

Development of a chewing simulator for food breakdown and the analysis of *in vitro* flavor compound release in a mouth environment

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Abstract

Flavor release during eating is highly dependent upon mouth parameters. Major limitations have been reported during *in vivo* flavor release studies, such as marked intra- and inter-individual variability. To overcome these limitations, a chewing simulator has been developed to mimic the human mastication of food samples. Several devices had already been developed for diverse applications, but they only reproduced certain oral functions and were therefore not characteristic of the natural mouth environment. The newly developed device faithfully reproduces most of the functions of the human mouth. The active part of the system is a special cell, precisely tooled using a biocompatible and inert material, which operates around three axes which are fully actuated and computer-controlled. The cell comprises several mobile parts that can accurately reproduce shear and compression strengths and tongue functions real-time, according to data collected *in vivo*. Flavor release can be monitored on-line using either API-MS or chemical sensors, or *off-line* using HPLC for non-volatile compounds. A preliminary study using peanuts was performed to test and validate the mechanical functionalities of the system. Comparable masticatory efficiencies were observed from *in vivo* and *in vitro* tests.

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1. Introduction

Mastication is a complex process involving both the orofacial muscles and the tongue. It is an important aspect of the food intake of solids and can be considered as a pre-digesting step. Whatever form the food takes, it must be converted into a bolus with a soft consistency by mastication so that it can be swallowed. One of the main consequences of this complex process is breakdown of the food matrix and impregnation with saliva, allowing flavor compounds to be released gradually in the mouth and thus reach the aroma and taste receptors, inducing perception.

Numerous variables need to be taken into account to measure the flavor of foods, including the effects of mouth warming, saliva composition and flow rate, frictional forces and mixing, pH and airflow, etc. Thus simple static headspace analysis for volatile compounds, or water extract analysis for non-volatile compounds, will only provide limited results which do not take account of all the dynamic phenomena in the mouth.

In particular, the chewing process, which leads to breakdown of the matrix, contributes markedly to flavor release. This process depends on the nature of the matrix and on the specific oral physiology of individuals.

Many of the studies carried out on temporal flavor release have been conducted *in vitro* using static or dynamic headspace analysis, or *in vivo* by analyzing the nosespace of

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a subject chewing foods or drinking liquids. The total nose-space was either trapped on Tenax TA (Ingham, Linforth, & Taylor, 1995; Linforth, Savary, Pattenden, & Taylor, 1994) or, more recently, directly analyzed on-line by connecting the subject's nose to an atmospheric pressure ionization mass spectrometer (API-MS) (Taylor, Linforth, Harvey, & Blake, 2000; Taylor & Linforth, 2003). This device also enables the real-time monitoring of several aroma compounds released during chewing.

Although temporal *in vivo* studies enabled the direct release-perception tests, numerous limitations were observed, such as major inter-individual differences, moderate intra-individual reproducibility, only a limited daily sample throughput and the food sample must be acceptable to panelists. Use of a chewing simulator to investigate *in vitro* temporal analyses could overcome these difficulties. Furthermore, temporal flavor release has been correlated to several masticatory parameters such as the chew rates, the number of chews, the work, the chewing time, the salivary flow and the masticatory performances (Neyraud, Prinz, & Dransfield, 2003; Pionnier, Chabanet, Mioche, Le Quéré, & Salles, 2004; Pionnier et al., 2004), but it remained difficult to understand the individual effect of each of these parameters as *in vivo*, many of them being related and varying simultaneously. So, the possibility of decoupling these parameters using a chewing simulator would be of great interest to better understand flavor release phenomenon.

In vitro studies have been carried out to evaluate the impact of oral processes on food breakdown and flavor release (Piggott & Schaschke, 2001). Several devices have been proposed to simulate human mastication but their functionalities are low. So, they are still far removed from the complex *in vivo* process. For example, Nassl, Kropf, and Klostermeyer (1995) proposed a simple system which could gradually add food to artificial saliva (100 mL). It consisted of a glass vessel equipped with a stirrer, septa on the top and two valves. The authors were able to study the temporal release of homologous series of hydrophobic volatile compounds from a complex fat/protein/water emulsion at different times of the chewing process. van Ruth, Roozen, and Cozijnsen (1994) studied three types of mouth model systems for the flavor release of rehydrated bell pepper cuttings, one of which consisted of a dynamic headspace system where mastication was only simulated by an oscillating plunger. Roberts and Acree (1995) designed a so-called "Retronasal Aroma Simulator" (RAS) to investigate the effects of saliva, temperature, shearing and fat on flavor release. This device was able to incorporate synthetic saliva and to apply controlled shearing to the mixture. Jensen, Beck, Jeppesen, Norrelykke, and Hansen (2003) presented a chewing simulator device coupled with a membrane inlet mass spectrometer (MIMS) allowing the on-line monitoring of flavor release during simulated eating. The device consisted of a chewing chamber in which gum was chewed by two horizontal and a vertical pistons with wavy surfaces, with controlled rotat-

ing around their own axis. However, there was no gas phase in this system. So, it could not simulate the progressive release of aroma compounds in the gas phase to give an idea of the "mouthspace" composition and concentration.

The objectives of this work were to develop a system which could reproduce as faithfully as possible the principal phenomena occurring in the mouth during eating (i.e. controlled compression and shearing, continuous saliva flow, applied strength, speed and frequency in the same range as reported *in vivo*...) and to demonstrate its feasibility by means of preliminary experiments.

