

Model cheese aroma perception is explained not only by in vivo aroma release but also by salivary composition and oral processing parameters

Elizabeth Guichard, Marie Repoux, E.M. Qannari, Hélène Labouré, Gilles

Feron

► To cite this version:

Elizabeth Guichard, Marie Repoux, E.M. Qannari, Hélène Labouré, Gilles Feron. Model cheese aroma perception is explained not only by in vivo aroma release but also by salivary composition and oral processing parameters. Food and Function, 2017, 8 (2), pp.615-628. 10.1039/C6FO01472K . hal-01564771

HAL Id: hal-01564771 https://u-bourgogne.hal.science/hal-01564771

Submitted on 22 Feb 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Model cheese aroma perception is not only explained by *in vivo* aroma

2 release but also by salivary composition and oral processing parameters

3 E. Guichard, *^a M. Repoux^a, E. M. Qannari,^b H. Laboure,^a and G. Feron,^a

- 4 ^aUMR CSGA (Centre des Sciences du Goût et de l'Alimentation): INRA, CNRS, Université de Bourgogne Franche-Comte,
- 5 AgroSupDijon, F-21000 Dijon, France.
- 6 ^bLUNAM University, ONIRIS, USC "Sensometrics and Chemometrics Laboratory", Nantes, France.
- 7 *Corresponding author. E. Guichard : elisabeth.guichard@inra.fr
- 8

9 Abstract

10 The aim of the present paper was to determine, on four model cheeses differing in fat content and firmness consumed by 11 fourteen well characterised subjects, the respective impacts of in vivo aroma release, bolus rheology, chewing activity, 12 mouth coating and saliva composition on dynamic aroma perception. The originality of the approach was to consider all the 13 parameters together and to be able to evaluate their relative contribution using multi-block partial least square (MB-PLS) 14 regression. Fruity aroma perception of the more hydrophilic compound (ethyl propanoate) was related to its dynamic 15 release parameters before swallowing whereas blue cheese aroma perception of the more hydrophobic compound (nonan-16 2-one) was related to its dynamic release parameters after swallowing and was highly impacted by mouth coating. 17 Moreover MB-PLS approach made it possible to evidence the combined effects of saliva composition and cross-modal 18 interactions to understand why in some cases dynamic aroma perception could not be explained by dynamic in vivo aroma 19 release data. Subjects with low sodium content in saliva perceived fruity aroma which is not congruent with saltiness as less 20 intense and salt- congruent (blue cheese) aroma as more intense, which was explained by their higher sensitivity to salt. 21 Subjects with a high lipolysis activity perceived fruity aroma which is not congruent to fat as less intense and fat-congruent 22 (blue cheese) aroma as more intense, which should be explained by the link between lipolysis activity and fat sensitivity. 23 These results could be considered for the reformulation of foods towards specific populations taking into account 24 nutritional recommendations.

25 Key words: aroma perception, aroma release, cheese, bolus rheology, saliva composition, chewing behaviour

26 Introduction

27 Aroma perception is an important aspect of food acceptability by consumers. However it is often very difficult to 28 directly explain aroma perception during food consumption by the amount of aroma compounds in the food. One 29 explanation is that aroma compounds have first to be released from the food matrix to the vapour phase in the 30 buccal cavity then transferred into the nasal cavity via the velum to reach the olfactory receptors¹. The partition of aroma compounds between the food matrix and the vapour phase depends on the affinity of aroma compounds 31 32 for the macromolecules present in the food² which can be determined by measuring the vapour/matrix partition 33 coefficients of aroma compounds. These coefficients depend both on the physico-chemical properties of the 34 aroma but also on the food matrix composition. As an example hydrophobic aroma compounds are more soluble 35 in fat than in water and thus are better released in the vapour phase from products with a reduced fat content³. 36 Proteins are able to form reversible or irreversible binding with aroma compounds which will impact aroma 37 release in the vapour phase^{4, 5}. In case of irreversible binding such as the formation of Schiff base between amino 38 groups of proteins and aldehydes⁶, the aroma compounds cannot be released in the vapour phase and thus cannot 39 be perceived. In case of reversible binding the aroma perception is lowered⁷. The major effect of hydrocolloids is 40 to increase food viscosity and thus decrease the transfer of aroma compounds from matrix to vapour^{8,9}, however 41 inclusion complexes are formed between amylose and specific aroma compounds which also impacts aroma 42 perception¹⁰. During consumption, food is mixed with saliva and broken down during the masticatory step to form 43 a swallowable bolus. During this step, aroma compounds are transferred from food to saliva before being released 44 in the nasal cavity. This transfer of aroma compounds is highly dependent on food composition and texture but also on subject's physiology¹¹. Thanks to the development of on line aroma release technologies, such as 45 46 atmospheric pressure chemical ionisation (APCI-MS)¹² or proton transfer reaction mass spectrometry (PTR-MS)¹³, it was possible to better understand the impact on dynamic in vivo aroma release of food matrix composition and 47 48 structure^{14, 15} and of physiological parameters such as chewing, swallowing, saliva flow^{16, 17}. Concerning the impact 49 of food matrix, increasing fat content results in a decrease in maximum intensity and overall in vivo aroma release together with an increase in aroma remanence as was observed in gelatin gels¹⁸, whey protein gels¹⁵ or model 50 cheeses¹⁹, which was explained by a lower solubility of hydrophobic aroma compounds in fat. Even if food 51 rheology could explain chewing activity during consumption²⁰, bolus properties and in-mouth bolus rheology 52 53 better explain *in vivo* aroma release²¹. The amount of cheese remaining in the mouth after swallowing has also 54 been found important to explain in vivo aroma release. A higher amount of fat product remaining in the mouth 55 leads to a lower release of the more hydrophobic compounds before swallowing due to their high affinity for the food bolus and to a longer aroma persistence in the breath^{16, 22}. The less hydrophobic compounds are less 56

impacted by mouth coating but are more quickly released from firmer cheeses, due to a higher food breakdown^{16,}
 ¹⁷. When consuming solid foods, differences in chewing activities between subjects are responsible for differences

59 in aroma release, an intense chewing work leads to a higher amount of aroma release¹⁷.

