry (PTR-MS); release; saliva

INTRODUCTION

Perception of flavor is a process that involves release from the food of odorants within the oral cavity and their transport via the retronasal route to the nasal cavity, where they interact with the receptors in the olfactory epithelium. Here, the receptors transform the sensory information into electrical signals, which are transported to the brain, and the flavor is perceived (*1*). However, behavioral responses to the same flavor can vary within and between individuals, depending on attitude, memory, expectations, experience, and physiology (*2*).

It is not an easy task to establish which parameters affect aroma release in the mouth, because the process involves several steps (namely, dilution with saliva, mastication, and swallowing), all of which change the volume, composition, and viscosity of the sample. When a food is taken into the mouth, it is directly covered by a thin film of saliva (*3*). From a solid food, the aroma is first released to the saliva and then to the gas phase. However, from a liquid food, the aroma compounds are already in the liquid phase and are transported directly to the gas phase (*3*). The constituents of saliva affect the headspace concentration of aroma compounds. Proteins (mucin, α-amylase) bind the more hydrophobic compounds, while smaller, more hydrophilic compounds are salted out. The saliva also changes the overall composition of the mixture, affects mastication through a change in saliva volume, and influences various factors on mass transfer (*3*, *4*). Mastication breaks down the product, increasing the surface area, and spreads out the food throughout the mouth, which increases the release of aroma compounds (*5*).

To gain insight into the kinetics of aroma release in vivo, online measurements of aroma concentrations have to be made. The PTR-MS is an online chemical ionization (CI) mass spectrometer suitable for these kinds of measurements. Headspace gas, which consists of air/inert gas and volatile compounds in trace amounts, is introduced into a CI cell, ionized by proton transfer from H_3O^+, and mass-analyzed (*6*). Protonated aroma compounds drift downstream toward the end of the drift tube, where the compounds are accelerated by an electrical field into a quadropole mass spectrometer. Water (H_2O) has a proton affinity of 166.5 kcal/mol, and volatile compounds with an affinity exceeding this value become ionized by proton transfer from H_3O^+. All constituents of air have an affinity of <166.5 kcal/mol and are therefore not ionized (*7*). The ion source operates at atmospheric pressure, which makes it safe and relatively easy to sample air from people.

Studies using in-nose measurements of aroma release from different kinds of gels have recently been performed, all with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) (*8*−*10*), another method available for online flavor measurements. Baek et al. (*8*) have shown that, due to different rates of gel breakdown in the mouth, aroma compounds from gelatin gels are released at different rates. The release of aroma compounds from mixed-phase gels with gelatin and agarose measured in vivo by APCI-MS has been shown to be dependent

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Table 1. Aroma Compounds Used in This Study

compound class	aroma compound
alcohols	1-butanol
	3-methyl-1-butanol
ketones	2,3-butanedione
	2-butanone
	2-heptanone
	2-octanone
	2-decanone
aldehydes	hexanal
	heptanal
esters	ethyl acetate
	ethyl butyrate

Table 2. Concentrations of White Syrup, Citric Acid, and Pectin in the Four Pectin Systems[a]

gel name	white syrup (%)	citric acid (%)	pectin (%)	δ (deg)	G' (Pa)	G'' (Pa)	F_{break} (N)
gel 1	90.0	3.0	1.50	4.6	1050	83.2	56
gel 2	79.0	1.0	1.85	20	91	34.1	33
gel 3	85.0	1.0	2.00	40	58	49.7	13
viscous soln	75.0	0.1	1.50	86	0.1	2.00	<0.5

[a] The values of the rheological parameters on these pectin systems, measured by oscillation at 1 Hz, in an earlier study (δ, G',G'') (*11*). Penetration measurements (The force required to break the surface (90 mm ϕ) of the gel with a probe (35 mm ϕ), F_{break}) performed earlier on these pectin systems (*17*).

on both the matrix and the properties of the aroma compound (*9*). Flavor release from whey protein gels was studied by Weel et al. (*10*), who found that the texture of the gels, rather than the in-nose flavor concentration, determines the perception of flavor intensity.

The aim of the present study was to investigate how, during eating, aroma compounds are released from pectin gels with different strengths and structures. Four pectin-containing systems were subjected to both model mouth analysis and in-nose measurements. The model mouth was used beacuse the conditions are more easily controlled than those in the human mouth, although it was important to compare the results with those from the in-nose measurements. Aroma concentrations were monitored with a PTR-MS in both cases.

MATERIALS & METHODS

Gel Preparation. The pectin gel system used basically consisted of water, high-methoxyl pectin (HMP), white syrup (34% w/w sucrose, 24% w/w glucose, 22% w/w fructose, 20% w/w water) (Danisco Cultor, Arlöv, Sweden), citric acid, and eleven aroma compounds. The aroma compounds are listed in **Table 1**. The pectin used was GRINDSTED Pectin CF 120 (Danisco Cultor, Aarhus, Denmark), an extra slow-setting, high-ester pectin standardized with sugars. As a first step in gel preparation, white syrup was mixed with water and heated. Pectin was mixed with sodium citrate and added to the syrup solution, which was then heated until boiling. After boiling for 2 min, citric acid was added to the mixture. The aroma compounds were added to water at a concentration of 0.005% (v/v) and finally added to the mixture as the last preparation step. For the in-nose measurements, the aroma concentration used was 0.020% (v/v). After preparation, the samples were placed in a refrigerator and kept at 4 °C for 24 h. Four different pectin-containing systems with different strengths (defined by the force value (F) of the highest peak according to texture analyzer measurements performed in an earlier study (*17*)) were mixed, each one prepared in triplicate (**Table 2**). The ingredients in the pectin systems are listed in **Table 2**. The samples were balanced with water to 100%.