2. Materials and methods

2.1. Development of the chewing simulator

The principal limitations of the existing systems (Piggott & Schaschke, 2001; Jensen et al., 2003) were that ratios between food (solid), liquid (saliva) and gas (aroma headspace) did not represent the situation *in vivo*, the volume of liquid phase remained constant throughout the process and the complex process of mastication was reduced to

Table 1
Description and specifications of the chewing simulator

| Functionalities | Specifications |
|--------------------------|--|
| Food breakdown | |
| • Upper jaw fixed | • Initial sample: 5–10 g |
| • Lower jaw mobile | • Cycle time from 800 ms |
| | • Chewing up to 180 s |
| | • Forces up to 250 N |
| | • Biting speed up to 75 mm/s |
| | • Maximum volume 100 mL |
| | • Working volume up to 50 mL |
| Compression, penetration | Vertical motion |
| Shearing, milling | Angular motion |
| Tongue | Vertical motion, concentric |
| Teeth | • Reproduction of a molar motif |
| | • Interchangeable jaws |
| Fluids | |
| • Gas | • Gas tightness (inlet and outlet) |
| • Liquid | • Artificial saliva inlet: up to 5 mL/min |
| Controls | |
| • Temperature | • Thermostatization of the cell |
| • Motion and forces | • Self tune based on food bolus breakdown degree |
| • Volume | • Indexed on saliva supply |
| • Fluids | • Saliva flow and composition (syringe pump) |
| | • Gas : mass flow controller |
| Sampling | • Possibility to connect gas outlet directly to analysis apparatus (on-line and real time) |
| | • Possibility to collect saliva sample manually |
| Practical aspects | • High chemical inertness |
| | • Easy dismantling and cleaning |
| | • No lubricant, no gaskets, no sealant (causes of sorption, release) |

simple stirring or compression by parts with a geometry very different from a real jaw. However, food breakdown in the mouth is due to the combined action of teeth, tongue and saliva, and physiological parameters such as the geometry of teeth, masticatory frequency, the force applied by the jaws, saliva flow and composition. Table 1 presents the specifications of the system we designed. These specifications mainly concern the mechanical parameters involved in food breakdown, which were ranked between the values reported during *in vivo* studies or specifically measured, and the quality of the materials used.

2.1.1. General description

The system includes an actuated cell where the mastication process takes place and sampling is performed, an electronic control box and a computer to monitor and tune each parameter. As presented in Fig. 1, the active cell comprises several mobile parts: an actuated lower mandible (displacement, revolution, force and torque control), and an actuated tongue (displacement and force control) and a fixed upper mandible. The teeth were tooled in the bulk of a ring-shaped polyetheretherketone (PEEK) cylinder, representing the lower mandible, translating in a vertical axis against the internal face of the PEEK cell wall, actuated by a motor allowing the vertical translation and a second one allowing a rotation. The tongue is made of a full PEEK cylinder of 4.5 cm diameter, ended with a conical upper part, and is actuated by a third motor and running inside the lower mandible. These are the lower parts of

the active cell. The complex movement of the tongue, as it collects food in the mouth, places it between the teeth and then assembles it in a bolus, is simplified as a linear movement of a concentric piston. The upper part is composed of a second ring-shaped PEEK cylinder, including 3D engraved teeth that constitutes the upper mandible. Only first molars are represented and duplicated in the jaws (Fig. 2). It might have been possible to reproduce all types of teeth in the jaw, but the functionality of the apparatus would have been extremely complicated; in particular, monitoring horizontal movements of the jaw containing teeth with very different geometries. However, the jaws can be dismantled and be replaced by jaws with other tooth motifs. The top of the palate, tooled in a cone shape corresponding to that of the tongue, is receiving the embedded sampling system which is described below. The prototype (Fig. 3) is able to reproduce shear and compression strengths together with tongue functions (up to 250 N), in the real mastication time. Strengths, shear forces and torques can be reproduced accurately according to the *in vivo* data collected, in terms of amplitude and frequency. Though this device is able to mimic important functions of the mouth, it was not designed to simulate certain other functions such as swallowing.

2.1.2. Mechanical aspects

For the three reducing motors of the device, the choice was made to use a brushless technology which would enable linear force measurements using DC current instead

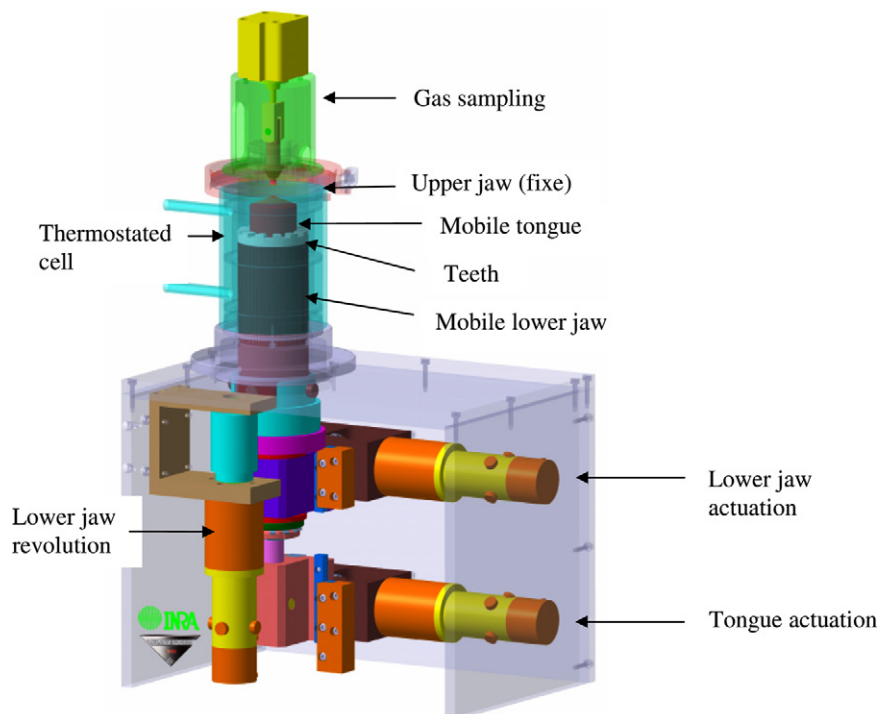


Fig. 1. Diagram showing mechanical parts of the device.