60 However despite the development of *in vivo* measurements, results in the literature in the field of dairy products 61 showed that no clear relationship exists between in vivo aroma release and aroma perception due to the co-62 existence of physicochemical and cognitive mechanisms²³. Cognitive mechanisms are due to cross-modal interactions which vary according to the type of texture²⁴. In the case of semi-liquid foods such as yoghurts, an 63 64 increase in viscosity induced a decrease in both aroma release and aroma perception²⁵, whereas for solid cheeses 65 aroma perception decreased with firmness without any noticeable difference in aroma release²⁶. This finding was 66 explained by the attention paid by the subjects to the texture perception which likely led to a decrease in the aroma perception. By delivering simultaneously different kinds of texture and different odorants, Bult et al.²⁷ 67 68 showed that the perceived aroma intensity was reduced with increasing viscosity when the odour was presented 69 either ortho or retronasally. Moreover the perceived thickness was increased only when the odour was presented 70 retronasally simulataneously with swallowing time. An adaptation phenomenon was also evidenced in gel candies 71 as being responsible for the influence of taste and texture on aroma perception²⁸, by comparing the *in vivo* aroma 72 release curves and sensory time intensity curves which were recorded simultaneously. This study showed that 73 time at maximum chewing activity came first followed by time at maximum in nose concentration then time at 74 maximum perceived intensity. The time delays between release and perception increased when the product 75 remained longer in the mouth. The difficulties to relate results from analytic and sensory approaches was also 76 pointed out in brandies²⁹ and explained by sensory interactions between fruity and woody aroma³⁰. In coffee, the 77 burnt sensory notes could be associated with pyrazines detected by in vivo PTR-MS analysis but the effect of 78 adding sugar produced a change in aroma perception from burnt to caramel which could not be associated to any 79 change in aroma composition³¹, confirming the existence of sweet-aroma interactions²³. Odour-taste interaction 80 basically depends on the capacity of the two stimuli to form an appropriate combination in a food product context 81 (congruency)³². Thus, odour-taste integrated perception highly depends on learned associations, the context in 82 which the food is consumed and the consumer's previous experience³³. In experimental situations, odour-taste 83 interactions were found to be affected by the so-called dumping and halo effects³⁴, which resulted from the 84 tendency for sensory panellists to dump their sensations on several available scales (taste and/or odour). 85 Moreover, in more complex cheese flavour mixtures, NaCl, lactic acid, and aroma were found to be able to 86 enhance cheese flavour intensity and to compensate each other towards cheese flavour intensity³⁵, thus 87 suggesting complex taste-taste and taste-aroma interactions involved in the overall cheese flavour. So far, no 88 study has investigated cross-modal interactions using parameters related to oral physiology.

89 During the last decade, saliva has gained more and more interest in the field of sensory science. Indeed, due to its 90 role in oral clearance, bolus moisturizing and hydrolytic properties, saliva promotes the disintegration of the 91 matrix and thus the release of palatable active substances during food consumption. For example the salivary flow 92 rate influences aroma release³⁶ by a dilution effect³⁷. Salivary flow has also been positively associated with liking 93 for fat³⁸. Moreover, amylase activity can modify the bolus rheological properties³⁹ and thus modulates the 94 perception of salt in starch-based matrices⁴⁰. Even subject to controversy, lipase might be responsible for hydrolysis of food triglycerides and thus fat perception^{41, 42}. Finally the catalytic activity of saliva against some 95 aroma compounds (e.g. esters, aldehydes) has been demonstrated in vivo and in vitro⁴³⁻⁴⁶ with consequences on 96 97 aroma release and thus on perception. Saliva is also composed of small and large molecules that contribute to 98 maintain a "salivary homeostasis" whose background level can regulate the dynamics of molecule release and thus their sensory impact (sensory or nutritional)⁴⁷. For instance, it has been shown that a human subject with a high 99 salivary sodium concentration is less sensitive to saltiness⁴⁸. 100

A previous paper²² highlighted the role of bolus rheology and composition of stimulated saliva on aroma release from cheeses varying in fat content and firmness. The aim of the present paper is to better understand the physicochemical and physiological parameters which drive dynamic aroma perception. For that purpose, a group of fourteen subjects were selected as representative of those participating to our previous study in order to evaluate aroma perception as a function of time and to determine, on these well characterised subjects, the respective impacts of *in vivo* aroma release, bolus rheology, chewing activity, mouth coating and saliva composition on dynamic aroma perception.

108 **Experimental**

109 Cheese products

110 Four processed model cheeses were used¹⁶. They were composed of a mixture of cheddar, soft cheese, butter, melting 111 salts, protein powder (casein), salt and water. Two levels of texture (S=soft, F=firm) were obtained by varying the water 112 content and two ratio of fat to dry matter, a low level at 25% for low fat cheeses (IfF and IfS) and a high level at 50% for high 113 fat cheeses (hfF and hfS). The pH ranged from 5.27 to 5.55. The rheological properties of the cheeses were measured in a 114 large deformation at a rotation of 0.01 rad.s⁻¹ for 240 s using a Haake Viscotester (VT550 - Thermo electron GmbH, Karlsruhe, Germany), as previously described¹⁶. The breakdown stress corresponds to the maximum strength necessary to 115 116 cause cheese breakdown, with the lowest values for the softest cheese (8129 ± 469 Pa for lfS and 8022 ± 1309 Pa for hfS) 117 and the highest values for the firmest cheese (15253 ± 1231 Pa for IfF and 15556 ± 2307 Pa for hfF). The critical strain at 118 breakdown corresponds to the maximum rotation angle required to cause breakdown, with the lowest values for cheeses

119	with the highest fat content (0.273 \pm 0.022 rad for hfS and 0.348 \pm 0.061 rad for hfF), and the highest values for cheeses
120	with the lowest fat content (0.804 \pm 0.056 rad for IfS and 0.836 \pm 0.036 rad for IfF). Two aroma compounds were added
121	during cheese production, a hydrophobic ketone, nonan-2-one (NO: logP= 2.9, 6 mg.kg ⁻¹) and a hydrophilic ester, ethyl
122	propanoate (EP: logP= 1.4, 25 mg.kg ⁻¹).

123

124 Subjects

125 In a first step, 48 subjects (23 female and 25 males) were selected from a group of 100 volunteers based on their good 126 dental and oral status, and on the repeatability of physiological parameters (salivary flow rate under resting and stimulated 127 conditions, respiratory flux, salivary composition)^{16, 20}. They were characterised for their oral volume, saliva composition 128 and flow, in vivo release measurement, chewing activity, bolus saliva content, bolus rheology and mouth coating. From this 129 group of 48 subjects, a subgroup of fourteen subjects (6 females and 8 males, average age: 40 years ± 9) was selected for 130 the sensory analyses, on their ability to detect and recognise the two aroma notes. The subjects were not allowed to 131 smoke, eat or drink starting at least one hour before the different test sessions. All the subjects were informed of the 132 observational nature of this study. They gave their signed consent and received a financial compensation for their 133 participation. The study protocol was submitted to an Ethics Committee and was approved on 17 April 2008 by the Comité 134 de Protection des Personnes Est-1 (N°2008/15) and on 8 August 2008 by the Direction Générale de la Santé - France (N° 135 DGS2008-0196).

136 Subjects were characterised for specific physiological parameters described in the following sections.

137

138 Oral volume

Oral volume was measured using an Eccovision[®] acoustic pharyngometer (Hood Laboratories, USA), as described previously⁴⁹. This device consists of two microphones and a horn driver mounted on a wave tube and connected to a PCcompatible computer with signal conversion capabilities. The signal was converted into the surface change (cm²) as a function of the length of the oral cavity (cm). The subjects held the mouthpiece in their mouth with their teeth against the flange and their tongue in a low position. To prevent air leaks, which could cause measurement errors, the subjects placed their lips over the flange, sealing the mouthpiece. The subjects were instructed to breath with their nose during the experiment. Values are expressed in cm³ and correspond to the average of 10 measures.