Model Mouth/PTR-MS. For isolation of the aroma compounds, 6 g of a pectin-containing sample were placed in a sample flask (70 mL) of the model mouth system (*3*). The temperature was kept constant at 37 °C by water circulating through the cavity wall of the flask. Artificial saliva (4 mL), consisting of distilled water, potassium phosphate dibasic trihydrate (1.369 g/L), sodium chloride (0.877 g/L), calcium chloride dehydrate (0.441 g/L), sodium nitrate (0.5 g/L), sodium bicarbonate (5.208 g/L), mucin (2.016 g/L), and α-amylase (200 000 units/L) (*12*), was added to half of the samples. Mastication was imitated by a plunger making up-and-down rotating movements, and the rate was varied from 0 to 26 and 52 rpm for all pectin-containing systems. The model mouth was connected directly to the PTR-MS and the headspace was withdrawn using a vacuum pump at a rate of 100 mL/min. A portion (15 mL/min) of the withdrawn headspace was lead into the PTR-MS through a heated transfer line. The aroma compounds were analyzed according to the method described by Lindinger et al. (*13*). Eleven mass fragments (*m*/*z* 57 (1-butanol), *m*/*z* 61 (ethyl acetate), *m*/*z* 71 (3-methyl-1-butanol), *m*/*z* 73 (2-butanone), *m*/*z* 83 (hexanal), *m*/*z* 87 (2,3-butanedione), *m*/*z* 97 (heptanal), *m*/*z* 115 (2-heptanone), *m*/*z* 117 (ethyl butyrate), *m*/*z* 129 (2-octanone), and *m*/*z* 157 (2-decanone) (*14*)) were analyzed, and their changes with time during artificial chewing in the model mouth were followed for 5 min. Unfortunately, the response for ethyl acetate and 2-decanone did not exceed the response for the baseline sufficiently to be used for evaluation. These two compounds were therefore not presented in the results. Aroma concentrations were transmission- and fragmentation-corrected and calculated according to a method developed by Lindinger et al. (*13*). Three replicates of each pectin-containing system were analyzed and used for statistical evaluation.

In-Nose Analysis/PTR-MS. For analysis of the aroma concentrations in the nose, the assessor (AH) had a u-shaped glass nosepiece inserted into the nostrils. During breathing, the air was withdrawn at 100 mL/min by a vacuum pump into the PTR-MS instrument. An orthogonal outlet on the nosepiece made it possible for the assessor to breathe normally with a constant air flow. Six grams of the pectin-containing samples were placed on a spoon and placed in the mouth. The assessor was breathing for 30 s at a rate of 4 s in and 4 s out before the sample was taken into the mouth. The samples were chewed for 1 min at a mastication rate of one bite/second and then swallowed. After swallowing, the nosespace for the assessor was further monitored for one minute. The eleven mass fragments, which has been monitored in the model mouth, were also followed in the nose, where the same calculations were applied. However, only four of the aroma compounds (2-butanone, 2,3-butanedione, 2-heptanone, and ethyl butyrate) showed a response above noise level. These are therefore the only compounds presented in the results. All pectin systems were analyzed in three replicates.

Statistical Analysis. Principal Component Analysis (PCA) was performed to gain an overview of how the mastication rate, saliva concentration, and gel type were correlated to the maximum concentration (C_{max}) of the aroma compounds, and of the time it took to reach this maximum (T_{max}). A biplot was used to show the correlation between the samples, the design variables, and C_{max}, T_{max}, and the aroma concentration at 250 s (C_{250}). The total variation (V_{tot}) between the samples exceeded the variation between the replicates (V_{rep}) ($V_{tot}/V_{rep} > 3$), which means that the systematic variation was larger than the experimental error. Validation of the PCA was established using cross-validation (*15*). All calculations were performed using Unscrambler Extended Version 7.5 (Camo ASA, Oslo, Norway) software. Student's *t*-test (two-sample test, equal variance) was used to further evaluate significant differences in the release of the aroma compounds regarding structural variations between the pectin-containing systems. A *p*-value of <0.05 was used throughout the study to indicate statistical significance.

RESULTS & DISCUSSION

Model Mouth. *Mastication.* The four different pectin-containing systems were placed in the model mouth and chewed at different rates, with and without saliva, resulting in different release curves, as exemplified by ethyl butyrate without any