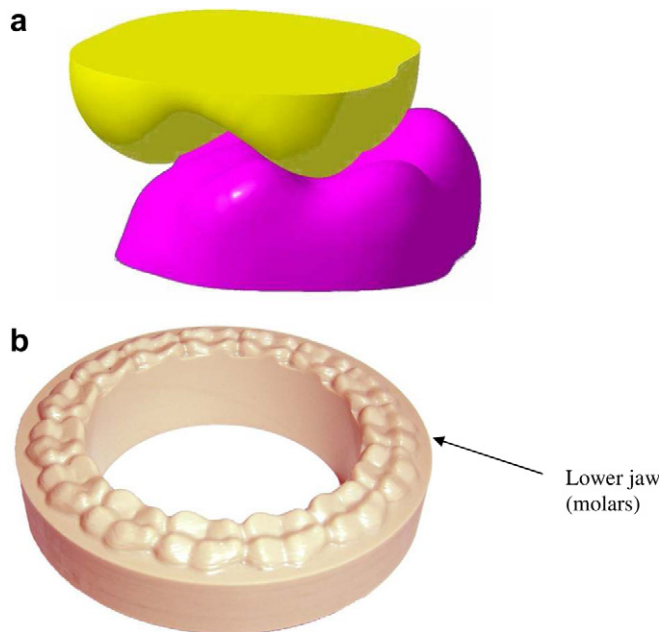


Fig. 2. Teeth and jaw designed for the device: (a) arrangement of molars between the upper and the lower jaws and (b) lower jaw.

of strain gauges. An optical rotary encoder (1024 pulses/revolution) directly coupled to the motor shaft allows the very precise measurement of motion ($1.44 \mu\text{m}$ or $0.00164^\circ/\text{pulse}$). For linear motions, a rack and pinion gearing system is linked to the motor shaft. Linear motion bearings ensure precise guiding together with minimum sliding friction. A set of gears is used to ensure the mandible revolution.

The teeth were 3D tooled (Ultra High Speed milling machine Huron KX8) and based on 3D scans of the first human molars (Minolta VI-910 scanner and Polygon Editing Tools software), after 3D modeling and rendering (RapidForm 2004 and Catia V5) for both the upper and lower dentures (Fig. 2). The upper and lower teeth are adjusted to ensure that their motifs have sufficient comple-

mentarities to allow an efficient crushing effect similar to that which occurs with human jaws. The cell temperature (generally around 35°C) is regulated by electrical heaters.

2.1.3. Gas and liquid sampling

Artificial saliva and the neutral carrier gas are introduced into the cell at variable and continuous flow rates via a valve system located at the top of the device (Figs. 1 and 3). The sampling system is embedded, mimicking the retronasal sniffing pathway by means of controlled flows and volumes. The saliva flow rate (between 0 and $5 \text{ mL}/\text{min}$) is ensured by a syringe pump and the gas flow rate (between 10 and $50 \text{ mL}/\text{min}$) is controlled by a mass flow controller. Valves are activated by an air cylinder so as to comply with the sampling time specifications. The period during the cycle when actuation takes place is extremely critical as gas sampling should not occur when the jaw or tongue is in the upper position, otherwise, the bolus may enter the sampling pathway and contaminate both the valve and tubing. Accurate synchronization of the sampling valve, tongue and lower jaw is essential for the system to function satisfactorily. Sampling parts and pipes are heated to prevent any condensation of the volatile compounds. Flavor release sampled in the headspace of the cell can be monitored on-line using either API-MS (Taylor & Linfoth, 2003; Taylor et al., 2000) or chemical sensor systems (Juteau, Mielle, & Guichard, 2003).

The liquid sampling is still under development. It is probable that this system will automatically mimic, during the chewing time, the method for the collection of saliva swabs (Davidson, Linfoth, Hollowood, & Taylor, 1999), using a modified automated sampler. After extraction, active compounds could be analyzed by liquid chromatography (Pionnier et al., 2004) or by chemical sensor systems for liquid phases (Legin et al., 2004).

2.1.4. Automation

Two National Instruments[®] cards contain the control parameters for the system (Fig. 4). The first card (Ref.

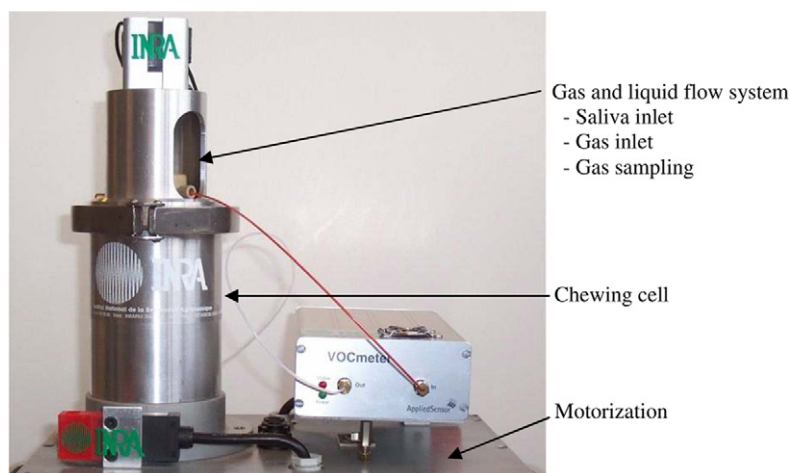


Fig. 3. Photograph of the device.

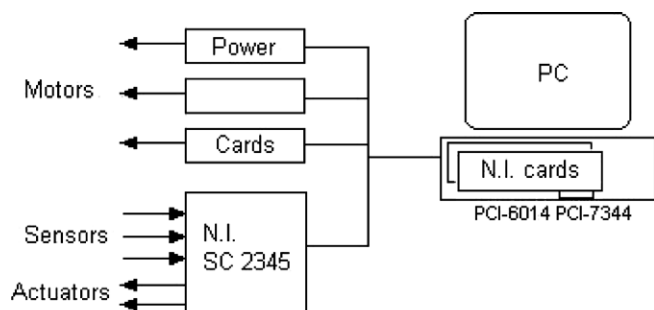


Fig. 4. Automation of the system.

PCI-7344) can control four axes that can be actuated either by a stepper or DC brushless motor. Three axes are used: one for the tongue translation, one for the mandible translation and the last for the mandible revolution (shearing). We used the DC motor control mode. Some input/output digital ports are also available on this card, that are used to forward information to the control software on the process security (motors overheating, incomplete caps closure). This device has also analog outputs that are used for the measurement of the motor currents and their position. The second card (Ref. PCI-6014) is used for the analog input/output, such as temperature or carrier gas flow-rate measurements and to setup the carrier gas flow-rate. There are also some input/output digital ports that are used for digital controls like the active cell heating. These two devices are located in the computer frame and are connected to the process through a National Instruments® signal conditioning system (Ref. SC-2345), both for the sensors and the digital input/output. The four axes card controls the motors through three analog power cards (one for each motor).

