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Effect of Apple Particle State on the Release of Volatile Compounds in a New Artificial Mouth Device

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Varying the crushing parameters in a model mouth apparatus gave different crushed apple samples, which were compared to apples crushed in the human mouth by six people. An image analysis method was developed to measure the similarity between apple particles after crushing in the artificial mouth and in the human mouth. Thus, experimental conditions were determined that produced fruit in a state closest to that obtained after mastication in a human mouth. The influence of these different conditions on the quantity of released volatile compounds was then studied.

KEYWORDS: Apple; artificial mouth; image analysis; volatile release

INTRODUCTION

To be perceived while eating a food, aroma compounds must be released from the food matrix before being transported to the receptors. In solid foods, a succession of events takes place, which influence volatile release. When the food is crushed and mixed with saliva, its structure is modified and the diffusion of its volatiles from the resulting bolus to the headspace is affected. With mastication, the food surface area exposed to the air increases, and the food matrix is separated from the water it contained initially. These processes involve not only the composition and structure of foods but also the conditions in the mouth, that is, temperature, presence of saliva, rate at which food is broken down during chewing, and possible adsorption by the mouth mucosa (*1–9*). Moreover, the disruption of tissues can induce the enzymatic generation of volatiles. Two experimental approaches have been developed to study volatile release in the mouth. The first one consists in analyzing the air exhaled from the subject's nose or mouth (*2, 10–13*). Another approach consists in reproducing human mouth conditions by the use of an "artificial mouth". The borderline between volatile analyses by dynamic headspace and by artificial mouths has sometimes been blurred. Indeed, the artificial mouths described in the literature vary in terms of realism. The most conclusive ones combine several processes imitating changes during eating. Generally, the temperature is the same as that of the human mouth, and the food sample is humidified by water or artificial saliva (*14–16*). Nassl et al. (*17*) showed the importance of stirring in the release of volatiles from oil/water emulsions. However, depending on the texture of the sample and the apparatus, it can be either stirred (*15, 17–19*) or crushed with a plunger (*16, 20*). The stripping gas can flow through or above the food sample, inducing different volatile compound releases (*21*). By comparing three model mouth systems, van Ruth et al. (*20*) showed the influence of the gas flow course and mastication on the release. The Retronasal Aroma Simulator designed by Roberts and Acree (*22*) revealed that both saliva volume and shear rate have an effect on the release of beverage aroma compounds (*22, 23*). Another study focused on the deglutition step (*24*), showing its importance in the release of volatiles from liquid foods. Extraction time can vary from a few seconds, when using online measurements by atmospheric pressure chemical ionization/mass spectrometry (APCI/MS) or proton transfer reaction/mass spectrometry (PTR/MS) (*13*), to longer times with "offline" measurements using trapping polymers. Online measurements are usually carried out on model matrices, containing a limited number of volatile compounds. In the study of a real food, it may be necessary to use trapping systems first to extract all of the volatiles and to identify those that greatly contribute to the aroma. Each of these studies focused on the release of volatile compounds from model foods or soft real foods. To our knowledge, very hard foodstuffs have not yet been studied. The crushed state of the food matrix after artificial mastication has never been described in flavor studies even though it can influence aroma release. In a study focusing on in vitro digestion, the particle sizes of starchy foods after in vivo mastication or mincing were compared by image analyses (*25*).

In the present work, an artificial mouth was designed in which we studied apples as a model of real foodstuffs with a complex structure and chewy texture. Using a previous artificial mouth (*26*), we showed that the amounts of extracted volatile compounds were not the same when apples were crushed, cut into slices, or reduced to a puree state. It follows that to study the aroma compounds responsible for global aroma perception, it

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is necessary to reproduce the changes that the foodstuffs undergo in the human mouth. Our objectives were, first, to find artificial mastication settings that best reproduce human mastication and, second, to determine if artificial mastication conditions have an effect on the release of volatile compounds. Our aim was not to reproduce human mouth conditions exactly, but to reproduce the result of mastication. Some control parameters of the artificial mouth were determined by comparison with in vivo mastication. Parameters that could not imitate exactly in vivo conditions were assigned different values, with an experimental plan. Fruit crushed in the artificial mouth were compared with fruit chewed in vivo by image texture analysis. Volatile compounds extracted in each experimental condition were analyzed. We did not compare volatile compounds released after in vitro and in vivo mastication in the present study.

MATERIALS AND METHODS

Materials. *Fruit.* One hundred apples of the Golden Delicious cultivar were purchased at a local supermarket 1 week before analysis. They were stored at 2 °C until the day before measurements and were brought to room temperature 24 h before analysis. Apples to be analyzed were selected on the basis of absence of damage or blemishes. They were peeled immediately before analysis.

Chemicals. Artificial saliva (*27*) was prepared by dissolving in 250 mL of water (purified by a Milli-Q system, Millipore Corp., Molsheim, France) 1.3020 g of $NaHCO_3$ (99.5%), 0.2193 g of NaCl (99.5%), 0.1250 g of NaN_3 (99.5%), 0.1193 g of KCl (99.5%), 0.1102 g of $CaCl_2 \cdot 2H_2O$ (97%), 0.2612 g of K_2HPO_4 (98%), mucin (0.540 g), and porcin α-amylase (3.2504 g or 50000 units) (pH adjusted to 7). All of the salts were purchased from Merck (Fontenay-sous-Bois, France), and mucin and porcin α-amylase came from Sigma-Aldrich Chemical Co (Saint-Quentin-Fallavier, France).

n-Alkanes from octane to nonadecane were used to determine volatile compound retention indices. They were purchased from Aldrich (Steinheim, Germany), except for octane, hexadecane, octadecane, and nonadecane, which came from Sigma (St. Louis, MO), and pentadecane, which was from Fluka (Steinheim, Germany). Ethanol, used as a solvent, was purchased from Vermapur (Fontenay-sous-Bois, France).