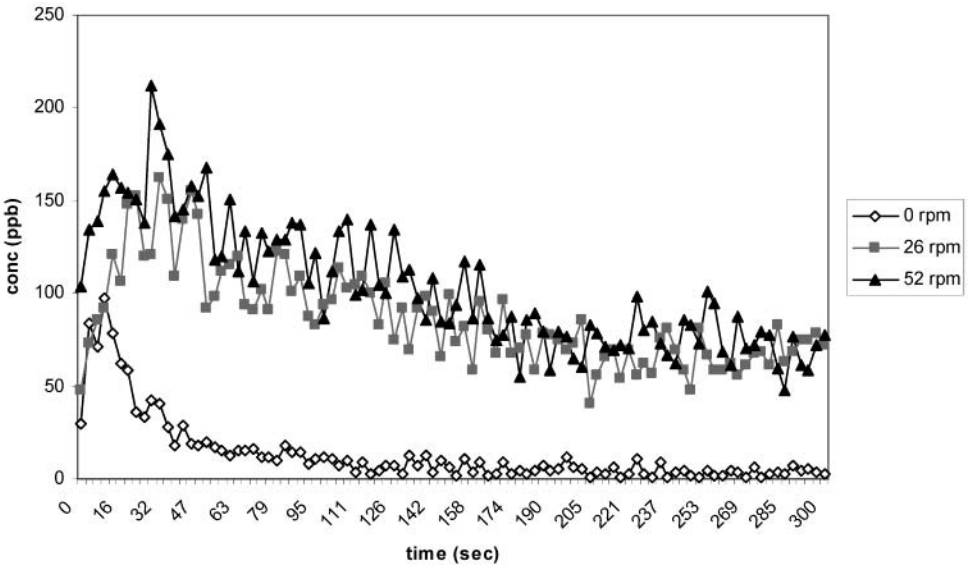

Figure 1. Release curves of ethyl butyrate from gel 3 at different mastication rates and without addition of saliva.

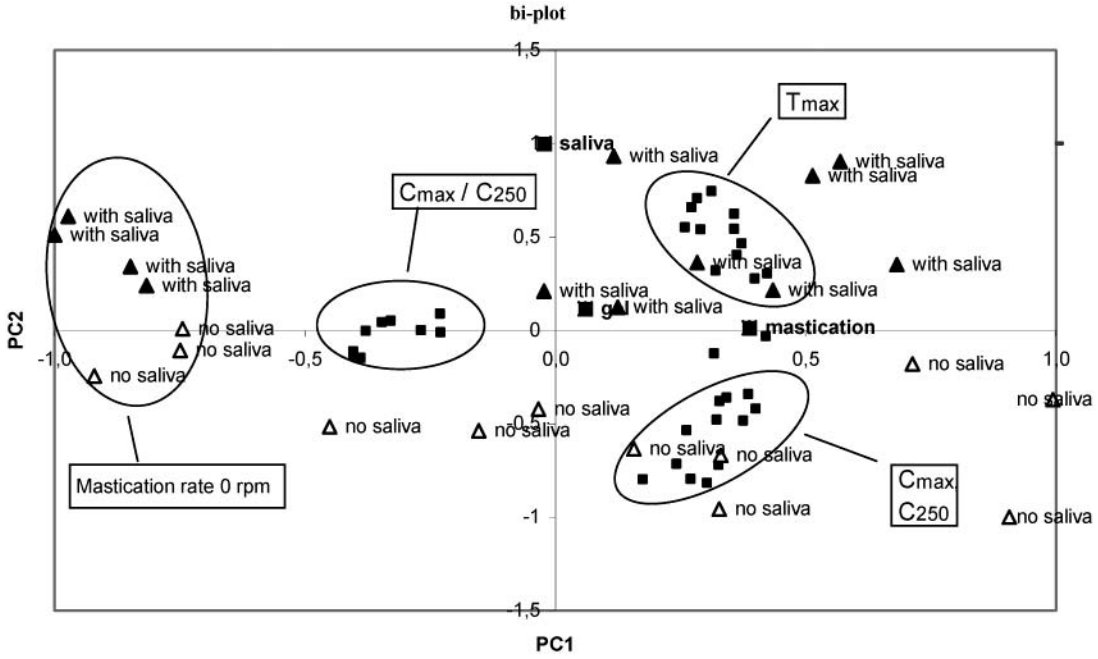

Figure 2. A PCA biplot of all samples and all variables. The degree of explanation was 50% for principal component 1 (PC1) and for 17% for PC2. △ = samples without saliva; ▲ = samples with saliva; and ■ = variables (C_{max}, T_{max}, C_{250}, C_{max}/C_{250}).

saliva (**Figure 1**). The loading plot showed that of all variables (mastication, saliva, and gel type), mastication rate was the parameter with the largest influence on aroma release (**Figure 2**). Mastication was positively correlated to C_{max}, T_{max}, and C_{250}. This means that an increase in mastication rate increased the aroma release due to the increase in surface area after mastication breakdown of the samples. Mastication has previously been reported to affect aroma release, because it disturbs diffusion gradients and creates fresh interfaces (*16*). The results are in agreement with Van Ruth et al. (*3*), who showed that mastication increases aroma release in general, although the effect of mastication rate differs among compounds, depending on their varying mass transfer coefficients. The positive correlation between mastication and T_{max} suggests that with mastication, C_{max} was reached later than without mastication. This was probably due to the increased release from new surfaces that were generated with each chew until a plateau of aroma concentration was reached.

In the PCA plot, it was also seen that C_{max} and C_{250} were positively correlated (**Figure 2**), which indicated that a sample with a high maximum concentration had a high aroma concentration throughout the measurement. This was a result of a higher release with mastication for all compounds than without.