The control software is written using the graphic programming language LabView®. This software uses specific subroutines, called Virtual Instruments (Vi) to control the

devices. The software allows all the necessary functions for running the process and data acquisition, either in the normal operation or maintenance mode. All sequences, alignment of the teeth, heating, tests, beginning of the cycle, emergency stop... can be activated by the operator very simply in clicking on various buttons, with are controlled for safety. A great numbers of parameters can be setup or modified by the operator, i.e: chewing speed, food, headspace and bolus volumes, temperature of the cell.

To protect the system against unauthorized changes, the critical parameters for a correct operation of the system can be changed only when entering a super-user password. All data collected during the cycle of chewing can be stored in text files for manipulation with external software. At the end of the experiment, data can be exported to MS-Excel®.

All the measurements, positions, forces and errors are reported in real-time on the screen. An animated synoptic allows monitoring of the mandible and tongue position during the cycle, and a waveform chart displays the chewing efforts.

2.2. Inertness of polyetheretherketone (PEEK)

Thin layers of PEEK (approximately 100 µm thick) covering a total surface area of 28 cm² were used. A 250 mL hermetically-sealed flask containing 100 mL of a water solution of aroma compounds (0.05 µL per L of each, listed in Table 2) (Sigma–Aldrich, St Quentin Fallavier, France) was maintained at 20 °C. The headspace was sampled into a Platform LCZ mass spectrometer fitted with a MS Nose interface (Micromass, Manchester, UK) at a flow rate of 31.8 mL min⁻¹. The transfer line temperature was 160 °C. The volatile compounds present in the gas phase were ionized by a 4 kV corona discharge in the source (75 °C) and were

Table 2
Inertness of polyetheretherketone for volatile compounds

| Compounds | <i>m/z</i> | <i>S</i> ₀ | <i>S</i> (5 min) | % Losses | <i>S</i> (1 h) | % Losses |
|---------------------|------------|-----------------------|------------------|----------|----------------|----------|
| 2-Butanone | 72.8 | 89 | 76 | 15 | 79 | 7 |
| 2-Heptanone | 114.8 | 134 | 111 | 17 | 131 | 10 |
| 2-Nonanone | 142.8 | 148 | 127 | 14 | 143 | 15 |
| 1-Octen-3-ol | 110.8 | 10 | 9 | 10 | 6 | 0 |
| Propanol | 42.8 | 18 | 18 | 0 | 15.5 | 9 |
| Ethyl butanoate | 116.5 | 198 | 149 | 25 | 198 | 21 |
| Ethyl octanoate | 173.2 | 325 | 250 | 23 | 249 | 45 |
| Phenylacetate | 104.7 | 12 | 11 | 8 | 10 | 5 |
| Propanoic acid | 74.8 | 5.5 | 6 | -9 | 3 | 14 |
| Diméthyldisulfure | 93.7 | 44.5 | 35 | 21 | 48.5 | 46 |
| S-méthylthioacetate | 90.8 | 168 | 131.5 | 22 | 163 | 14 |
| Pyrazine | 81.0 | 11 | 12 | -9 | 5 | 0 |
| Acetaldehyde | 44.8 | 38 | 34.5 | 9 | 28 | 3 |
| Hexanal | 82.9 | 121 | 130.5 | -8 | 123 | 10 |

% Losses = $(S_0 - (S_0 + S)) * 100 / S_0$, *S*₀: signal height obtained for volatile compound solution alone, *S*: signal height obtained for volatile compound solution when PEEK layers are added.

then analyzed in the quadrupole. The cone voltage was optimized for each analyzed ion. MH^+ ions were monitored in a selected ion mode (dwell time 0.01 s). Calibration was achieved by comparison of the signal intensities obtained for samples with those of diluted solutions of volatile compounds in hexane or cyclohexane which was volatilized in the MS Nose gas stream. Measurements were collected for the aroma solution alone and then aroma solution to which PEEK layers were added. Percentages of losses were calculated by comparing the strength of the signal with or without PEEK for each volatile compound. PEEK layers were then successively rinsed with 2×100 mL hot tap water (50°C), cold deionized water, 2×50 mL pure ethanol and 4×200 mL cold deionized water before being dried in an oven at 100°C for 20 min. To control the efficiency of cleaning, PEEK layers were placed alone in a 250 mL hermetically-sealed flask, which was maintained for 1 h at 20°C and then connected to the API-MS to analyze the headspace content, as described above.

2.3. Validation of the chewing system

2.3.1. Chewing simulator tests

First of all, a series of tests were performed during which the principal functional parameters were adjusted separately: maximum bite force, maximum shearing force, biting speed, shearing speed, saliva flow and the number of cycles, and the quantity of food was also varied. For the subsequent validation study, three peanuts were placed in the chewing simulator, and “chewed” samples were obtained after 4 and 8 cycles. Different procedures were studied, varying in terms of the maximum bite force (250, 300 and 350 N) or the shearing angle value (0, 3 and 6°). Three replicates were performed for each procedure.

2.3.2. Human chewing tests

The subject group consisted of four volunteers (three women, one man) without any functional mastication problems. They were asked to eat three peanuts (2.0 ± 0.2 g) and after 4 and 8 chews, to spit the sample into a coffee filter and rinse their mouth with water. The rinsing water was also collected in the filter. The chewed sample was spread on paper and dried for 24 h at room temperature. This procedure was carried out three times for each number of chews.

2.3.3. Particle size measurements

The degree of fragmentation of both *in vivo* and *in vitro* chewed samples was studied by measuring the weight of masticated peanuts that could pass through both 4 mm and 2 mm aperture size sieves, and then the weight percentages were calculated of particles larger than 4 mm, particles between 2 and 4 mm and particles smaller than 2 mm.