In Vivo Mastication. *Determination of the Apple Quantity That Can Be Studied in the Artificial Mouth.* Nineteen people (5 men and 14 women, 21−44 years old) were asked to bite an apple as naturally as possible and to spit out the resulting piece without masticating it. The apple piece was weighed. Each subject was then asked to take a maximal amount of water in his/her mouth and to spit it out for its weight to be measured. Each measurement was carried out in triplicate for each subject, and a ratio was calculated between the average amount of water (considered to be the mouth volume) and the average weight of apple pieces. This ratio was then used to determine the apple quantity to introduce into the artificial mouth container for each experiment.

Determination of the Apple/Saliva Ratio To Introduce into the Artificial Mouth. The same 19 people chewed an apple piece previously cut and weighed. No indications were given concerning the mastication conditions and the time, except that they were not allowed to swallow. They were asked to spit out the entire bolus when swallowing would normally have been triggered. All apple fragments were collected by rinsing the mouth with a known quantity of water and spitting it out. Saliva quantity was determined as follows: saliva weight = total weight − apple weight − rinsing water weight.

Determination of the Breakdown State of Apples Chewed in the Human Mouth. Six people (three males and three females, aged between 20 and 60 years to take into account a possible evolution of dentition with age) were asked to peel an apple, crunch a piece of it, chew it normally, and then spit out the bolus at the point when swallowing would normally have been triggered. To take into account apple texture variability, four randomly selected apples were used, and two apple pieces from each of them were crunched. In total, each subject chewed eight apple pieces, which were mixed before being immediately photographed. Two sessions were carried out.

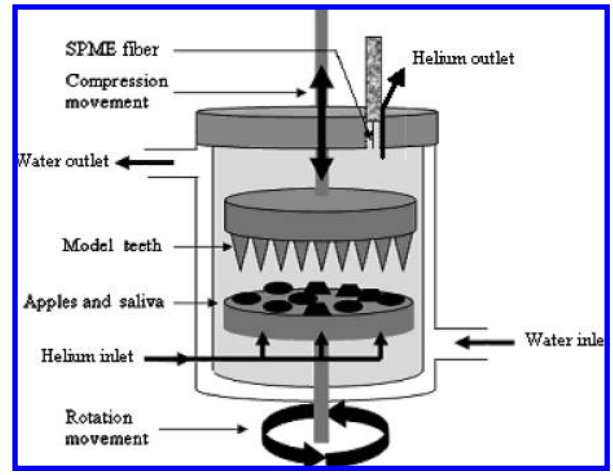

Figure 1. Schematic representation of the artificial mouth apparatus.

In Vitro Mastication. The artificial mouth (**Figure 1**) is composed of a sample container (600 mL), a notched plunger, and variable-speed motors to control precisely the speed of compression and rotation movements. The container is maintained at 37 °C by means of a laboratory thermostat (Bioblock Scientific) via an outer layer. The container is sealed with a cap maintained by a circlip. All the parts of the artificial mouth that can be in contact with the analyzed food are made of inert materials (treated stainless steel and PEEK). At the same time as food is broken down, helium flows through the device at a sequential rate to reproduce breathing phenomena.

Equal longitudinal slices (8 mm thick and 2 cm long) were cut from four peeled Golden Delicious apples to take into account the variability of fruit volatile composition. Apples slices and artificial saliva were introduced into the container of the artificial mouth.

Trials were carried out according to an experimental procedure designed to obtain different breakdown states of apple slices. The studied parameters were compression movement frequency (25, 50, and 75 chews per minute), rotation movement speed (10 and 50 rotations per minute), and time (5, 10, 20, and 30 min) (**Table 1**).

The crushed apples were transferred and spread in a Petri dish to present a flat surface and a regular thickness. Two digitized images (one picture on each side of the box) of the apple mixture surface were immediately acquired, and apple color was measured in each set of conditions.

Extraction of Apple Volatile Compounds by the Artificial Mouth. The helium flow carried away volatile compounds, released by the breakdown of the fruit, to an opening in the cap, where a solid-phase microextraction (SPME) fiber (Supelco, Bellefonte, PA) coated with Carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm thickness) was located. This fiber was compared in previous experiments with another fiber of different selectivity (Car/PDMS/DVB, 75 μm). It was chosen as being the most sensitive and having a greater repeatability. The extracted volatile compounds were immediately desorbed from the SPME fiber into a splitless mode injection port for 2 min to be analyzed by chromatography. The same fiber was used for all experiments.

To check the repeatability of the experiment, one set of conditions was performed in triplicate.

Comparison of in Vivo and in Vitro Conditions. *Image Analysis.* After each experiment in vivo and in the artificial mouth, images of the resulting apple state were acquired. The imaging system was composed of a CCD camera (Sony DFW-SX910, NOESIS, France) equipped with a lens (focal length of 9 mm) and mounted on a photographic bench. The camera was connected to a PC with a bus IEEE-1394. The lighting system was composed of two white light tubes inside two semispherical black chambers, thus providing a homogeneous direct visible light. Digitized images of 512 × 512 pixels corresponding to a sample surface of 5 × 5 cm were chosen. We considered this surface to be representative of the whole apple sample. Two images were acquired per sample. We obtained 12 pictures for apple breakdown in the human mouth and 48 pictures for apple breakdown in the artificial mouth.

Images, initially acquired in an RGB (red, green, blue) system, were transformed into grayscale images. Hence, an image was stored in a

Table 1. Experimental Conditions for the Extraction of Apple Volatile Compounds in an Artificial Mouth: Varying Parameters, Number of Tested Conditions, and Explanation of the Experiment Codes

rotation speed (rpm)	compression frequency (movements min^{-1})	expt duration (min)	expt code[a]
10	25	5	G10-25-5
10	25	10	G10-25-10
10	25	20	G10-25-20
10	25	30	G10-25-30
10	50	5	G10-50-5
10	50	10	G10-50-10
10	50	20	G10-50-20
10	50	30	G10-50-30
10	75	5	G10-75-5
10	75	10	G10-75-10
10	75	20	G10-75-20
10	75	30	G10-75-30
50	25	5	G50-25-5
50	25	10	G50-25-10
50	25	20	G50-25-20
50	25	30	G50-25-30
50	50	5	G50-50-5
50	50	10	G50-50-10
50	50	20	G50-50-20
50	50	30	G50-50-30
50	75	5	G50-75-5
50	75	10	G50-75-10
50	75	20	G50-75-20
50	75	30	G50-75-30

[a] G stands for Golden Delicious.

matrix containing brightness values associated with each pixel, ranging from 0 (black) to 255 (white). The images of the chewed apples can be characterized by their smoothness, or their coarseness, which depends on the number and the size of the apple pieces more or less distinguishable from a puree. From a mathematical point of view, we aimed to characterize the texture of the images, which has been described by some authors as an attribute representing the spatial arrangement of the gray levels of the pixels in a region of the image (*28, 29*). The methodology used, which is probably the most frequently cited method for statistical texture analysis, was based on the extraction of various textural features from a gray level co-occurrence matrix (GLCM).