146

147 Saliva samples and flow

Since our previous study²² highlighted that resting saliva poorly contributed to explaining aroma release whereas stimulated saliva significantly impacted *in vivo* aroma release from soft cheeses, only results obtained with stimulated saliva

are taken into account in the present paper. Stimulated saliva was collected as previously described⁴⁹. The subjects chewed a piece of Parafilm^M (0.5 g ± 0.2 g) for a period of 5 min and spit out the saliva every 30 seconds into a pre weighed cup over a period of 5 minutes. The cups were weighted and the salivary flow rates were expressed in mL.min⁻¹. Immediately after collection, the saliva samples were standardized by a first step of centrifugation for 30 min at 15000-x g to remove bacteria and cellular debris. Thereafter the supernatants were stored at -80°C to stop metabolism until subjected to biochemical analyses.

156

157 Biochemical analyses of saliva samples

Protein concentrations. Protein concentrations (Prot) were measured with a standard Quick Start Bradford protein assay
 (Bio-Rad, France) with bovine serum albumin as the calibration standard.

Enzyme activities. All enzyme activities were expressed in International Enzyme Activity Units (U) per ml of saliva. One U is
 defined as the amount of enzyme that catalyses the conversion of 1 micromole of substrate per minute. The lipolytic
 (Lipolysis), proteolytic (Proteolysis), lysozymal (Lysozyme) and amylolytic (Amylase) activities were determined as
 previously described^{42, 50, 51}.

Sodium analysis. The saliva samples were diluted to 1/20 (50 µL saliva in 950 µL filtered 18 mΩ Milli-Q-water (Millipore, Bedford, MA, USA)) and filtered through a membrane (pore size = 0.45 µm, C.I.L., Sainte-Foy-La-Grande, France). The amounts of sodium (Na) in saliva were determined by HPLC ionic chromatography using a Dionex ICS2500 ion chromatographic system (Dionex, Voisins le Bretonneux, France) as previously described⁵². Quantifications were performed using calibration curves realised with sodium standard solutions ranging from 0.1 to 10 mM in 22 mM sulfuric acid (R^2 =0.999).

170

171 Sensory analyses

All sessions took place in an air-conditioned (21 ± 2°C) sensory testing room of the ChemoSens platform (Centre des Sciences du Goût et de l'Alimentation, INRA, Dijon) using standardized booths equipped with computers. Subjects were instructed to place each piece of cheese (6 g) in the mouth, and freely consume it while keeping the lips closed. The products were presented in a random order at 17° C. All measurements were done in triplicate. Bread, apple and water were used as mouth cleansers between two tests.

177 The subjects were firstly trained during eight sessions to recognise the odour of the two aroma compounds, blue cheese for 178 nonan-2-one (NO) and fruity for ethyl propanoate (EP), using triangular tests and recognition tests. Other training sessions 179 were conducted to familiarize the subjects with the discontinuous time-intensity methodology used. The last training

180 sessions were dedicated to the use of a continuous scale (0 to 10) to score the intensity of the two aroma compounds. Two 181 test sessions were then done using Fizz ${
m I\!R}$ software to score the intensity of the two aroma notes during the mastication, to 182 indicate the time of the first swallowing and then to score the intensity of the two aroma notes after each swallowing 183 event, during a total time of 3 min. During mastication and at each swallowing time, the intensity of each aroma note was 184 scored on the continuous scale (0-10). Each of the four cheeses was presented three times, in a random order, at 17°C. 185 From the aroma intensity perception at each swallowing event and the time of each swallowing event, time-intensity curves 186 were reconstituted for each aroma note and the following parameters were extracted: Imax S for maximum intensity, 187 Tmax_S for time to reach maximum intensity, Ideg_S for intensity at the first swallowing and Tend_S for time to reach the 188 end of perception (Fig. 1). The rate of perception (Vmax_S = Imax_S/Tmax_S) was then calculated as a fifth variable.

189 Insert Figure 1

190 In vivo aroma release measurement

191 The same protocol was applied for cheese consumption than that described for sensory analysis. The release of the two 192 aroma compounds was followed simultaneously in the nasal cavity as previously described ¹⁶ using Atmospheric Pressure 193 Chemical Ionisation-Mass Spectrometry (APCI-MS) with an ion trap Esquire-LC mass spectrometer (Bruker Daltonique, 194 Wissembourg, France) according to their protonated molecular ion (MH⁺), which is the main ion: ethyl propanoate 195 (m/z=103) and nonan-2-one (m/z=143). Air was sampled from the nose at an average flow rate of 37 mL.min⁻¹ via a fused 196 silica capillary tubing (i.d. = 0.53 mm) heated at 150 °C and to which a 5 kV positive ion corona pin discharge was applied. 197 Each subject was asked to position the plastic tube in one nostril (the same for all the experiments) and to breathe 198 normally. This period (breath-blank phase) was used to record the potential residual signal of the previous sample until 199 return to the baseline and to control the regularity of breathing.

200 The curves were smoothed using a wavelet decomposition method to eliminate signal fluctuations due to the subjects' 201 breathing patterns. Two release phases were identified, the chewing phase (1) extended from placing the cheese in the 202 mouth to the first swallowing, and the post-swallowing phase (2) extended from the first swallowing to the time at which 203 the signal returned to its baseline level. For both release phases and for each aroma compound, four main parameters were 204 extracted from each individual release curve: the area under the curve (A1_P and A2_P (a.u.: arbitrary unit)) representing 205 the quantity of aroma released, the maximum intensity (IMax1 P and IMax2 P (a.u.)), the time to reach maximum intensity 206 (tMax1_P and tMax2_P (min)) and the release rate (Vmax_P= IMax1_P / tMax1_P (a.u./min)). These data, also not 207 quantitative, allow a direct comparison of the different release curves.

208

209 Chewing activity

Food & Function Accepted Manuscript

210 Chewing activity was monitored during cheese consumption, simultaneously to aroma release. The muscle activity of the 211 superficial masseter and temporis muscles (left and right) during chewing was recorded by electromyography (EMG) using 212 gold surface electrodes (Grass technologies, West Warwick, RI, U.S.A), at 382 Hz, then the signal was amplified and 213 digitalized⁵³. The following parameters were extracted from EMG data: number of chewing cycles (Nber Cycle), chewing 214 duration (Chew_time expressed in s), total muscle work (W_tot expressed in mV.s-1) and mean amplitude of contraction 215 (Ampl expressed in mV) which corresponds to a mean calculated from the amplitude values of each chewing cycle 216 registered in a whole chewing sequence²⁰.