The value C_{max} divided by C_{250} (C_{max}/C_{250}) was introduced to describe the shape of the release curve after the maximum. In the biplot, it was seen that this ratio correlated negatively to mastication (**Figure 2**). This indicated that when the samples were not chewed a higher C_{max} in relation to C_{250} was reached, leading to a more sharply decreasing release curve after the maximum than seen with higher mastication rates (**Figure 1**). The samples that were not chewed, with or without saliva, were positively correlated to the C_{max}/C_{250} ratio (**Figure 2**), which confirmed this statement. As seen in a release curve for samples evaluated without mastication, C_{max} was reached after about 15 s, after which the aroma concentrations successively leveled off (**Figure 1**). The release profile without mastication reflects

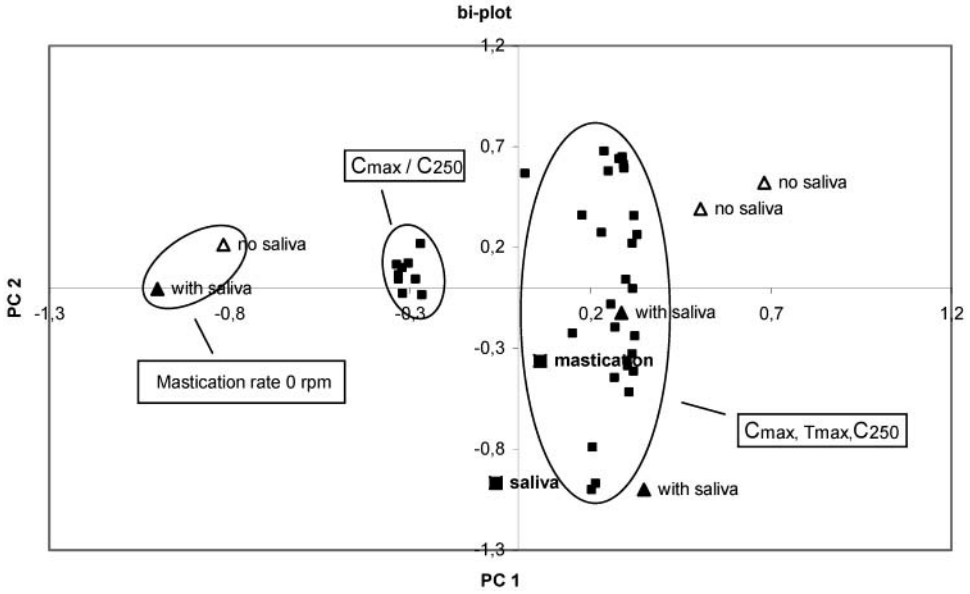

Figure 3. A PCA biplot of all variables and the viscous solution samples. The degree of explanation was 66% for PC1 and 13% for PC2. △ = samples without saliva; ▲ = samples with saliva; and ■ = variables (C_{max}, T_{max}, C_{250}, C_{max}/C_{250}).

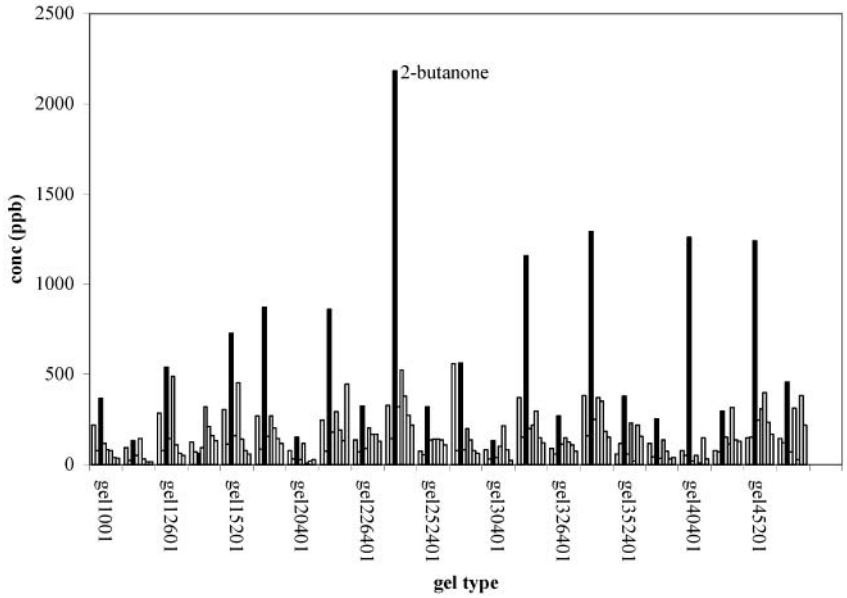

Figure 4. C_{max} for all aroma compounds from all samples. Example of sample codes: gel22640 = gel 2, 26 rpm, 40% saliva.

a dynamic procedure, where the aroma compounds in the headspace are replenished more rapidly than they can be replaced. It also reflects the affinity of the compounds for the pectin-containing systems (*3*).

Saliva. The samples that were chewed at a mastication rate of 26 and 52 rpm without saliva correlated positively to C_{max} and C_{250} (**Figure 2**). When saliva was added to the samples chewed at 26 and 52 rpm, there was a positive correlation between the release from these samples and T_{max}. These results showed that at higher mastication rates, addition of saliva decreases the release of aroma compounds. This is in agreement with Van Ruth et al. (*3*), who observed that aroma release decreases with an increased volume of saliva, partly due to dilution. However, the relative decrease was different for some aroma compounds when rehydrated French beans were analyzed.

Gel Type. From the PCA plots, it was seen that gel type had a relatively small influence on aroma release. This could have been due to the fact that mastication and saliva had such a large influence on the release that the effect of gel structure was somewhat negligible. When PCA plots were performed for each pectin-containing system separately, some differences were obtained. For the gels (gels 1, 2, and 3) it was seen that mastication was positively correlated to C_{max}, C_{250}, and T_{max}, as seen in the overall PCA plot (**Figure 2**). Samples chewed with saliva correlated positively to T_{max}, whereas the release from samples chewed at the same rates without saliva correlated positively to C_{max}. This indicates that the total aroma release was lower when saliva was added to the samples. Aroma release from samples without mastication correlated positively to C_{max}/C_{250}, which was shown by a strong decrease in the release curve after the C_{max} was reached.