A GLCM is a matrix of size $N_g \times N_g$ (N_g is the number of gray levels) defined for a displacement of a distance δ along a given direction θ. The entry $P_{\delta,\theta}(i, j)$ of this matrix is the number of occurrences of a pair of gray levels, i and j, for the specified displacement.

Once the GLCM is defined, various textural descriptors can be evaluated. We considered the most widely used ones, that is, the *energy*, the *entropy*, the *contrast*, and the *inverse differential moment* as well as the *correlation*, which are defined as

$$\text{energy} = \sum_{i=1}^{N_g}\sum_{j=1}^{N_g} P_{\delta,\theta}(i,j)^2$$

$$\text{entropy} = \sum_{i=1}^{N_g}\sum_{j=1}^{N_g} P_{\delta,\theta}(i,j)\ \log(P_{\delta,\theta}(i,j))$$

$$\text{contrast} = \sum^{N_{g-1}} k^2 \left[\sum_{i=1}^{n_g}\sum_{j=1}^{n_g} P_{\delta,\theta}(i,j)\right] \quad |i-j| = k$$

$$\text{inverse differential moment} = \sum_{i=1}^{N_g}\sum_{j=1}^{N_g} \frac{1}{1+(i-j)^2} P_{\delta,\theta}(i,j)$$

$$\text{correlation} = \sum_{i=1}^{N_g}\sum_{j=1}^{N_g} \frac{(i\mu_x)\,(j\mu_y)\,P_{\delta,\theta}(i,j)}{\sigma_x\,\sigma_y}$$

where μ_x (respectively, μ_y) and σ_x (respectively, σ_y) are the mean and the standard deviation of the GLCM marginal distributions by row (respectively, by column).

Moreover, these textural parameters were evaluated for different displacements. As the chewed apple images seemed to be isotropic, we always considered a horizontal displacement direction ($\theta = 0°$) but with several distances, varying from 1 pixel ($\delta = 1$, about 0.1 mm on the images) to 150 pixels (about 15 mm). Due to the high redundancy of the information obtained between some distances, we finally selected $\delta = 1$, 10, and 30 pixels to evaluate the texture of the images at different levels of sharpness.

Another aspect deals with the pretreatment of the images. Unlike the images obtained from the human mouth, those obtained from the artificial mouth needed to be pretreated. Indeed, the addition of water to the apparatus, to mimic saliva, produced reflected light, which had a great influence on the textural parameters, such as the contrast. The amount of added water had been determined to correspond to the volume of saliva used by people to chew apple, but subjects swallowed the saliva before spitting out chewed apple, whereas, in the artificial mouth, we could not remove the water. The pretreatment of this collection of images consists, in summary, of detecting the edges in an image (using the function "edge" with the Sobel method, included in the Image toolbox of Matlab 7.0, The Mathworks), then identifying, from each pixel linked to an edge, a zone where the gray levels are higher than that of the reference pixel, and finally applying a median filter on the region surrounding the delineated zone. The mathematical correction was applied to all of the images of the collection.

Volatile Compound Analysis. The identification and quantification of volatile compounds were carried out with a gas chromatograph Agilent 6890N coupled to a flame ionization detector (FID) and a quadrupole mass detector (MS) Agilent 5973 network. Volatile compounds were separated on a nonpolar capillary column (DB-5MS, J&W Scientific, Folsom, CA) of 30 m × 0.32 m i.d., 0.5 μm thickness.

The injector temperature was set at 260 °C in split 1:1 for 2 min and was then put in splitless mode until the end of the run time. The GC oven temperature was increased by 10 °C min^{-1} from 40 to 105 °C, held for 5 min, and then raised to 250 °C by 10 °C min^{-1}, and held for 10 min. Volatile compounds were pulled by a helium carrier gas with a flow rate of 26 mL min^{-1} (pressure = 32 kPa). The mass detector was at 280 °C and operated in scan mode, with electronic impact ionization (ionization energy = 70 eV), and a mass range of 33−300 amu and a scan rate of 2.72 scans s^{-1} were used to detect the formed ions. Compound identification was based on mass spectra identification (comparison with standard MS spectra databases, Wiley 6) and injection of standards.

It was previously shown that the addition of a standard is not reliable when different extraction conditions in an artificial mouth are compared, because the extraction of the standard depends on the crushed state of the apple (*26*). As the volatile compound release is not influenced in the same way by the crushing and extraction conditions, it would have been impossible to compare two experiments if the results had been expressed as the ratio to the internal standard. Quantitative results were thus expressed in peak area per kilogram of apple.

Apple Color Measurements. The coloration of apples after in vitro (with the artificial mouth) and in vivo chewing was measured using a Minolta CR300 colorimeter (Carrières-sur-Seine, France) which displayed the *L*C*h* color parameters for every sample. Nine measurements were taken at different places on the surface of the crushed apples. The mean values of *L*C*h* were calculated, and the color difference between in vivo chewed apples and in vitro chewed apples was evaluated according to the equation

$$\Delta E = \Delta L^2 + \Delta a^2 + \Delta b^2$$

$$a = \frac{b}{\tan h}$$

$$b = \sqrt{\frac{C^{*2} \times (\tan h)^2}{1 + (\tan h)^2}}$$

where ΔE corresponds to the color variation between the reference and the sample, L corresponds to the clearness, C^* corresponds to the saturation, and h corresponds to the shade angle.