217

218 **Bolus saliva content**

219 The percentage of dry matter and water content were determined using an infrared dryer for all cheeses and boluses 220 obtained just before swallowing. The percentage of moistening (Moist %) into the bolus was calculated from the bolus 221 water content (Bwc %), the bolus dry matter (Bdm %), the cheese dry matter (Cdm %) and the cheese water content (Cwc 222 %) as follows:

223
$$Moist(\%) = \left(\frac{Bwc}{Bdm} \times Cdm\right) - Cwc$$

224 Three replicates per cheese and per subject were performed.

225

226 **Bolus rheology**

227 The subjects were instructed to chew the cheese samples until swallowing and to spit out the bolus into a truncated 228 syringe. Bolus rheological properties were measured using a compression test on an aliquot of 3 mL of bolus²⁰. The test was 229 performed using a mobile circle upper plate and a fixed circle lower plate as compression device, with a compression rate of 230 1 mm.s⁻¹. The bolus was subjected to a force F ranging between 0.01 N and 50 N. From the compression curve, particularly 231 two phases were highlighted. A "flow phase" during which the suspension begins to flow and the particles move 232 significantly in relation to one another at a height denoted as hflow (mm). Yield stress and viscous effects were described 233 respectively by the parameters sflow (Pa) and Kflow (Pa.s). A "particle phase" during which the mechanical response is 234 governed by the particle size which is represented by a height denoted hpart (mm) and the yield stress component denoted 235 spart (Pa). At the end of the compression, hend (mm) denotes the final height and Send (mm²) the area generated under 236 the maximal force. All measurements were done in triplicate.

237

238 Mouth coating

8

The amount of food that sticks to the oral surface after food ingestion (QRB) was quantified by the "mouth rinse" method⁵⁴. Curcumin (Naturex, France) was added during cheese production (30 mg.kg⁻¹). Each subject was asked to place a piece of cheese (6 g, at 17°C) in the mouth and to chew normally until swallowing. The subjects swallowed without cleaning movement and then rinsed their mouth (with cleaning movements) with 4 mL of warm water at 50°C for 30 s, and spat it into a vial. This rinsing procedure was applied two times consecutively and the spittle was cumulated in the same vials. The fluorescence intensity of curcumin was quantified using a Perkin Elmer 1420 Multilabel Counter Victor 3V at an excitation wavelength of 450 nm and an emission wavelength of 510 nm. All measurements were done in triplicate.

246

247 Statistical analyses

Analyses of variance (ANOVA) and Principal Component Analysis (PCA) were performed using XLSTAT[®] Software (Excel 97, version 8.0, Paris, France). When a significant effect (p<0.05) was revealed by applying ANOVA, the Student-Newman-Keuls test was used to compare the differences in least-squares (LS) means.

Statistical treatments for Partial lest Square (PLS) analysis were performed using the free software R 3.3.0 (http://cran. rproject.org/), as already described for the treatment of *in vivo* release data obtained with the same cheeses and 48 subjects ²². The main R package used for multivariate data analyses was «pls 2.5-0» ⁵⁵. In a preliminary stage, the statistical treatment consisted in a pre-processing step ⁵⁶. More precisely, all the variables are mean centered; then the blocks of variables are set to the same total variance. Finally, in order to explore the systematic variation patterns in the X blocks which are likely to predict the systematic variation patterns in Y, Multiblock -PLSR (MB-PLSR) is applied.

The different variables used in the MB-PLS approach are presented in Table 1. They have been divided in six blocks. The Y block corresponds to the variables to be explained that is, the sensory variables. The five other blocks correspond to the explanatory variables (X1-X5). X1 is related to aroma release variables before (1) and after swallowing (2) as previously described. The variables in the subsequent blocks were selected as highly impacting the *in vivo* aroma release²². X2 is related to bolus rheological variables and bolus moistening. X3 is related to mouth coating and oral volume. X4 is related to masticatory variables extracted from the EMG signals. X5 is related to the properties of stimulated saliva.

263 Insert Table1

264 Results

265

266 Subjects' physiology

The 14 subjects were selected from the 48 subjects participating to the *in vivo* release measurements¹⁶. Their physiological
 characteristics (Table 2) cover the range of variability observed for the 48 subjects as presented in the PCA representation

269 (Fig.2). The principal plan represents 47% of the information. Axis 1 (26.76%) is explained by the salivary flow (Flux_S) and

the amount of sodium (Na S) on its positive part, which are higher for subjects S027 and S094. Axis 2 (20.14%) is explained

271 by the amount of amylase (Amylase_S), the amount of salivary proteins (Prot_S) and the oral volume (Oral_vol) on its

- positive part, in relation with subjects S001, S052 and S086. At the opposite, S004 has a very low amount of amylase.
- 273

274 Insert Table 2.

275 Insert Figure 2

276 Time intensity perception

277 From the time intensity curves obtained for the four cheeses, 14 subjects and two odour notes, four variables 278 were extracted namely, maximum intensity (Imax_S), time to reach the maximum intensity (Tmax_S), intensity at 279 the first swallowing (Ideg_S) and time at the end of perception (Tend_S). The fifth variable, rate of perception 280 (Vmax_S = Imax_S/Tmax_S) was calculated. Due to the high inter-individual differences between subjects only two 281 variables show significant differences between the cheeses. Tmax_S is significantly higher (p = 0.002) for the blue 282 cheese aroma (NO) detected in low fat cheeses (IfS and IfF) and Vmax_S is significantly higher (p = 0.07) for the 283 fruity aroma (EP) detected in high fat soft cheese (hfS). Means and standard deviations for the different cheeses 284 are reported in supplementary Table S1.

The high inter-individual variability was also observed on the *in vivo* release variables for the 48 subjects, which was explained by differences in physiological parameters. Results of *in vivo* aroma release have already been published¹⁶, However the data obtained for the 14 subjects selected for the sensory analysis are available in supplementary Table S2. In order to better highlight the subject effect, a multiblock PLS analysis was performed on these 14 subjects to explain sensory perception for each cheese and each aroma compound by the *in vivo* release variables together with the physiological variables (Table 1).

291

292 Relating sensory perception to in vivo aroma release and physiological parameters using MB-PLS

MB-PLS is an extension of the PLS method, a class of regression models attempting to find relationships between explanatory and response variables. In MB-PLS, the predictor variables are separated into subsets or blocks that are standardized in order to balance for the size effect due to the measurement scale. It is a statistical approach particularly relevant when different data sets reflecting different dimensions (physiology, physic, chemistry ..) and with a different number of variables in each set are considered.

298 MB-PLS analyses were conducted on the different data sets to assess the extent to which the X blocks of variables 299 explain sensory perception during cheese matrix consumption (Fig. 3 and Fig. 5). The four cheeses and the two

aroma compounds nonan-2-one (NO) and ethyl propanoate (EP) were considered separately for the statisticaltreatment.

302 Two different results are presented from MB-PLS analysis in the following sections, the importance of the block 303 and the projection of the variables in the correlation circle. At first, the importance of the blocks is calculated from 304 the sum of the beta-weight of the different variables constitutive of the block related to the corresponding 305 component and is expressed in %. It thus reflects the contribution of the block of variables to the determination of 306 the component. Higher is the percentage, higher is the contribution of the block for explaining sensory data. 307 Complementary to the importance of the blocks, the projections correspond to the depiction in a correlation circle 308 of the variables belonging to the different blocks. They thus represent the importance of the variables to the 309 components. Higher a variable is correlated to the components, higher it contributes to the model and thus to 310 explain perception.

The choice of the number of MB-PLS components to be retained for the importance of the block and the projections was based on the total variance of block Y recovered by these components. For more details regarding this aspect, we refer to a previous paper²². We restrict ourselves to the first two components because they explain between 45.3% and 56% of the total variance of Y for the high fat cheeses and the two aroma compounds and between 36.5 and 50.4% for the low fat cheeses and the two aroma compounds.