The viscous solution showed a positive correlation between mastication and C_{max}, T_{max}, and C_{250}. However, in a biplot, it was seen that aroma release from samples that were chewed, with and without saliva, were positively correlated to both C_{max} and T_{max} (**Figure 3**). This could be explained by the fact that from a liquid food, the aroma compounds are already in the liquid phase and are transported directly to the gas phase (*3*).

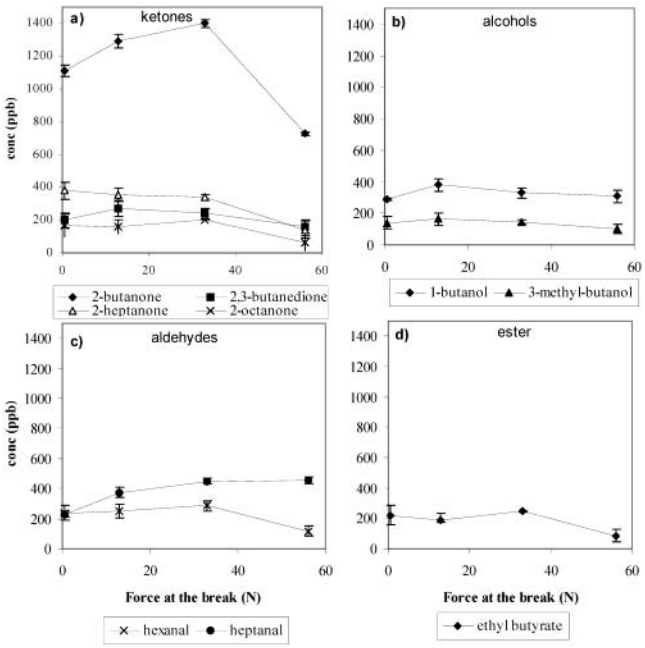

Figure 5. C_{max} for all aroma compounds from the four pectin-containing systems at a mastication rate of 52 rpm, without addition of saliva. (a) ketones, (b) alcohols, (c) aldehydes, and (d) ester.

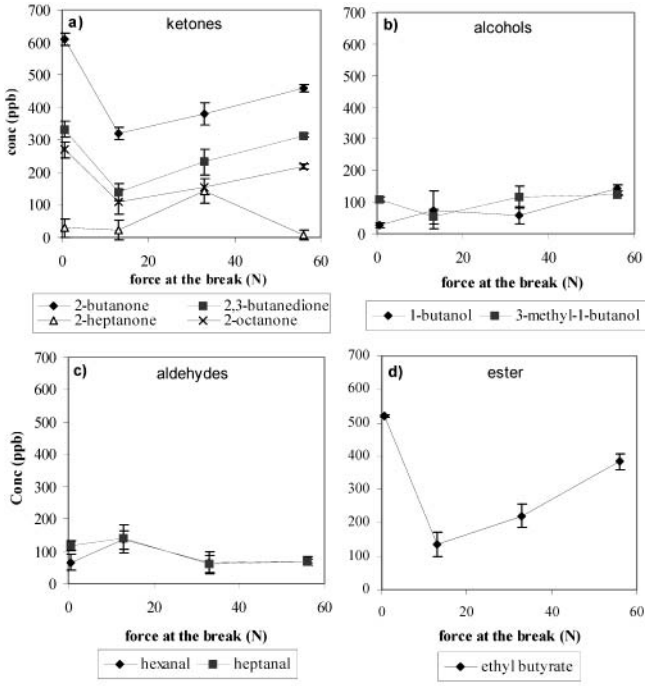

Figure 6. C_{max} for all aroma compounds from the four pectin-containing systems at a mastication rate of 52 rpm, with addition of 40% saliva. (a) ketones (b) alcohols (c) aldehydes (d) ester.

Therefore, viscous solutions did not show any differences in T_{max} when adding saliva than when no saliva was used.

Effects on Aroma Compounds. The PCA biplot of all samples showed that Cmax of all aroma compounds was correlated (**Figure 2**). However, when the aroma release was studied with regard to gel type, differences in Cmax for the different compounds could be seen. In a line plot, it was seen that, from most of the samples, release of 2-butanone was the highest (**Figure 4**). This could have been due to its high volatility or polarity (log $P = 0.29$), which favors its concentration in the gas phase, as opposed to its concentration in the more nonpolar gel matrix. For the samples that contained saliva, this effect could perhaps have been enhanced by a "salting-out" effect caused by saliva on hydrophilic compounds (*4*). For the other compounds, the release varied with the type of pectin-containing system, mastication rate, and saliva concentration. The mass transfer coefficient of each aroma compound probably influenced the release as well.

With a mastication rate of 52 rpm and no saliva used, gel 1 showed a significantly lower C_{max} of 2-butanone, hexanal, 2,3-butanedione, 2-heptanone, ethyl butyrate, and 2-octanone compared with gel 2 (**Figure 5**). Gel 3 and the viscous solution also showed a higher C_{max} of these compounds, although it was not significantly different from that of gel 1. These results are in agreement with findings reported in earlier studies, where a stronger gel was seen to give a lower aroma concentration in the headspace, though these studies were performed in a static system (*11*, *17*). Another reason was probably that this gel was not so easily broken down during mastication.