Statistical Analysis. Data were subjected to the analysis of variance (ANOVA) using Statgraphics Plus 5.1 software (Manugistics, Rockville,

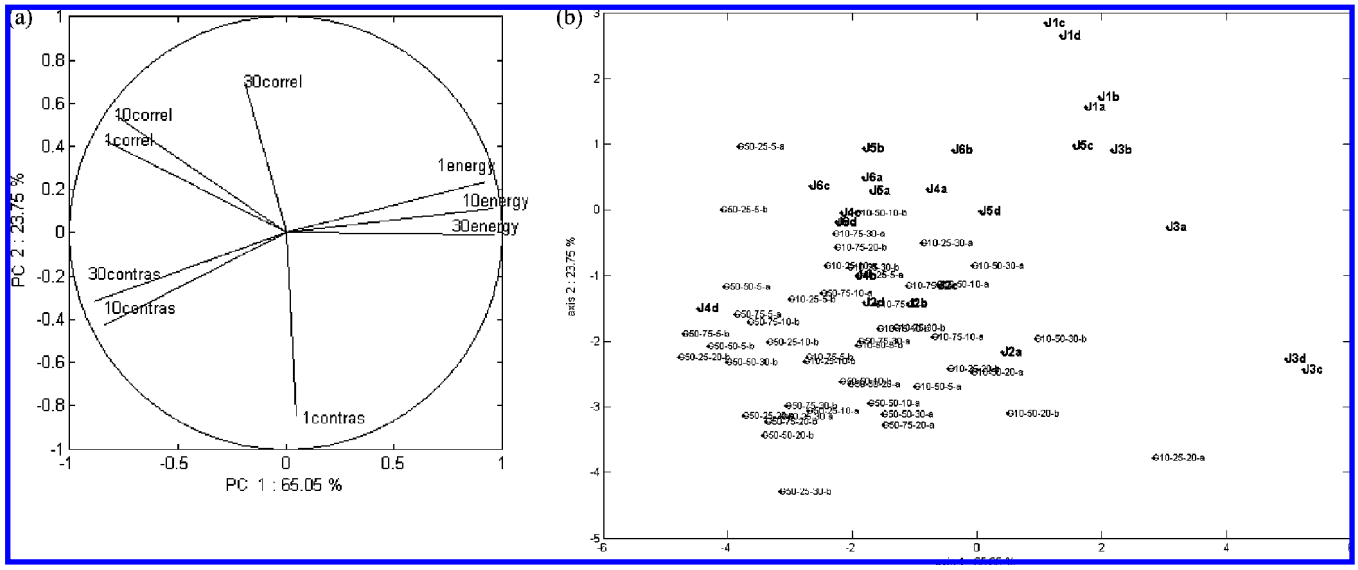

Figure 2. Correlation plot of image texture analysis parameters obtained for apples chewed in the human mouth (**a**) and projection of images of apples chewed in the artificial mouth on the image plot obtained for apples chewed in the human mouth (**b**), in the two first dimensions. G*x-y-z*: Golden Delicious apple analyzed in the artificial mouth with *x* turns per minute, *y* compression movements per minute, during *z* minutes. J*x*a: Golden Delicious apple chewed by subject *x*, picture a (four pictures per subject, coded a−d).

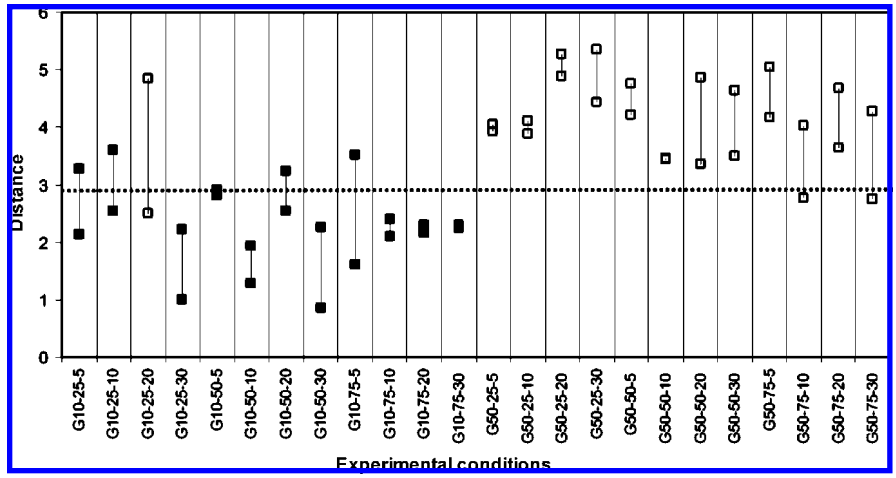

Figure 3. Distances between the baricenter of images of apples chewed in the human mouth and the images obtained for each set of artificial mouth conditions. G*x-y-z*: Golden Delicious apple analyzed in the artificial mouth with *x* rotations per minute, *y* compression movements per minute, during *z* min. The two distances obtained for each condition are represented. The dotted line represents the baricenter of all artificial mouth conditions. Black symbols belong to the same group defined by an LSD test at 5% risk; open symbols belong to other groups.

MD). Least significant differences (LSD) were chosen for multiple-comparison tests with $\alpha = 5\%$. A principal component analysis (PCA) using Uniwin Plus 5.1 software (Sigma Plus, Paris, France) was performed to exhibit the typology of apple images according to the experimental conditions.

RESULTS AND DISCUSSION

In Vivo Measurements. The mean ratio measured between mouth volume and apple weight was 0.113 mg mL^{-1}. The mean ratio between saliva and chewed apple mass was 0.22. The artificial mouth container volume being 600 mL, 68 g of apple and 15 mL of artificial saliva were used for each experiment.

Image Analysis. The selection of GLCM parameters was performed on apples chewed in vivo. Images of apple chewed in the human mouth were first analyzed by each of the parameters described under Materials and Methods: entropy, energy, correlation, inverse differential moment, and contrast, with distances of 1, 10, and 30 pixels. According to a previous PCA (data not shown), Pearson's correlation coefficients showed that some of these parameters were redundant: energy and entropy (correlation coefficients = −0.98, −0.94, and −0.94 for 1, 10, and 30 pixels, respectively); and contrast and inverse differential moment (correlation coefficients = −0.91, −0.91, and −0.92 for 1, 10, and 30 pixels, respectively).