In order to avoid cumbersome graphical displays on the projections, only the variables with a correlation coefficient with one of the two first components above 0.5 are depicted. The font size for each variable on the projection (Fig. 4 and Fig. 6) reflects the importance of the correlation coefficient with the first two components; with large font size indicating large correlations.

320 The results from the MB-PLS are presented and discussed successively for high fat cheeses and for low fat cheeses.

321

322 Relative Importance of the blocks in the projection for high fat cheeses

323 The importance of the blocks of variables for the two first MB-PLS components is shown in figure 3 for the two 324 high fat cheeses and the two aroma compounds. The release block is important for the high fat firm cheese (hfF) 325 and both molecules and this on the two components (31.7% for NO on component 1 and 37% for EP on 326 component 2) and less important for high fat soft cheeses (with a maximum of contribution of 18.5%). The bolus 327 rheology block is mainly reflected by the second component for EP (25.9% for hfF-EP and 31.5% for hfS-EP) and 328 less reflected for NO. The importance of coating and oral volume is higher for EP than for NO, on the first 329 component. The masticatory variables take an important part in the explanation for both cheeses and both aroma 330 compounds. Notice also the high contribution of stimulated saliva, mainly for hfS-NO (48.9% on component 1 and

331 24.8% on component 2) for which the masticatory variables are less important (12.5% on component 1 and 21.3%

332 on component 2).

333 Insert Figure 3

334 Insert Figure 4

335 Projection of the main variables for high fat cheeses

336 Figure 4 shows the projection of the variables from each block for the two high fat cheeses (hfF and hfS) and the two aroma 337 compounds (EP and NO). The sensory variables from the Y block (variables to be explained) are negatively correlated with 338 component 1 and for the high fat soft cheese, the rate of perception is negatively correlated with component 2. The other 339 variables (explanatory variables) are projected differently according to both the cheese and the aroma compound. For the 340 high fat firm cheese and ethyl propanoate (hfF-EP), the masticatory parameters are correlated with component 1, with the 341 chewing time (Chew_time) and number of chewing cycles (Nber_Cycle) on the positive part and the amplitude (Ampl) on 342 the negative part, the release parameters are correlated with component 2, with a higher correlation coefficient for 343 Vmax_P and Imax1_P, the amount of product remaining in the mouth (QRB) is projected at the opposite of the maximum 344 intensity perceived (Imax_S). Among the salivary parameters, the amount of proteins (Prot_S) is positively correlated with 345 component 1, opposite to the amount of sodium (Na_S) and of amylase (Amylase_S). The amount of lipolysis (Lipolysis_S) is 346 projected close to the QRB. For the high fat soft cheese and ethyl propanoate (hfF-EP), the most correlated explanatory 347 variables are the bolus rheology properties, with Send and Moist negatively correlated with component 1 together with the 348 sensory variables, opposite to hend and hpart. The masticatory parameters are also correlated with component 1, with 349 Chew time and Nbe Cycle on the positive part and Ampl on the negative part. Among the release parameters, only 350 Imax1_P and A1_P are positively correlated with component 1 and Vmax_P with a lower correlation. QRB is also projected 351 opposite to Imax_S. Concerning the high fat firm cheese and nonan-2-one (hfF-NO), the masticatory parameters 352 (Chew_time and Nbe_Cycle) are also positively correlated with component 1 and negatively with component 2, the release 353 parameters before swallowing (Imax1_P and A1_P) are positivele correlated with component 1 whereas the release 354 parameters after swallowing (Imax2 P, A2 P) are negatively correlated with component 2. Concerning the high fat soft 355 cheese and nonan-2-one (hfS-NO), the salivary parameters are well represented on the projection, mainly Flux_S and Na_S, 356 positively correlated with component 1 and component 2 and opposite to the maximum intensity perceived. The release 357 parameters have only low correlations with these two components as represented by their small font size, but the time to 358 reach maximum intensities both before and after swallowing (tmax1_P and tmax2_P) are positively correlated with 359 component 2, opposite to the rate of perception. The masticatory parameters and QRB are negatively correlated with 360 component 1.

362 Relative Importance of the blocks in the projection for low fat cheeses

12

Food & Function Accepted Manuscript

363 The importance of the blocks of variables for the two first MB-PLS components is shown in figure 5 for the two low fat 364 cheeses and the two aroma compounds. The relative importance of the release block is always below 28%, it is higher for 365 nonan-2-one and the firm cheese (IfF-NO, 27.5% on component 1 and 14.9% on component 2) and for ethyl propanoate 366 and the soft cheese (IfS-EP, 20.7% on component 1 and 19.8% on component 2). The bolus rheology block is reflected on 367 the two components with a contribution of 19.2% on component 1 and 6.8% on component 2 for IfF-NO, 17.4% on 368 component 1 and 29% on component 2 for IfS-NO and intermediates for EP. Coating and oral volume are highly important 369 for IfF-NO (25.2% on component 1 and 29.8% on component 2) and IfS-EP (48.7% on component 2). The masticatory 370 properties have a high impact on fruity aroma (EP) in the low fat firm cheese (46.5% on component 1 and 47% on 371 component 2), and in the low fat soft cheese (41.3% on component 1) but they seem to impact less blue cheese aroma 372 (NO). For nonan-2-one the salivary parameters are more relevant to explain sensory perception with 22.2% of contribution 373 on component 1 and 28.5% on component 2 for low fat firm cheese and 14.8% on component 1 and 46.5% on component 2 374 for low fat soft cheese.

375 Insert Figure 5

376 Insert Figure 6

377 Projection of the main variables for low fat cheeses

Figure 6 shows the projection of the variables from each block for the two low fat cheeses and the two aromacompounds on the two first MB-PLS components.

380 For the low fat firm cheeses (IfF), the sensory variables related to aroma persistence (Tmax_S and Tend_S) are 381 negatively correlated with component 1, whereas the rate of perception (Vmax S) and the maximum intensity of 382 perception (Imax_S) are negatively correlated with component 2 for ethyl propanoate and positively for nonan-2-383 one. For the low fat soft cheeses, the rate of perception (Vmax_S) is positively correlated with component 1, the 384 maximum intensity (Imax_S) negatively with component 2, whereas the times (Tmax_S and Tend_S) are negative 385 correlated with component 1 for ethyl propanoate and negatively correlated with component 2 for nonan-2-one. 386 Concerning the explanatory variables, the masticatory parameters (Chew time and Nber Cycle) are negatively 387 correlated with component 1 for IfS cheese and the 2 aroma compounds and for IfF cheese and ethyl propanoate 388 but negatively correlated with component 2 for IFF cheese and nonan-2-one, whereas the amplitude is negatively 389 correlated with component 2 only for IfF cheese and ethyl propanoate. Concerning the bolus rheology parameters 390 Hend, hpart and Kflow are always positive correlated with component 1 and component 2 whereas Moist and 391 Send are negatively correlated with component 1 and component 2. Concerning the release parameters, tmax2_P 392 is always negative correlated with component 1, the other parameters have lower correlations on this plan, except 393 Vmax P on the positive part of component 1 for IfF cheese and ethyl propanoate, Imax1 P on the positive part of 394 component 1 and negative part of component 2 for IfF cheese and nonan-2-one, A2_P and A_P for both cheese

395	and nonan-2-one. The salivary parameters have only small correlations on this plane for the low fat soft cheese
396	but for the low fat firm cheese, the salivary flow (Flux_S) and the amount of sodium (Na_S) are negatively
397	correlated with component 2.