3. Results and discussion

3.1. PEEK inertness towards volatile compounds

A variety of materials were screened so that a choice could be made of those which could be tooled precisely and would be inert towards aroma compounds, taking into account both chemical inertness and adsorption onto the material surface. Different types of stainless steel (raw, polished and coated with a thin layer of nickel or chrome) and PEEK were tested. As reported in Table 2, the loss of volatile compounds in the headspace due to the presence of PEEK layers in an aqueous aroma solution was relatively small, with the exception of dimethyldisulfide and ethyl octanoate, losses of which reached around 40% after 1 h of contact. After a contact time of only 5 min (which is more representative of a chewing time for food) (1–1.5 min), losses were less than 25% for the most sensitive compounds and insignificant for most of them. After the cleaning of PEEK layers, no aroma compound release was detected, demonstrating the efficiency of this simple procedure. These results demonstrated the relatively satisfactory inertness of PEEK with respect to volatile compounds. It was thus deemed an acceptable material with which to construct the device, and would be used for parts in direct contact with volatile compounds. In contrast, major aroma losses by adsorption were observed for metallic materials such as raw, nickel-plated or chrome-plated stainless steel (results not shown). Moreover, PEEK is hard enough to be easily tooled and the limited wear between two moving parts enables a good seal without any gasket if high-precision engineering is used to produce them.

3.2. Food breakdown trials

Once the prototype had been tooled, preliminary trials with food were performed to verify that its different functions worked as had been expected in theory, and also to study the key parameters affecting the food breakdown process. Furthermore, the degrees of food breakdown achieved by applying different modes of oral processing were compared with those of different human boli in order to validate the chewing simulator as a true simulator of human mastication. Peanuts were chosen for this preliminary study as they mainly break down by fracture and the degree of fracture can be determined easily by sieving (van der Bilt & Fontijn-Tekamp, 2004).

3.2.1. Functional parameters

During each experiment, the mastication process in the apparatus was monitored by recording data corresponding to most of the functions: vertical position and force of both tongue and mandible, and angular position and shearing force of the mandible. As an example, the mandible force and position values recorded during two different experiments with peanuts are plotted in Fig. 5.

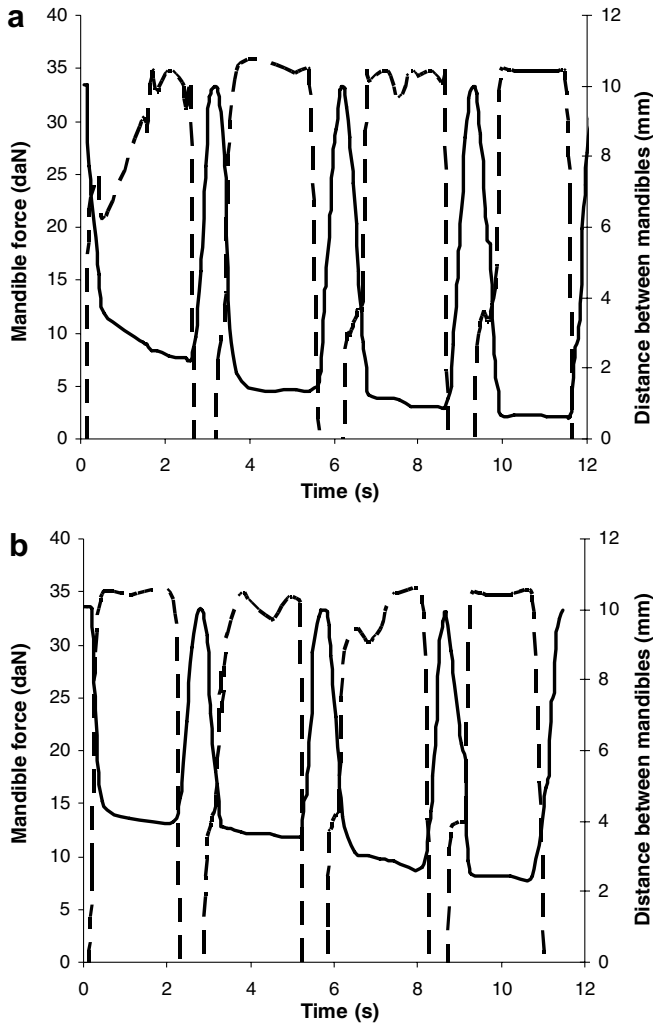


Fig. 5. Changes to mandible force (----) and mandible position (—) in the chewing simulator throughout the mastication process of 1 (a) and 3 (b) peanuts.

During each chewing cycle, as the mandible was displaced to join the upper mandible, the force increased rapidly until it reached the preset force value, remained constant during the shearing period and finally decreased rapidly during the aperture period. The distance between the mandibles, which had rapidly decreased during closure, also decreased (but only slightly) during the shearing period. Furthermore, as the number of cycles increased, the distance between the mandibles at the end of the cycle was reduced, indicating that a reduction in the size of the peanuts was being achieved by both vertical and shearing forces. Thus the course of the breakdown of peanuts could be followed indirectly by monitoring the reduction in the distance between the mandibles after each cycle. As an example, Fig. 6 shows the evolution in these distance values in line with the number of chews, for the different degrees of shearing applied. Similar differences in the distance between mandibles were observed when the mandible force and the quantity of peanuts were changed.

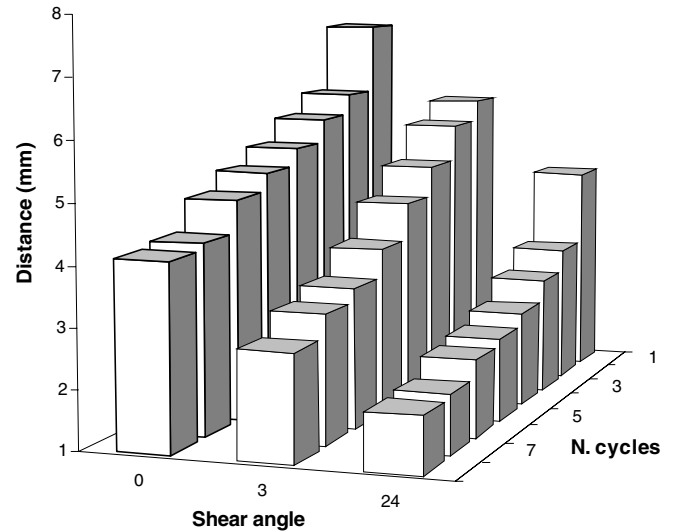


Fig. 6. Distance between mandibles achieved at the end of each cycle in the chewing simulator. Experiments were performed with 5 peanuts and applying different shearing angle.