Thus, further data analyses were based only on the energy, contrast, and correlation parameters, with distances of 1, 10, and 30 pixels.

Energy measures image homogeneity according to the distribution of gray levels. The higher the energy value, the more homogeneous parts are present in the image. Contrast measures local variations in gray levels. The higher the contrast is, the more heterogeneous parts are present in the image. Correlation corresponds to the similarity between the gray levels of a couple of pixels. The higher the correlation, the more the image contains large homogeneous parts along the horizontal direction.

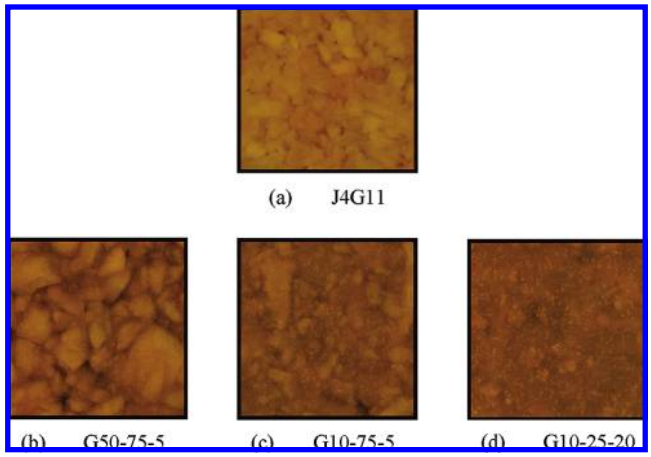

Figure 4. Images of Golden Delicious apple chewed in the human mouth (**a**) and in the artificial mouth (**b**−**d**). G*x*-*y*-*z*: Golden Delicious apple analyzed in artificial mouth with *x* turns per minute, *y* compression movements per minute, during *z* minutes. Condition c G10-75-5 was shown to be close to the human mouth chewing conditions, whereas conditions b and d were shown to be very different, most probably because in condition b apple was insufficiently chewed, whereas in condition d it was chewed too much, so pieces cannot be distinguished.

A normalized PCA was carried out on the nine parameters (energy, contrast, and correlation, with 1, 10, and 30 pixel distances) to characterize apples chewed in the human mouth (**Figure 2**).

The first two principal components, shown in **Figure 2**, enable 89% of the total variance to be explained. The correlation plot shows that the first axis opposes the three energy variables (1, 10, and 30 pixels) to the contrast at 10 and 30 pixels and to the correlation for 1 and 10 pixels. The second axis is negatively correlated with contrast (1 pixel), whereas it is positively correlated with correlation (30 pixels).

The images can be differentiated according to these variables. Indeed, images located at the bottom right-hand corner of **Figure 2** (for instance, J3c and J3d) are characterized by homogeneous texture and thin apple particles. This explains their higher energy value, whereas their contrast for long distances and correlation for small distances are lower. These images are opposed along the first axis to those that are characterized by a heterogeneous texture and rather large particles (for example, J4d). Lower energy values and higher contrast and correlation values are thus obtained for these images. This illustrates the fact that people chew food differently, producing food pieces of different sizes.

Images of apple chewed in the artificial mouth were then projected on this plot (**Figure 2**). The figure shows that images from the human mouth and those from the artificial mouth are superposed to a certain extent. The degree of overlap depends on the actual values of the control parameters. We can thus make the hypothesis that some artificial mouth conditions are more relevant to mimic the chewing performed in the human mouth.

The baricenter of images of apples chewed in the human mouth was then calculated. The distances between this baricenter and the images obtained for each set of artificial mouth conditions were determined (**Figure 3**). Two groups of images can be distinguished according to ANOVA and LSD tests at 5% risk (**Figure 3**).

Apples chewed in the artificial mouth are closer to apples chewed in the human mouth when the rotation speed is 10 rpm than when it is 50 rpm, except for the condition of 25 compressions per minute for 20 min. The conditions that led to apple images far from those obtained in the human mouth correspond to either insufficiently or excessively chewed apples