Samples chewed at a mastication rate of 52 rpm and with the addition of 40% saliva showed a significantly higher C_{max} for most compounds from the viscous solution compared with the gels (**Figure 6**). 2-Butanone, 2,3-butanedione, 2-octanone, ethyl butyrate, and heptanal showed a significantly higher release above the viscous solution compared with gels 1 and 2. For 1-butanol, 3-methyl-1-butanol, and ethyl butyrate, C_{max} was significantly higher above the viscous solution than above gel 3. The viscous solution did not contain any network, and therefore, it also gave the highest release (*11*). However, for hexanal and 2-heptanone, C_{max} was higher above gel 2 than above the viscous solution and gel 1.

When a mastication rate of 26 rpm was used on the samples no significant differences in aroma release were found between the pectin-containing systems, either with or without saliva. The pectin systems were probably not broken down to the same extent as at the rate of 52 rpm and therefore gave more or less the same aroma release from all of the pectin-containing samples. However, most compounds had a slightly lower C_{max} than at 52 rpm.

At mastication rates of 26 and 52 rpm and without any saliva added, the T_{max} for most of the compounds was reached significantly later for the viscous solution (**Figure 7h**) than for the gels (**Figure 7**, parts b, d, and f). However, C_{max} then stayed at a higher level than that for the gels. This was probably an effect of the quicker transportation of the aroma compounds through the viscous solution to the interface, keeping the aroma release at a higher level.

When no mastication and no saliva were used, the C_{max} of most of the compounds from the viscous solution was significantly lower than that from the gels (**Figure 8**). However, the release of 2-butanone, heptanal, ethyl butyrate, and 2-octanone was not significantly higher above gel 1 than above the viscous solution. C_{max} of most compounds was significantly higher for gel 2 and 3 than for the strong gel and the viscous solution. This was probably due to the weakness of gels 2 and 3. When pieces of these gels were cut to be placed in the model mouth, the damage to the network during cutting could have made the transport of the aroma molecules from these gels easier than that from the strong gel and the viscous solution. No significant differences were seen between the pectin-containing systems for T_{max} (**Figure 7**, parts b, d, f, and h).

With no mastication and addition of 40% saliva, only a few significant differences in C_{max} and T_{max} between the different pectin-containing systems were seen for the aroma compounds. Heptanal, 2-heptanone, and ethyl butyrate showed a significantly