It was possible to draw some preliminary conclusions from initial trials during which the principal parameters were varied separately. Firstly, it was shown that the degree of peanut breakdown depended mainly on the size of the sample (Figs. 5 and 7), but major variations were also observed due to the size and shape of the peanuts. For a fixed amount of peanuts, the biting force and shearing angle were the parameters which most affected the degree of peanut breakdown. Changes in the saliva flow did not modify the degree of peanut breakdown during the period studied (Fig. 7). The fact that with hard food such as peanuts, fragments are not really dissolved in saliva might explain why the presence of saliva did not have any clear effect on peanut breakdown. The biting speed and shearing

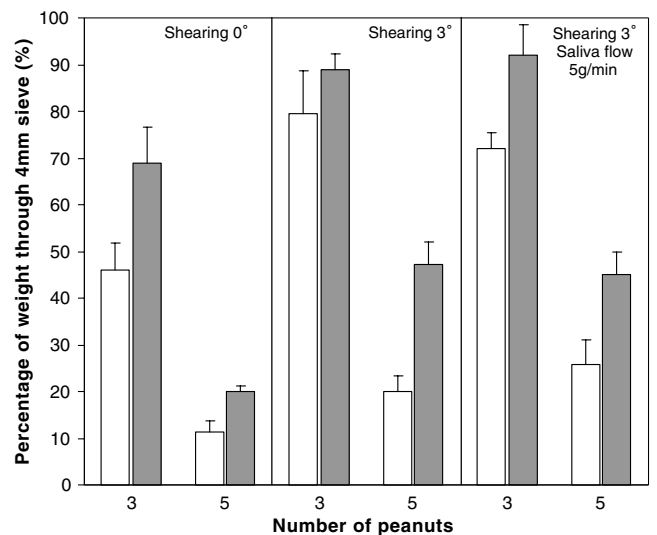


Fig. 7. Differences in the degree of peanut breakdown achieved in the chewing simulator after 4 (□) and 8 cycles (■) when the number of peanuts, shearing angle and saliva flow were varied.

speed did not modify the degree of peanut breakdown achieved after a determined number of chews, but of course variations to both parameters will be necessary to reproduce different chewing rates during time-intensity experiments.

3.2.2. Effects of mandible force and shearing angle on peanut breakdown

It was hoped that different patterns of food breakdown could be reproduced in the apparatus, mainly by varying certain parameters such as mandible force and the shearing angle. In order to study the effect of varying these two parameters on the degree of breakdown, differences in the particle size distribution of fragmented peanuts obtained after 4 and 8 chews were studied (Figs. 8 and 9, respectively). In general, and as might have been expected, an increase in both the shearing angle and bite force resulted in an increase in the degree of peanut breakdown. The effects of both parameters on the percentages of small sized

particles (<2 mm) and large sized particles (>4 mm) were studied by applying two-tailed ANOVA (Table 3). The results revealed a significant interaction between the two factors regarding both the quantity of small particles after 4 cycles and large particles after 8 cycles, and in all cases the effects of both factors were significant. As can be observed from Fig. 9, the effect of mandible force on the degree of breakdown depended on the shearing angle applied, and vice versa. In general, the effect on particle size distribution of changing the mandible force was greater when no shearing was applied than when shearing angles of 3° and 6° were applied.

3.2.3. Comparison with human mastication

The course of peanut fragmentation in the human mouth when chewed by four subjects was also studied. The particle size distributions of fragmented peanuts after 4 and 8 chews are represented in Fig. 10a and b, respectively. In general, the number of particles larger than

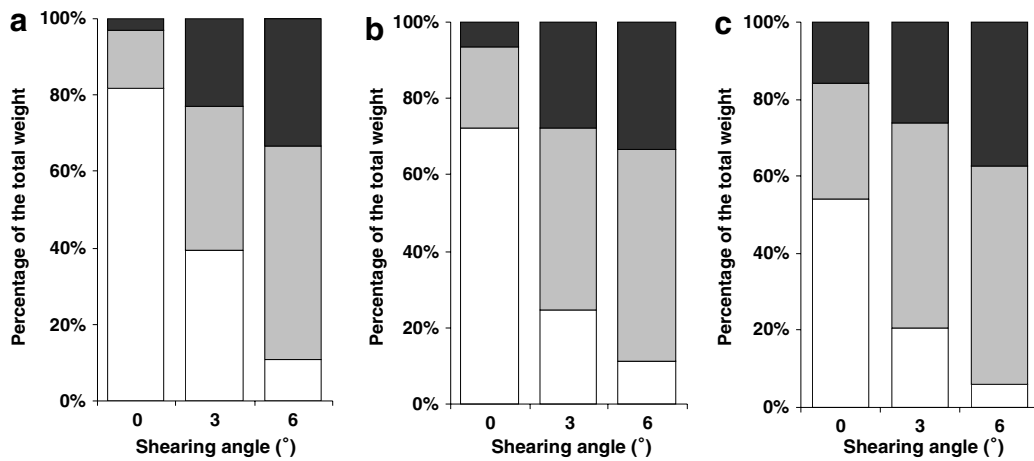


Fig. 8. Particle size distribution of peanut fragments after 4 cycles of chewing in the chewing simulator when applying different vertical force values: 250 (a), 300 (b) and 350 N (c) and different shearing angles. Particles smaller than 2 mm (■), between 2 and 4 mm (▒) and larger than 4 mm (□).