398

399 Discussion

400 Considering the results of MB-PLS on high fat cheeses (Fig. 4), different trends are observed for firm and soft cheeses. For 401 the firm cheeses, the perception of fruity aroma is more related to the release of ethyl propanoate (EP) before swallowing 402 whereas the perception of blue cheese aroma is more related to the release of nonan-2-one (NO) after swallowing. Ethyl 403 propanoate which is less hydrophobic is released faster from the fat cheese matrix¹⁶, and thus perceived at the beginning of 404 the oral processing for the firm cheese whereas nonan-2-one which is hydrophobic is released later and mainly after 405 swallowing¹⁹, which explains the longer time to reach maximum intensity and time to reach the end of perception. For the 406 soft cheeses the same correlations are found for nonan-2-one whereas no such correlation is found between sensory 407 parameters and release parameters for ethyl propanoate. Regarding the masticatory variables, it appears that the mean 408 amplitude of contraction (Ampl) is well correlated with the rate of fruity perception (EP) on component 1 for firm cheese 409 and component 2 for soft cheeses. This variable was already highlighted in our previous paper²² as responsible for a higher 410 rate of EP release. Thus, we confirm that subjects with high amplitude per burst will release more rapidly the hydrophilic 411 compound (EP) and we demonstate that this higher release rate induce a more rapid perception. The other masticatory 412 variables better explain the blue cheese perception (NO) and more specifically aroma persistence. Subjects with a longer 413 chewing time (Chew_time) and larger number of bursts (Nber_Cycle) have a longer duration of blue cheese aroma (Tmax_S 414 and Tend_S) for both cheeses. This could also be explained by a longer time to reach the maximum NO release intensity 415 after swallowing (tMax2_P). The bolus rheology properties impact fruity perception (EP) more, with a positive relationship 416 between bolus moistening (Moist) and time to reach the end of perception (Tend-S), which could be explained by an 417 important bolus spreadability (Send). Bolus moistening (Moist) impacts EP release intensity (Imax1_P), a high bolus 418 moistening decreases the amount of release before swallowing. This could be explained by dilution with saliva which 419 decreases the rate of release of hydrophilic aroma compounds as already proposed using *in vitro*⁵⁷ and *in silico* models⁵⁸ 420 and thus will delay the perception by increasing the persistence. The amount of product remaining in the mouth (QRB) 421 explains the perception of both aroma notes. QRB corresponds to the fat coating at the surface of the tongue and oral 422 mucosa after swallowing⁵⁴. For blue cheese perception (NO), it could be thus due to the retention of NO, hydrophobic 423 aroma compounds, in the fat, which delays its release and then enhances its perception. Conversely, the fruity perception 424 (EP) is projected opposite to fat coating in the mouth, i.e. higher is the coating lower is the perception. Many salivary 425 parameters impact aroma perception. Sodium concentration in saliva (Na_S) positively influences fruity perception (EP) and 426 negatively blue cheese perception (NO), whereas it was not found to have a strong impact on in vivo aroma release²².

Salivary lipolysis (Lipolysis_S) was depicted in the same direction of QRB in three projections (hfF-EP, hfF-NO and hfS-NO)
witnessing a different impact on sensory perception depending on the fruity (EP) or blue cheese (NO) note. Salivary flow
(Flux_S), protein content (Prot_S), amylase activity (Amylase_S) also impact sensory perception but differently according to
the cheese and the aroma notes.

431

432 As for high fat cheeses, different trends are observed between firm and soft low fat cheeses (Fig. 6). For the low 433 fat firm cheese (IfF) aroma persistence (Tmax S and Tend S) can be explained by the time to reach maximum 434 aroma release after swallowing (tMax2 P), the amount of product remaining in the oral cavity (QRB) and the oral 435 volume (Oral_Vol) for the two aroma compounds whereas the chewing time (Chew_time) and the number of 436 chewing cycles (Nber_Cycle) mainly explain fruity perception (EP). These correlations are logical because a higher 437 amount of product remaining in the mouth increases the amount of aroma released after swallowing²² which 438 should increase aroma persistence. Moreover a longer chewing time increases the total amount of aroma 439 released¹⁷ and thus aroma perception. The mean amplitude of contraction (Ampl) is depicted very similarly to the 440 rate of perception (Vmax_S) of the fruity note as it was the case for high fat cheeses. Similar to high fat cheeses, 441 stimulated saliva composition and in particular the sodium content (Na_S) impact aroma perception. High sodium 442 content is related to a high fruity aroma (EP) and low blue cheese aroma (NO) intensity.

For the low fat soft cheeses (IfS), similar trends are observed for the fruity aroma (EP) but not for blue cheese aroma (NO). The blue cheese aroma perception (NO) cannot be logically explained by NO release but is related to saliva composition and more precisely to lipolysis (Lypolysis_S). Bolus moistening and bolus rheology highly contribute to the perception of the two aroma notes. A higher aroma perception is related to a higher moistening (Moist) and higher bolus spreadability which is explained by the area at the end of compression (Send). These results are also in line with the higher salivary flow (Flux_S).

449

450 Thanks to MB-PLS statistical approach, some general trends can be highlighted concerning the explanation of 451 sensory perception by release and physiological properties. When consuming solid foods containing fat such as 452 cheeses, masticatory behaviour highly impacts the dynamic of aroma release and thus aroma perception. A large 453 mean amplitude of contraction increases rate of release and rate of perception. A longer chewing time increases 454 the time to reach maximum intensity after swallowing and the time to reach maximum perceived intensity. 455 Concerning the variables from the other blocks, the relationships are not always so simple to interpret, which may 456 be due to interactions between these variables. A high salivary flow leads to a high bolus moistening, a large bolus 457 area at the end of compression indicating a high bolus spreadability, which results in a lower rate of aroma 458 release²² but the direct consequence on aroma perception is not clearly evidenced.

459 However some variables were found to be related to sensory perception and not to aroma release. This is the case 460 for specific variables of saliva composition. The most striking finding is that lipolysis which is depicted in the same 461 direction to fat coating for NO and EP, is also depicted in the NO projection in the same direction to sensory 462 variables, while the opposite is observed for EP. This was particularly emphasized for high-fat cheeses. Salivary 463 lipolysis has been described as a marker of fat sensitivity and liking in human⁴¹. For example, a positive correlation 464 was found between the level of lipolysis and perceived fat intensity while the opposite was observed for liking in subjects⁵¹ with a normal weight. On obese subjects, *in vivo* inhibition of lingual lipase led to a significant 465 466 enhancement of their sensory threshold for triolein⁵⁹. Moreover, compared to normal-weight subjects, obese 467 individuals exhibited a significant low level of salivary lipolysis thus reflecting the higher liking for fat as reported in this population^{48, 60}. Concerning coating, fatty perception of oil in water emulsions has been previously related to 468 the level of fat retention at the surface of the tongue after consumption^{61, 62}. Altogether, these findings tend to 469 470 substantiate a positive correlation between the level of salivary lipolysis, fat coating and fat perception. These 471 findings support our hypothesis of cross-modal interactions between aroma perception and fattiness that differs 472 depending on the aroma. For NO (blue cheese aroma), the similar depiction of lipolysis, fat coating and sensory 473 variables should indicate a congruency between aroma perception and fat perception. Contrariwise, for EP (fruity 474 aroma), the depiction between lipolysis, fat coating and sensory variables should indicate a negative interaction 475 because of the non-congruency between this aroma and fat perception.