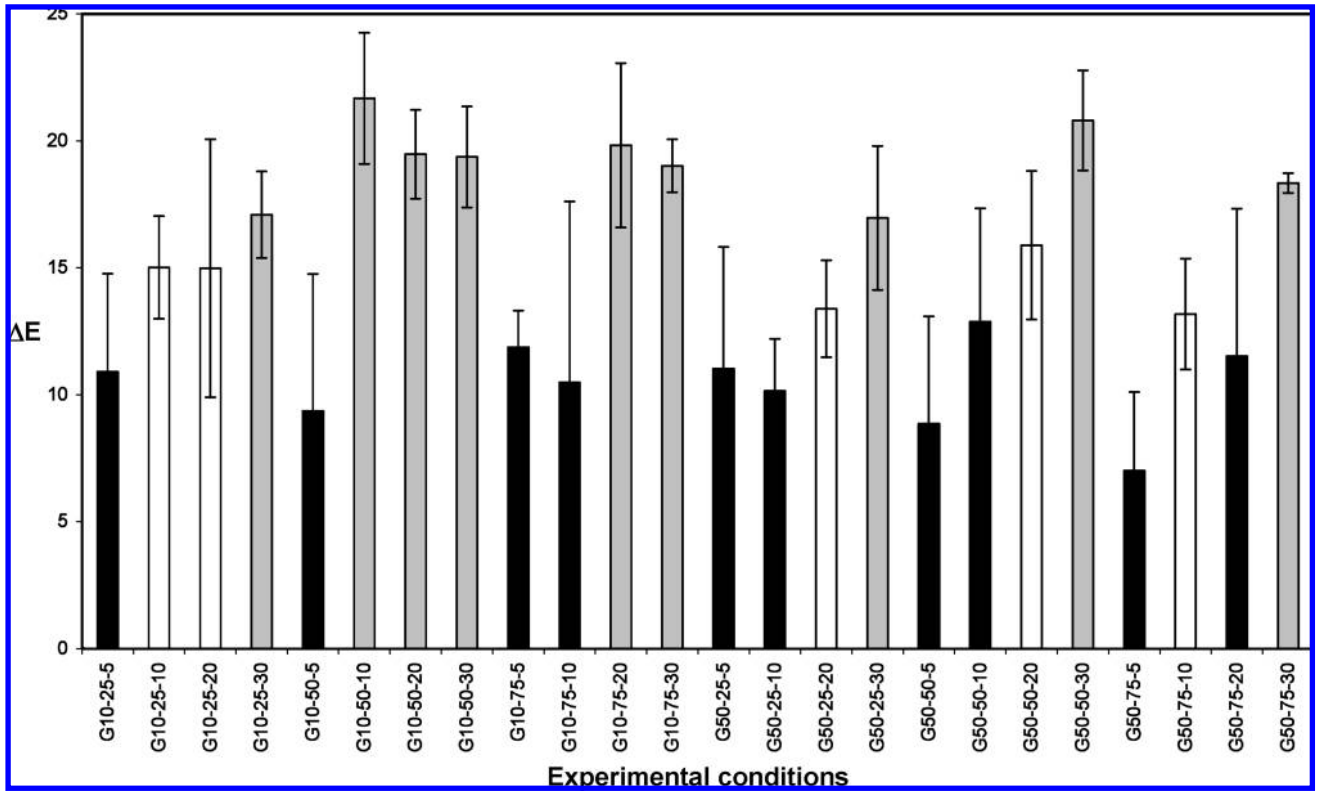

Figure 5. Color difference between apples chewed in the artificial mouth and apples chewed by assessors. Black bars belong to the same first LSD group, gray bars belong to the last LSD group, at 5% risk and white bars belong to other groups. G*x*-*y*-*z*: Golden Delicious apple analyzed in the artificial mouth with *x* turns per minute, *y* compression movements per minute, during *z* minutes.

Table 2. Volatile Compounds Identified from Golden Delicious Apples

RI[a]	compound	code	identification[b]	odor descriptor (*ref*)	supplier (purity) of standard molecules used for identification
583	2-propanol	Pol2	a, c, d	alcohol (*41*)	Carlo Erba (99.7)
592	1-propanol	Pol1	a, c, d	alcohol (*41*)	Fluka (99.9)
600	1-butanal	Bal1	a, b, c, d	green, pungent (*42*)	Fluka (99)
611	ethyl acetate	EA	a., b, c, d	solvent-like, fruity (*42*)	Sigma-Aldrich (99.5)
630	2-methyl-1-propanol	M2Pol1	a, b, c	solvent-like (*42*)	Sigma-Aldrich (99.5)
659	1-butanol	Bol1	a, b, c, d	fruity (*42*)	Sigma-Aldrich (99.5)
713	propyl acetate	PA	a, b, d, c	fruity, floral (*31*)	Sigma-Aldrich (99)
737	2-methyl-1-butanol	M2Bol1	a, b, c, d	malty (*42*)	Sigma-Aldrich (99)
765	1-pentanol	Pentol1	a, b, c	balsamic (*43*)	Sigma-Aldrich (99)
768	isobutyl acetate	IBA	a, b, c	fruity, apple-like (*31*)	Fluka (99.8)
801	1-hexanal	Hal1	a, b, c, d	green (*31*)	Sigma-Aldrich (98)
809	butyl acetate	BA	a, b, c, d	sweet-like, fruity (*31*)	Sigma-Aldrich (99.7)
851	(*E*)-2-hexen-1-al	E2Hxal	a, b, c, d	green (*31*)	Sigma-Aldrich (98)
863	1-hexanol	Hol1	a, b, c, d	fresh, green (*31*)	Sigma-Aldrich (98)
871	2-methyl-1-butyl acetate	M2BA	a, b, c, d	fruity, apple-like (*31*)	Sigma-Aldrich (94)
902	butyl propanoate	BP	a, b, c, d	red fruit-like (*31*)	Sigma-Aldrich (99)
905	amyl acetate	AA	a, b, c, d	fruity, banana-like (*31*)	Sigma-Aldrich (99)
947	(*E*)-2-hepten-1-al	E2Hpal	a, b, c	fatty, almond-like (*42*)	Sigma-Aldrich (90)
973	6-methyl-5-hexen-2-one	MHone	a, b, c	fruity, citrus-like, strawberry (*31*)	Sigma-Aldrich (99)
981	butyl butanoate	BB	a, b, c, d	rotten fruit-like (*31*)	Sigma-Aldrich (98)
1000	hexyl acetate	HA	a, b, c, d	fruity, pear-like (*31*)	Sigma-Aldrich (99)
1018	2-ethyl-1-hexanol	E2Hol1	a, b, c, d	sweet floral (*41*)	Sigma-Aldrich (99)
1030	butyl 2-methyl butanoate	B2MB	a, b, c, d	fruity, apple-like (*44*)	Fluka (98)
1065	1-octanol	Ool1	a, b, c	grass, pepper (*31*)	Riedel de haëns (99.5)
1094	1-nonanal	Nal1	a, b, c	soapy, citrus-like (*45*)	Sigma-Aldrich (95)
1180	hexyl butanoate	HB	a, b, c, d	green (*31*)	Sigma-Aldrich (98)
1197	*p*-allylanisole	pAol	a, b, c, d	aniseed and liquorice-like (*43*)	Fluka (98.5)
1226	hexyl 2-methyl butanoate	H2MB	a, b, c, d	apple and grapefruit-like (*44*)	Sigma-Aldrich (95)
1379	hexyl hexanoate	HH	a, b, c, d	apple and cucumber-like (*31*)	Sigma-Aldrich (97)
1504	(*E, E*)-α-farnesene	EEaF	a, b, c, d	green, herbaceous (*46*)	Sigma-Aldrich (unknown)

[a] RI obtained on DB-5MS column. [b] Every compound identification was checked by at least three methods: mass spectra identification (comparison with standard MS spectra database Wiley 6) (a); RI were compared with retention indexes found in the bibliography (b); analyses of standard molecules on DB-5MS (c); analyses of standard molecules on DB-Wax (d).

(**Figure 4**). The conditions leading to the smallest distance are 10 rpm, 25 movements per minute, and 30 min and 10 rpm, 50 movements per minute, and 10 and 30 min.