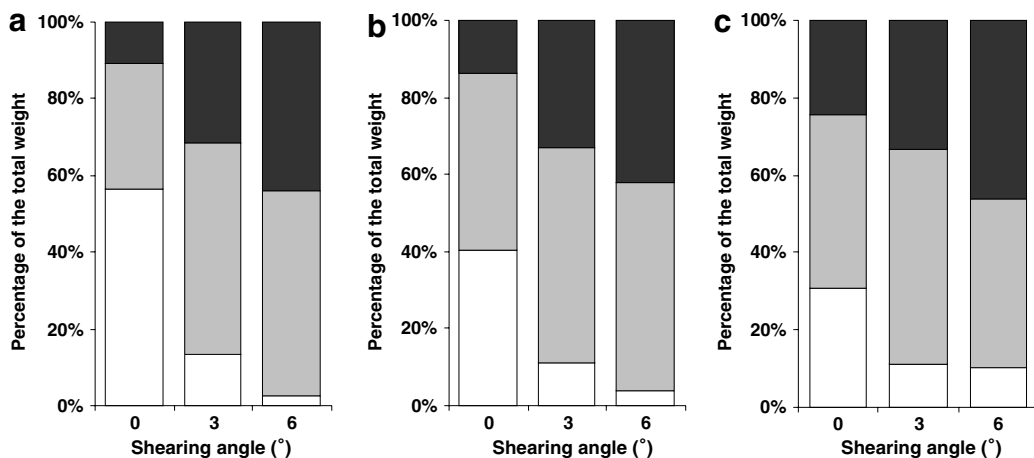


Fig. 9. Particle size distribution of peanut fragments after 8 cycles of chewing in the chewing simulator when applying different vertical force values: 250 (a), 300 (b) and 350 N (c) and different shearing angles. Particles smaller than 2 mm (■), between 2 and 4 mm (▒) and larger than 4 mm (□).

Table 3

Effects of mandible force and shearing angle on the percentage weight of small sized particles (<2 mm) and large sized particles (>4 mm) of chewed peanuts in the chewing simulator

| Samples obtained after | 4 cycles | | | | 8 cycles | | | |
|---------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| | <2 mm | | >4 mm | | <2 mm | | >4 mm | |
| Particle size | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> |
| <i>Main effects</i> | | | | | | | | |
| Mandible force | 11.81 | 0.0005 | 13.90 | 0.0002 | 5.67 | 0.0123 | 3.94 | 0.0380 |
| Shearing angle | 190.35 | 0.0000 | 177.18 | 0.0000 | 99.81 | 0.0000 | 126.21 | 0.0000 |
| <i>Interaction</i> | | | | | | | | |
| Mandible force × Shearing angle | 4.21 | 0.0140 | 2.61 | 0.0702 | 2.08 | 0.1258 | 7.77 | 0.0008 |

F and *p* values.

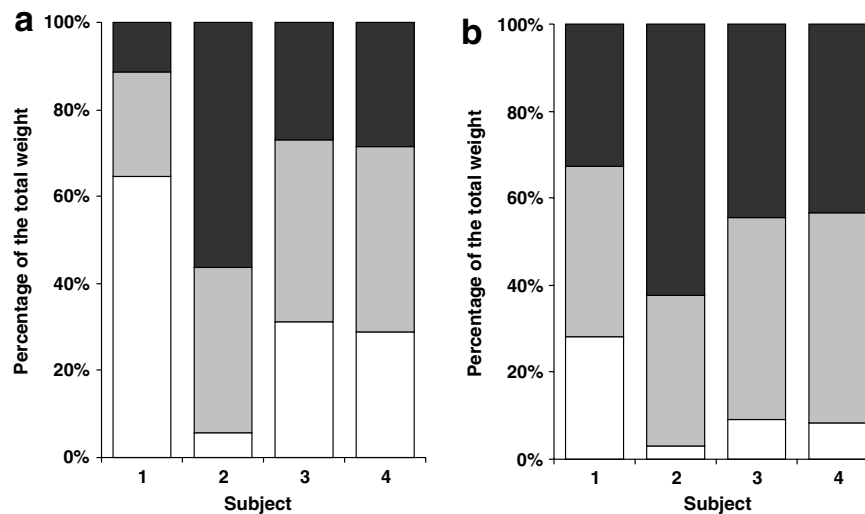


Fig. 10. Particle size distribution of human boli from 4 different subjects collected after 4(a) and 8 (b) chewing strokes. Particles smaller than 2 mm (■), between 2 and 4 mm (■) and larger than 4 mm (□).

4 mm decreased with the number of chews, and the number of those smaller than 2 mm increased, but clear differences were observed between subjects. Subject 2 (a man) exhibited greater efficiency than the other subjects, with 56% and 62% of the bolus total weight consisting of particles smaller than 2 mm after 4 and 8 chewing strokes, respectively. Subject 1 was the least efficient in terms of mastication, as 65% and 25% of particles remained larger than 4 mm after 4 and 8 chewing strokes, respectively. Finally, the degree of bolus fracture was intermediate and similar in subjects 3 and 4.

The data for 4 and 8 cycles were then combined, and the particle size distribution of samples obtained during chewing simulator experiments (Figs. 8 and 9) and human tests (Fig. 10) were compared. It could be observed that the particle size distribution in boli from subject 1 were very similar to those achieved by the chewing simulator when the mandible force was 350 N and no shearing force was applied. In the same way, the characteristics of boli from subjects 3 and 4 were similar to those obtained by applying 3° of shearing and a mandible force of 300 or 350 N. The degree of peanut breakdown achieved by subject 2 was greater than those obtained by the chewing

simulator under the conditions tested, but it could be obtained by applying greater force or higher shear angle values.

These results indicate that the course of peanut breakdown in the human mouth and related inter-individual differences could be reproduced in the chewing simulator. However, the range of variations of the main parameters in order to reproduce different oral breakdown patterns will differ as a function of the mechanical properties of the foodstuff considered (Kohyama & Mioche, 2004; Peyron, Mishellany, & Woda, 2004; Yven, Culioli, & Mioche, 2005, 2006). These ranges thus need to be determined for different food groups, and further study of physiological mastication parameters during chewing will enable a better fit for chewing simulator parameters.