476 A similar hypothesis can be proposed for the effect of sodium content in stimulated saliva on aroma perception. 477 This property is depicted in the same direction as sensory variables for EP whereas it is in the opposite direction 478 for NO. The amount of sodium in saliva has already been described as an important factor which contributes in the sensitivity for salt, i.e. the higher the concentration, the higher the detection threshold⁶³⁻⁶⁶. As for lipolysis, it is 479 480 likely that subjects exhibiting a high sodium concentration had a lower saltiness perception. Following our 481 hypothesis regarding the existence of salty-aroma interactions and considering that blue cheese aroma, unlike 482 fruity aroma, could be congruent to saltiness, subjects with a low salivary sodium content may perceive the 483 cheeses as being saltier. Since the subjects were instructed to rate the aroma intensity only and not the salty taste 484 in the cheese products during the sessions, they should have reported their salty perception on the blue cheese 485 aroma which is congruent with salty perception, due to a dumping effect³⁴. Contrariwise, the perception of a fruity 486 note may be increased for the subjects with high sodium content who perceived the cheeses as less salty, because 487 this aroma is not congruent with salty.

488 Conclusions

489 As a conclusion, this study allowed us to highlight the respective impacts of in vivo aroma release and physiological 490 properties on sensory perception. The originality of the approach is to consider all the variables together and 491 evaluate their relative contribution. Our study confirms the important role of masticatory variables for firm 492 cheeses and that of salivary properties for soft cheeses. The perception of the fruity aroma of the more 493 hydrophilic compound (ethyl propanoate) is related to its dynamic release parameters before swallowing whereas 494 the blue cheese aroma of the more hydrophobic compound (nonan-2-one) is related to its dynamic release 495 parameters after swallowing and is highly impacted by mouth coating. Moreover, it was evidenced that dynamic in 496 vivo aroma release does not always explain sensory perception. MB-PLS approach made it possible to evidence the 497 combined effects of saliva composition and cross-modal interactions to understand why in some cases dynamic 498 aroma perception could not be explained by dynamic in vivo aroma release data. Our study confirms the key role 499 of salivary sodium content and lipolysis activity in sensory perception. The main finding is that subjects with a high 500 sodium content in saliva perceive aromas which are not congruent with saltiness as more intense and salt-501 congruent aromas as less intense and that subjects with a high lipolysis activity perceive aromas which are not 502 congruent to fat as less intense and fat-congruent aromas as more intense. These findings could help to better 503 understand the inter-individual differences in aroma perception and could be considered for the reformulation of 504 foods targeted for specific populations taking into account nutritional recommendations.

505 Acknowledgements

506 The acknowledgements come at the end of an article after the conclusions and before the notes and references.

507 This work received financial support from the French National Research Agency (ANR-07-PNRA-014), the Regional 508 Council for Burgundy and FEDER (European Union). The authors thank Etienne Sémon from ChemoSens Platform 509 (CSGA) for APCI experiments, Vincent Gigot from CSGA for APCI curve smoothing, Claude Yven for 510 electromyography, Chantal Septier for subjecst's physiology and sensory analysis, Elsa Ropiteau for sensory 511 analysis, Laboratoire de Rhéologie (Université Joseph Fourier Grenoble, France) for bolus rheology, Fromageries 512 Bel SA, Soredab (groupe Soparind Bongrain) and the panellists.

References

- 1. A. Buettner, S. Otto, A. Beer, M. Mestres, P. Schieberle and T. Hummel, *Food Chemistry*, 2008, **108**, 1234-1246.
- 2. M. Kopjar, I. Andriot, A. Saint-Eve, I. Souchon and E. Guichard, *Journal of the Science of Food and Agriculture*, 2010, **90**, 1285-1292.
- 3. L. Boisard, C. Tournier, E. Semon, E. Noirot, E. Guichard and C. Salles, *Flavour and Fragrance Journal*, 2014, **29**, 95-106.
- 4. S. Lubbers, P. Landy and A. Voilley, *Food Technology*, 1998, **52**, 68-214.
- 5. A. Tromelin, I. Andriot and E. Guichard, in *Flavour in food*, eds. A. Voilley and P. Etiévant, Woodhead Publishing Limited, Cambridge, CB1 6AH, UK, CRC Press edn., 2006, vol. Part 2 Flavour retention and release from the food matrix, pp. 172-207.
- 6. A. Meynier, V. Rampon, M. M. Dalgalarrondo and C. Genot, *International Dairy Journal*, 2004, **14**, 681-690.
- 7. I. Andriot, M. Harrison, N. Fournier and E. Guichard, *Journal of Agricultural and Food Chemistry*, 2000, **48**, 4246-4251.
- 8. J. Delarue and P. Giampaoli, in *Flavour in food*, eds. A. Voilley and P. Etievant, Woodhead Publishing Limited and CRC Press LLC, Cambridge, CB1 6AH (GBR), CRC Press edn., 2006, pp. 208-228.
- 9. S. Lubbers and E. Guichard, *Food Chemistry*, 2003, **81**, 269-273.
- 10. C. Heinemann, M. Zinsli, A. Renggli, F. Escher and B. Conde-Petit, *LWT Food Science and Technology*, 2005, **38**, 885-894.
- 11. C. Salles, M. C. Chagnon, G. Feron, E. Guichard, H. Laboure, M. Morzel, E. Semon, A. Tarrega and C. Yven, *Critical Reviews in Food Science and Nutrition*, 2011, **51**, 67-90.
- 12. A. J. Taylor, R. S. T. Linforth, B. A. Harvey and A. Blake, *Food Chemistry*, 2000, **71**, 327-338.
- 13. W. Lindinger, A. Hansel and A. Jordan, *International Journal of Mass Spectrometry and Ion Processes*, 1998, **173**, 191-241.
- 14. E. Aprea, F. Biasioli, F. Gasperi, T. D. Märk and S. van Ruth, *Flavour and Fragrance Journal*, 2006, **21**, 53-58.
- 15. M. Mestres, N. Moran, A. Jordan and A. Buettner, J. Agric. Food Chem., 2005, 53, 403-409.
- 16. M. Repoux, H. Laboure, P. Courcoux, I. Andriot, E. Semon, C. Yven, G. Feron and E. Guichard, *Flavour and Fragrance Journal*, 2012, **27**, 414-423.
- 17. A. Tarrega, C. Yven, E. Sémon and C. Salles, *International Dairy Journal*, 2008, **18**, 849-857.
- 18. A. B. Boland, C. M. Delahunty and S. M. van Ruth, *Food Chemistry*, 2006, **96**, 452-460.
- 19. L. Boisard, I. Andriot, C. Martin, C. Septier, V. Boissard, C. Salles and E. Guichard, *Food Chemistry*, 2014, **145**, 437-444.
- 20. C. Yven, J. Patarin, A. Magnin, H. Labouré, M. Repoux, E. Guichard and G. Feron, *Journal of Texture Studies*, 2012, **43**, 309-318.
- 21. S. Prakash, D. D. Y. Tan and J. S. Chen, *Food Research International*, 2013, **54**, 1627-1635.
- 22. G. Feron, C. Ayed, E. M. Qannari, P. Courcoux, H. Labouré and E. Guichard, *PLoS One*, 2014, **9**, 1-15.
- 23. C. Tournier, C. Sulmont-Rosse and E. Guichard, in *Food*, ed. G. S. Books, Global Science Books LtD., Royaume-Uni (GBR), 2007, vol. 1, pp. 246-257.
- 24. I. Gierczynski, E. Guichard and H. Laboure, *Flavour and Fragrance Journal*, 2011, **26**, 141-152.
- 25. A. Saint-Eve, N. Martin, H. Guillemin, E. Sémon, E. Guichard and I. Souchon, *Journal of Agricultural and Food Chemistry*, 2006, **54**, 7794-7803.
- 26. I. Gierczynski, H. Laboure and E. Guichard, *Journal of Agricultural and Food Chemistry*, 2008, **56**, 1697-1703.