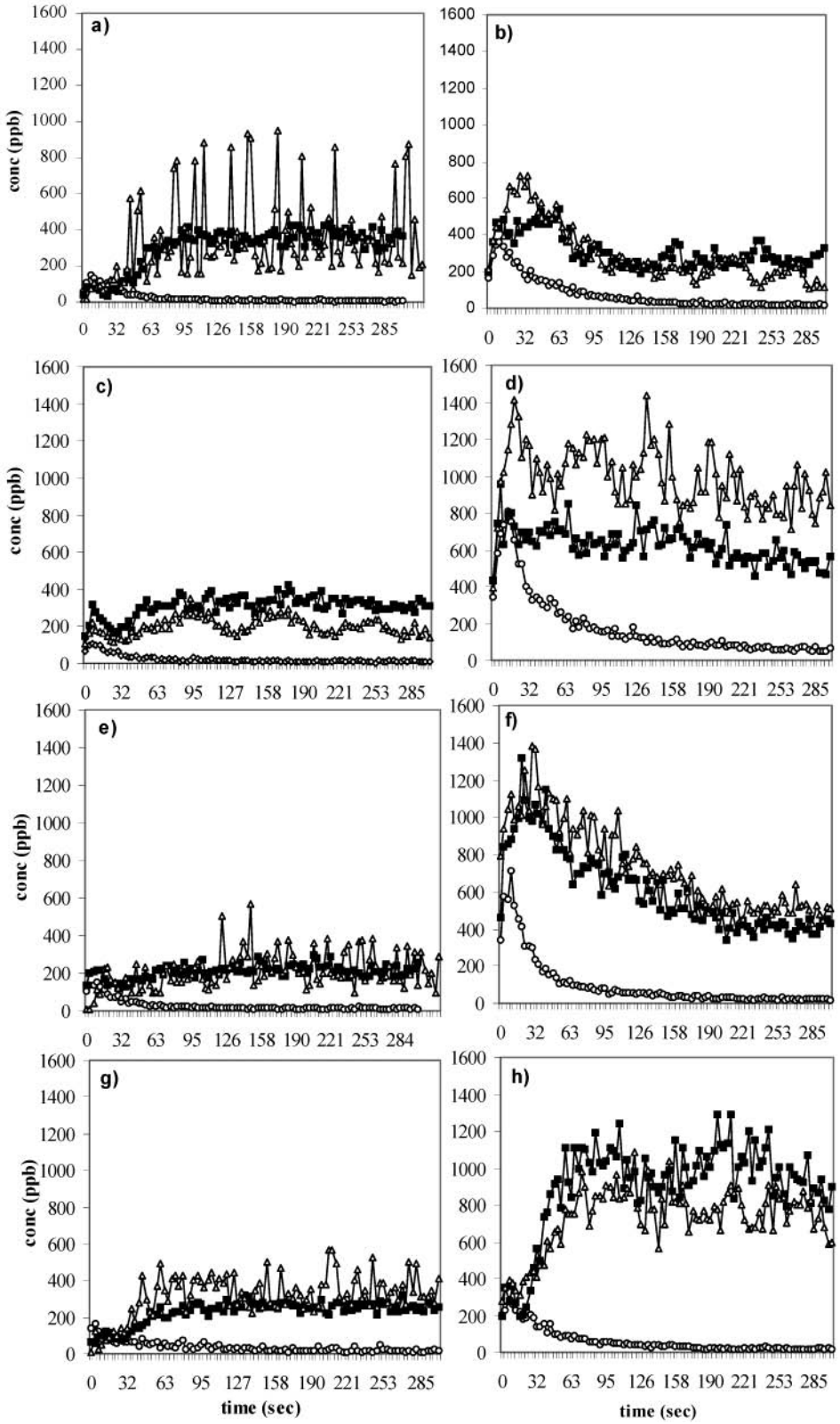

Figure 7. Release curves for 2-butanone from gel 1–3 and the viscous solution with and without addition of saliva, (a) gel 1 with saliva, (b) gel 1 without saliva, (c) gel 2 with saliva, (d) gel 2 without saliva, (e) gel 3 with saliva, (f) gel 3 without saliva, (g) the viscous solution with saliva, and (h) the viscous solution without saliva.

lower release above gel 1 than that above gels 3 and the viscous solution. However, most compounds showed no significantly different release from the various pectin-containing systems, as exemplified by 2-butanone in **Figure 7**, parts a, c, e, and g.

The results showed that release of aroma compounds is affected by the gel matrix as well as by the different properties of the aroma compounds, such as volatility and polarity. It was seen that some compounds are little affected by changes in gel composition and that others are not affected at all, which is in agreement with Taylor et al. (*9*).

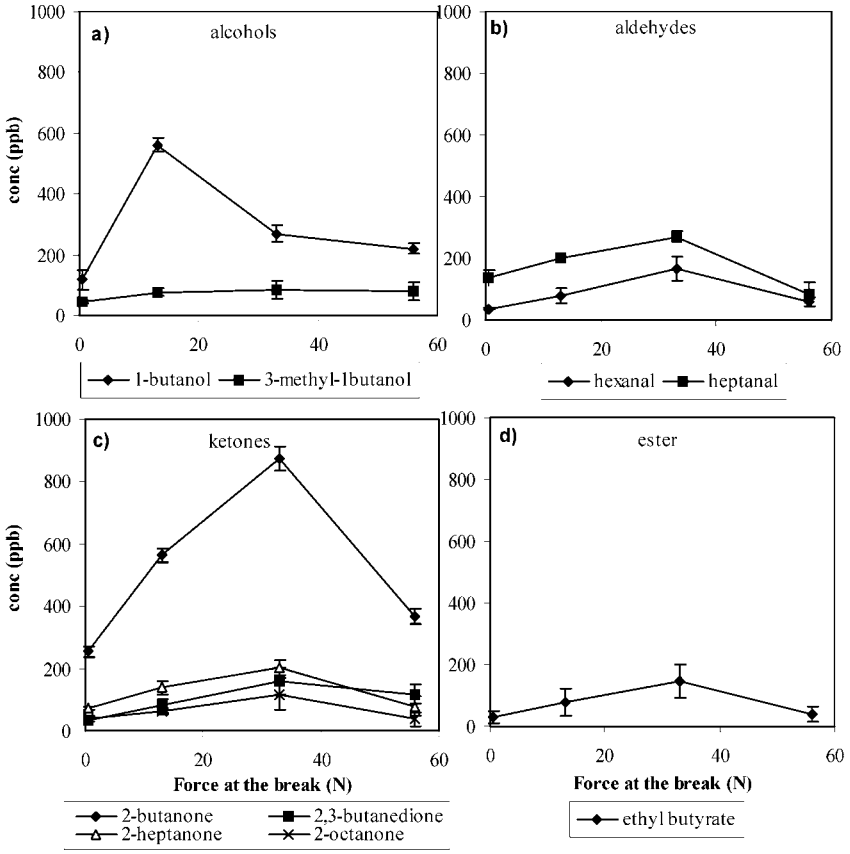

Figure 8. C_{max} for all aroma compounds from the four pectin-containing systems without mastication and without addition of saliva. (a) alcohols (b) aldehydes (c) ketones (d) ester.