We have thus developed a functional device which can precisely reproduce the compressive and shear strengths of a human jaw causing the breakdown of food with sufficient reliability when compared with in vivo measurements. The gradual addition of saliva and the collection of both the non-volatile compounds released in saliva and the volatile compounds released in the headspace during chewing are also possible. Our next step is to validate the on-line

sampling of volatile compounds with this device, connected to API-MS.

In terms of perspectives, we have planned to validate the functionality of the system using a variety of foods such as dairy, meat and bakery products. The use of image analysis will be investigated, as using such hydrated products, the application of simple sieving during chewing may not be sufficient to evaluate the status of the bolus. The mimicking of physiological functions such as swallowing and throat movements (which were not taken into account with this prototype because of their considerable complexity) are very important to the study of flavor release (Buettner, Beer, Hannig, Settles, & Schieberle, 2002; Hodgson, Linforth, & Taylor, 2003) and will be investigated in preparation for the next prototype.

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References

- Buettner, A., Beer, A., Hannig, C., Settles, M., & Schieberle, P. (2002). Physiological and analytical studies on flavor perception dynamics as induced by the eating and swallowing process. *Food Quality and Preference*, *13*, 497–504.
- Davidson, J. M., Linforth, R. S. T., Hollowood, T. A., & Taylor, A. J. (1999). Effect of sucrose on the perceived flavor intensity of chewing gum. *Journal of Agricultural and Food Chemistry*, *47*, 4336–4340.
- Hodgson, M., Linforth, R. S. T., & Taylor, A. J. (2003). Simultaneous real-time measurements of mastication, swallowing, nasal airflow, and aroma release. *Journal of Agricultural and Food Chemistry*, *51*, 5052–5057.
- Ingham, K. E., Linforth, R. S. T., & Taylor, A. J. (1995). The effect of eating on the rate of aroma release from mint-flavoured sweets. *Food Science and Technology – Lebensmittel – Wissenschaft und Technologie*, *28*, 105–110.
- Jensen, K. D., Beck, H. C., Jeppesen, L., Norrelykke, M. R., & Hansen, A. M. (2003). A new system for dynamic measurements of flavour release: A combined artificial mouth and membrane inlet mass spectrometer. In J. L. Le Quéré & P. X. Etievant (Eds.), *Flavour research at the dawn of the twenty-first century* (pp. 228–231). Lavoisier, Paris.
- Juteau, A., Mielle, P., & Guichard, E. (2003). Flavour release as a function of time and structure of matrices: an application for gas sensor technology. In J. L. Le Quéré & P. X. Etievant (Eds.), *Flavour research at the dawn of the twenty-first century* (pp. 232–235). Lavoisier, Paris.
- Kohyama, K., & Mioche, L. (2004). Chewing behavior observed at different stages of mastication for six foods, studied by electromyography and jaw kinematics in young and elderly subjects. *Journal of Texture Studies*, *35*, 395–414.
- Legin, A., Rudnitskaya, A., Clapham, D., Seleznev, K., Lord, K., & Vlasov, Y. (2004). Electronic tongue for pharmaceutical analytics - quantification of tastes and masking effects. *Analytical and Bioanalytical Chemistry*, *380*, 36–45.
- Linforth, R. S. T., Savary, I., Pattenden, B., & Taylor, A. J. (1994). Volatile compounds found in expired air during eating of fresh tomatoes and in the headspace above tomatoes. *Journal of the Science of Food and Agriculture*, *65*, 241–247.
- Nassl, K., Kropf, F., & Klostermeyer, H. (1995). A method to mimic and to study the release of flavour compounds from chewed food. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, *201*, 62–68.
- Neyraud, E., Prinz, J., & Dransfield, E. (2003). NaCl and sugar release, salivation and taste during mastication of salted chewing gum. *Physiology and Behavior*, *79*, 731–737.
- Peyron, M.-A., Mishellany, A., & Woda, A. (2004). Particle size distribution of food boluses after mastication of six natural foods. *Journal of Dental Research*, *83*, 578–582.
- Piggott, J. R., & Schaschke, C. J. (2001). Release cells, breath analysis and in-mouth analysis in favour research. *Biomolecular Engineering*, *17*, 129–136.
- Pionnier, E., Chabanet, C., Mioche, L., Le Quéré, J. L., & Salles, C. (2004). In vivo aroma release during eating of a model cheese: relationships with oral parameters. *Journal of Agricultural and Food Chemistry*, *52*, 557–564.
- Pionnier, E., Chabanet, C., Mioche, L., Taylor, A. J., Le Quéré, J. L., & Salles, C. (2004). In vivo nonvolatile release during eating of a model cheese: relationships with oral parameters. *Journal of Agricultural and Food Chemistry*, *52*, 565–571.
- Roberts, D. D., & Acree, T. E. (1995). Simulation of retronasal aroma using a modified headspace technique: investigating the effects of saliva, temperature, shearing, and oil on flavor release. *Journal of Agricultural and Food Chemistry*, *43*, 2179–2186.
- Taylor, A. J., & Linforth, R. S. T. (2003). Direct mass spectrometry of complex volatile and non-volatile flavour mixtures. *International Journal of Mass Spectrometry*, *223–224*, 179–191.
- Taylor, A. J., Linforth, R. S. T., Harvey, B. A., & Blake, B. (2000). Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chemistry*, *71*, 327–338.
- van der Bilt, A., & Fontijn-Tekamp, F. A. (2004). Comparison of single and multiple sieve methods for the determination of masticatory performance. *Archives of Oral Biology*, *49*, 155–160.
- van Ruth, S. M., Roozen, J. P., & Cozijnsen, J. L. (1994). Comparison of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings. In H. Maarse & D. G. Van Der Heij (Eds.), *Trends in flavour research* (pp. 59–64). Amsterdam: Elsevier.
- Yven, C., Bonnet, L., Cormier, D., Monier, S., & Mioche, L. (2006). Impaired mastication modifies the dynamics of bolus formation. *European Journal of Oral Sciences*, *114*, 184–190.
- Yven, C., Culioli, J., & Mioche, L. (2005). Meat bolus properties in relation with meat texture and chewing context. *Meat Science*, *70*, 365–371.