Color Measurements. The difference in color between each sample of apple chewed in the artificial mouth and the mean of the samples chewed in human mouths was measured by means of ΔE calculation. These results are shown in **Figure 5**. The shorter crushing time conditions are all in the same LSD group, containing the apples with low ΔE, that is, the least different from apples chewed in human mouths. This was obviously expected, because browning depends on the time of exposure of apples after cutting. This browning may have been reduced by the use of helium as the flow gas for all conditions. In contrast, all 30 min extractions and most 20 min extractions are characterized by high ΔE values. The LSD analysis shows that 10 min conditions induce very different browning states: although G10-75-10, G50-25-10, and G50-50-10 are in the same group as 5 min experiments, G10-25-10 and G50-75-10 show intermediate results, and G10-50-10 is comparable to 20 and 30 min experiments. Apple browning seems to be greater with 10 rpm rotation speed than with 50 rpm rotation speed.

Volatile Release. Thirty compounds were identified in Golden Delicious, namely, 13 esters, 9 alcohols, 5 aldehydes, 1 ketone, 1 phenol, and (*E,E*)-α-farnesene (**Table 2**). All of these compounds have previously been found in apples of different cultivars (*30–36*). However, mouth conditions may influence the diffusion of the compounds in fruit tissues and therefore speed the release of volatiles and/or influence the distribution of volatiles in the extract. It is well-known that the quantity of each volatile odorant compound may have an effect on the resulting perception. That is why the effect of each extraction parameter (rotation speed, mastication frequency, and extraction time) on apple compound extraction was checked by a multiple-factor variance analysis (**Table 3**).

The multifactor variance analysis showed that compression frequency had no effect on most compounds, except 2-ethyl-1-hexanol and *p*-allylanisole. Time had a significant effect on 12 compounds, including the 7 less volatile ones. Rotation speed had a significant effect on 21 compounds, whether they were very volatile or not, and this did not seem to depend on their chemical function.

The experimental conditions had a significant effect on 27 of 30 extracted compounds. PCA was computed on the quantity of volatile compounds extracted in each set of conditions (**Figure 6**). The 3 compounds for which amounts did not differ (1-propanol, propyl acetate, and 6-methyl-5-hepten-2-one) were not represented on the PCA. The first two principal components explained 67% of the total sum of squares.

In **Figure 6b**, the experimental conditions performed in triplicate (i.e., G10-50-5, corresponding to 10 rpm, 50 compressions per minute, and 5 min) are grouped on the plot. It can be concluded that the obtained results are close and the repeatability of the device is satisfactory. The distribution of experimental conditions on this representation distinguishes conditions mostly according to time and rotation speed. The three 30 min experiments performed with 50 rpm rotation speed are on the right side of the first axis. As shown on the correlation plot, these extraction conditions are characterized by higher quantities of C6 and C7 aldehydes, C4−C6 alcohols, butyl acetate, 2-methyl-1-butyl acetate, isobutyl acetate, hexyl acetate, butyl 2-methylbutanoate, ethyl acetate, butyl butanoate, and butyl propanoate. The experiments carried out with a 10 rpm rotation speed, situated on the left side of the first axis, were thus less concentrated in these compounds. The 30 min experiments (and

Table 3. Effect of Experimental Parameters on the Quantity of Golden Delicious Apple Volatile Compounds Extracted, Determined by Multifactor ANOVA[a]

compound	compression frequency	duration	rotation speed
2-propanol	NS	**	***
1-propanol	NS	NS	NS
1-butanal	NS	NS	*
ethyl acetate	NS	NS	*
2-methyl-1-propanol	NS	NS	**
1-butanol	NS	NS	***
propyl acetate	NS	NS	NS
2-methyl-1-butanol	NS	NS	***
1-pentanol	NS	*	**
isobutyl acetate	NS	NS	***
1-hexanal	NS	NS	***
butyl acetate	NS	NS	***
(*E*)-2-hexen-1-al	NS	*	***
1-hexanol	NS	**	***
2-methyl-1-butyl acetate	NS	NS	***
butyl propanoate	NS	NS	*
amyl acetate	NS	NS	**
(*E*)-2-hepten-1-al	NS	**	NS
6-methyl-5-hepten-2-one	NS	NS	NS
butyl butanoate	NS	NS	*
hexyl acetate	NS	NS	***
2-ethyl-1-hexanol	*	NS	NS
butyl 2-methyl butanoate	NS	NS	**
1-octanol	NS	*	***
1-nonanal	NS	***	NS
hexyl butanoate	NS	**	NS
p-allylanisole	*	***	NS
hexyl 2-methyl butanoate	NS	**	NS
hexyl hexanoate	NS	***	**
(*E*,*E*)-α-farnesene	NS	**	*

[a] NS, not significant; *, significant at 5% risk; **, significant at 1% risk; ***, significant at 0.1% risk.

to a lesser extent the 20 min experiments), performed with 10 rpm rotation speed, are located on the negative part of the second component, which means that these conditions are characterized by larger amounts of α-farnesene, hexyl hexanoate, hexyl 2-methylbutyrate, *p*-allylanisole, nonanal, and hexyl butyrate. These six compounds are the heaviest among all of the identified compounds. On the contrary, the two most volatile compounds are the more positively correlated with axis 2, even if this correlation is not very great. The third axis did not provide any further information.

It can be observed that the experiments with the same time and the same rotation speed are located in the same part of the PCA, which confirms the absence of an effect of the compression movement frequency. This contradicts previous studies, which have shown an effect of mastication rate on volatile release (*2, 16, 22, 37*). This may be due to the difference in time, because these earlier studies were carried out with very short extraction times compared to our experiment. The shape of artificial mouths may also be responsible for this difference, because in the previous studies, the movement was provided by a plunger without teeth.