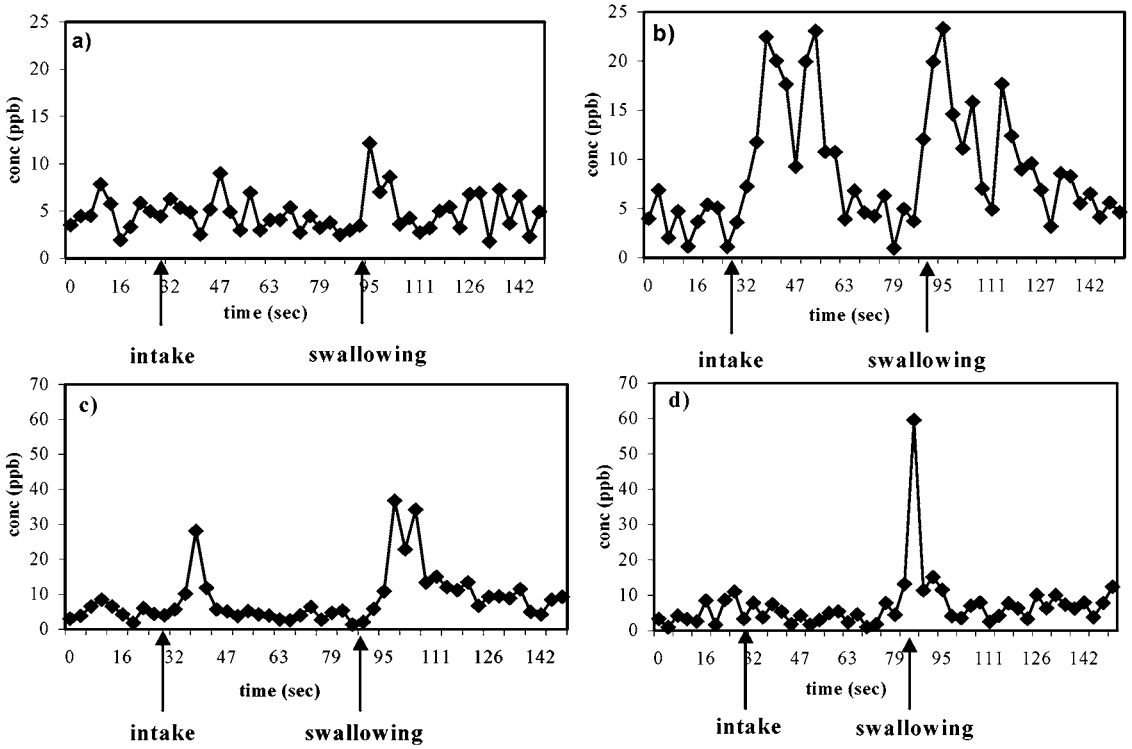

Figure 9. (a) Release curve for 2-butanone when gel 1 was being held in the mouth of the assessor. The coefficient of variance (CV) for the three replicates was 59%. (b) The release curve of 2-butanone when gel 1 was chewed by the assessor. CV, 58%. (c) Release curve of 2-butanone when the viscous solution was being held in the mouth of the assessor. CV, 65%. (d) The release curve of 2-butanone when the viscous solution was chewed by the assessor. CV, 57%.

In-Nose Analysis. In-nose analyses were conducted on a strong gel and a viscous solution. When the strong gel (i.e., gel 1) was held in the mouth of the assessor without chewing, no increase in aroma release was seen after intake (at 30 s) of the

gel into the mouth, as shown here for 2-butanone (**Figure 9a**). However, after chewing for 1 min, the assessor swallowed the gel (at 90 s), after which the aroma release increased.

While the assessor was chewing on the same gel, another aroma release pattern was seen, however. At the time of gel intake, there was a large increase in release of all four compounds, as shown for 2-butanone (**Figure 9b**). The concentrations of the aroma compounds in the nose were at a high level for about 30 s, whereafter a gradual decrease was seen during the next 30 s. After swallowing, the aroma concentrations were increased to the same extent. During the last minute of breathing only, the aroma concentrations again successively decreased (**Figure 9b**).

The viscous solution showed a similar release pattern as seen with gel 1. However, when the viscous solution was held in the mouth without chewing, there was an increase in the aroma concentration already at the intake (**Figure 9c**). Another increase in release was seen for the compounds at the time of swallowing. With the viscous solution, there was no increase in aroma concentration in the nose at the time of intake (**Figure 9d**). However, a large increase was seen after swallowing.

These results are in agreement with Buettner et al. (*18*), who showed that when a liquid food is consumed, the mouth can be regarded as a closed system, as long as no swallowing occurs. In a resting position, a tight closure is formed by the soft palate and the pharyngeal part of the tongue. No air flow can pass from the mouth to the nose, and therefore, aroma compounds are not released to the nasal cavity. During swallowing, the connection between the soft palate and the tongue opens, and the aroma compounds can reach the nose (*1*). In the present study, even though the viscous solution was chewed, the tongue−soft palate border did not open, because the viscous solution was not supposed to be swallowed. The increase in aroma concentration seen at the intake was probably also an effect of an open tongue−soft palate border.

When the strong gel was eaten and chewed, there was a high concentration of aroma compounds in the nose even before swallowing. The reason for this was that the soft palate-tongue border was opened each time the gel was chewed, making it possible for the aroma compounds to reach the nose (*1*).

The aroma concentration in the nose when the strong gel was consumed was lower than that when the viscous solution was consumed. This shows that the strength or structure of pectin gels is important with regard to the quantity of aroma compounds that reach the nose. It was also seen that chewing affected the aroma release and that the aroma concentrations were about twice as high when the pectin-containing systems were chewed compared with when they were just being held in the mouth, both at the time of intake and after swallowing. Consequently, mastication has a large influence on how much of the aroma compounds are released to the nose.

The model mouth used was one of the models that best simulates the oral conditions in the human mouth (*19*). However, the results from the model mouth were still not exactly in accordance with the in-nose measurements. There remained some differences between the systems, as for example, absorption of aroma compounds by the soft palate in the real mouth. Swallowing, which has been shown to have a large effect on the concentrations of aroma compounds that reach the nose, could not be observed in the model mouth analysis either. In addition, the amount and composition of the saliva, which vary in the human mouth during the year, may be another aspect to be taken into account when comparing model mouth data with results from in-nose measurements. Yet another difference is the mastication, which in a real mouth is probably not performed in the same way each time, and the breathing, which most likely varies as well.

CONCLUSION

Mastication was found to have a large influence on the release of aroma compounds from pectin-containing systems in the mouth and to the nose. A higher aroma release was shown when mastication was applied to the samples, although the release was also dependent on the gel type and the properties of the aroma compounds. Addition of saliva reduced the release of aroma compounds in general, and furthermore changed the release profiles for the samples that were chewed.

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