The longer experiments allowed the extraction of larger quantities of compounds. It is not surprising to observe such an effect. On the one hand, compounds could be produced by apple tissue disruption. On the other hand, the longer apples were chewed, the more volatile compounds were released and the more the fiber caught them. Longer experiments seem to be suitable conditions to attain our objective of finding artificial mastication settings that best reproduce human mastication. Indeed, among the three sets of conditions leading to the more representative mastication results, two lasted 30 min. Nevertheless, the prime need for the artificial mouth is to ensure volatile release from foods as in the human mouth. The time scale of an analytical procedure is often much too long compared to the length of time a food is likely to be in the mouth. In the study of fruits or vegetables, it is particularly important to take this parameter into account, because some compounds are known to be formed by oxidation and enzymatic reactions. This is underscored by ΔE values that show that apple browning may be correlated to experiment duration. This parameter must be reduced in further studies.

The rotation movement in the artificial mouth was programmed to play the role of the tongue and jaw in the human mouth. Its aim was to position the foodstuff differently between two teeth compression movements. We previously observed that, without this movement, apples could not be chewed properly, and large pieces remained (*26*). It appears that this movement is necessary not only to induce efficient chewing but also to allow the release of volatile compounds. The rotation speed can be considered to act as a stirring and may thus influence volatile release. Furthermore, because of its important effect on the

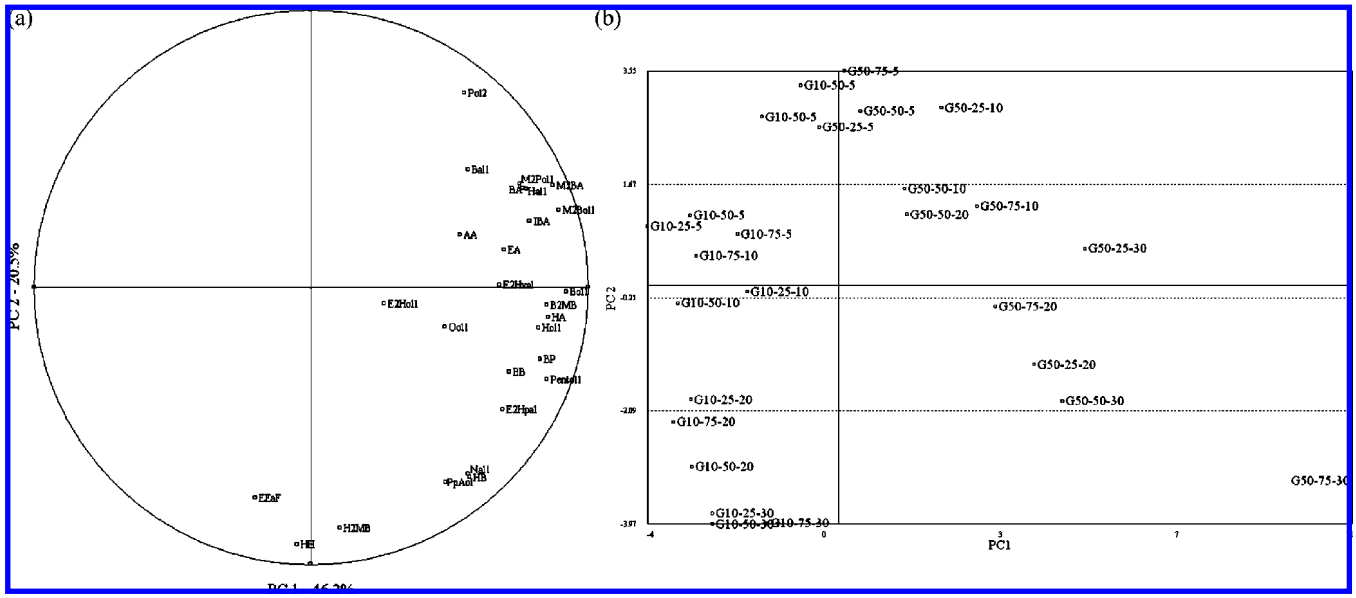

Figure 6. Factorial map (axes 1 and 2) of PCA for aroma analyses of Golden Delicious apple: (**a**) correlation plot; (**b**) experimental conditions plot according to the volatile composition of extracts. G*x*-*y*-*z*: Golden Delicious apple analyzed in the artificial mouth with *x* turns per minute, *y* compression movements per minutes, during *z* minutes.

crushed state of apple, it may indirectly affect volatile release, which may depend on the surface of apple particles in contact with the flow gas. The control of time and rotation speed enabled the artificial mouth to simulate a range of chewed states and volatile release conditions. Varying the rotation speed could be a way of decreasing extraction time without reducing the quantities of extracted volatile compounds too much.

The amount of enzyme-produced volatile odorant compounds depended on both the extraction time (**Table 3**) and the rotation speed. Considering 10 min experiments, (*E*)-2-hexen-1-al was significantly more released at 50 rpm and (*E*)-2-hepten-1-al was never extracted by 5 min extractions or by 10 min extractions with 10 rpm rotation. These compounds are produced by lipoxygenase action when the fruit is broken down (*38*). Production of α-farnesene has been related to the development of superficial scald (*39*). Our results seem to indicate that the production of α-farnesene also depends on the tissue state. Thus, not only the extraction time but also the rotation speed had an effect on the quantity of α-farnesene extracted. The rotation time was shown to be the determinant factor in the crushed state of apple. The notched plunger attacked the apple flesh differently at 10 rpm and at 50 rpm and may have caused different conditions for oxidation and enzymatic reactions. It has been previously shown that 10 rpm rotation speed induces particularly extensive browning.

6-Methyl-5-hepten-2-one is known as one of the oxidation products of α-farnesene (*40*). It is therefore surprising that its release did not depend on experimental conditions. It could be an argument for the formation of α-farnesene during extraction, due to the effect of experimental conditions. The fact that experiments were conducted in a helium atmosphere could explain why the quantity of 6-methyl-5-hepten-2-one was not affected.

Now that a method to characterize apple state has been successfully applied, the next steps will concern the optimization of aroma release. To know which artificial mouth settings best mimic the human mouth, it will be necessary to compare the volatile profile obtained with volatiles released in vivo. The compounds responsible for the aroma of fresh apple may then be distinguished from those produced during the extraction. It can be stated that, coupled with more sensitive analysis conditions or with online analysis such as APCI-MS or PTR-MS, the present device would allow a real improvement in extraction from hard foods.

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