- 27. J. H. F. Bult, R. A. de Wijk and T. Hummel, *Neurosci. Lett.*, 2007, **411**, 6-10.
- 28. S. Leclercq and G. Blancher, *Chem. Senses*, 2012, **37**, 689-700.
- 29. G. Fiches, A. Saint Eve, S. Jourdren, I. Deleris, P. Bruneriea and I. Souchon, *Flavour and Fragrance Journal*, 2016, **31**, 31-40.
- 30. B. Atanasova, T. Thomas-Danguin, D. Langlois, C. Chabanet, S. Nicklaus and P. Etievant, *Chem. Senses*, 2005, **30**, A28.
- 31. M. Charles, A. Romano, S. Yener, M. Barnaba, L. Navarini, T. D. Mark, F. Biasoli and F. Gasperi, *Food Research International*, 2015, **69**, 9-20.
- 32. H. N. J. Schifferstein and P. W. J. Verlegh, Acta Psychologica, 1996, 94, 87-105.
- 33. R. J. Stevenson, R. A. Boakes and J. Prescott, *Learning and Motivation*, 1998, **29**, 113-132.
- 34. C. C. Clark and H. T. Lawless, *Chem. Senses*, 1994, **19**, 583-594.
- 35. J. Niimi, A. I. Eddy, A. R. Overington, P. Silcock, P. J. Bremer and C. M. Delahunty, *International Dairy Journal*, 2014, **39**, 222-228.
- 36. A. Tarrega, C. Yven, E. Semon and C. Salles, *International Dairy Journal*, 2011, **21**, 358-364.
- 37. M. Doyennette, C. de Loubens, I. Deleris, I. Souchon and I. C. Trelea, *Food Chemistry*, 2011, **128**, 380-390.
- 38. C. Méjean, M. Morzel, E. Neyraud, S. Issanchou, C. Martin, S. Bozonnet, C. Urbano, P. Schlich, S. Hercberg, S. Péneau and G. Feron, *PLoS One*, 2015, **10**, e0137473.
- 39. L. Engelen, P. A. M. van den Keybus, R. A. de Wijk, E. C. I. Veerman, A. V. N. Amerongen, F. Bosman, J. F. Prinz and A. van der Bilt, *Archives of Oral Biology*, 2007, **52**, 518-525.
- 40. A. L. S. Ferry, J. R. Mitchell, J. Hort, S. E. Hill, A. J. Taylor, S. Lagarrigue and B. Valles-Pamies, *Journal of Agricultural and Food Chemistry*, 2006, **54**, 8869-8873.
- 41. G. Feron and J. Poette, *Oilseeds and fats, Crops and Lipids*, 2013, **20**, 102-107.
- 42. J. Poette, J. Mekoué, E. Neyraud, O. Berdeaux, A. Renault, E. Guichard, C. Genot and G. Feron, *Flavour and Fragrance Journal*, 2014, **29**, 39-49.
- 43. A. Buettner, *Journal of agricultural and food chemistry*, 2002, **50**, 3283-3289.
- 44. A. Buettner, *Journal of Agricultural and Food Chemistry*, 2002, **50**, 7105-7110.
- 45. S. Pagès-Hélary, I. Andriot, E. Guichard and F. Canon, *Food Research International*, 2014, **64**, 424-431.
- 46. P. Piombino, A. Genovese, S. Esposito, L. Moio, P. P. Cutolo, A. Chambery, V. Severino, E. Moneta, D. P. Smith, S. M. Owens, J. A. Gilbert and D. Ercolini, *PLoS One*, 2014, **9**, e85611.
- 47. R. Matsuo, Crit. Rev. Oral Biol. Med., 2000, 11, 216-229.
- 48. L. M. Bartoshuk, V. B. Duffy, J. E. Hayes, H. R. Moskowitz and D. J. Snyder, *Phil. Trans. R. Soc. B*, 2006, **361**, 1137-1148.
- 49. A. Mishellany-Dutour, A. Woda, H. Laboure, P. Bourdiol, P. Lachaze, E. Guichard and G. Feron, *PLoS One*, 2012, **7**, e41276.
- 50. S. R. Drago, M. Panouille, A. Saint-Eve, E. Neyraud, G. Feron and I. Souchon, *Food Hydrocolloids*, 2011, **25**, 659-667.
- 51. E. Neyraud, O. Palicki, C. Schwartz, S. Nicklaus and G. Feron, *Archives of Oral Biology*, 2012, **57**, 556-566.
- 52. M. Emorine, C. Septier, T. Thomas-Danguin and C. Salles, *Food Research International*, 2013, **51**, 641-647.
- 53. L. Mioche, P. Bourdiol and S. Monier, *Archives of Oral Biology*, 2003, **48**, 193-200.
- 54. M. Repoux, C. Septier, O. Palicki, E. Guichard, G. Feron and H. Labouré, *Archives of Oral Biology*, 2012, **57**, 81-86.
- 55. B. H. Mevik and R. Wehrens, *J. Stat. Softw.*, 2007, **18**, 1-24.
- 56. S. Hassani, H. Martens, E. L. Qannari, M. Hanafi and A. Kohler, *Chemometrics and intelligent laboratory systems*, 2012, **117**, 42-53.
- 57. S. Odake, J. P. Roozen and J. J. Burger, *Nahrung*, 1998, **42**, 385-391.
- 58. M. Doyennette, I. Déléris, G. Féron, E. Guichard, I. Souchon and I. C. Trelea, *Journal of Theoretical Biology*, 2014, **340**, 209-221.

- 59. M. Y. Pepino, L. Love-Gregory, S. Klein and N. A. Abumrad, *Journal of Lipid Research*, 2012, **53**, 561-566.
- 60. C. Vors, J. Drai, L. Gabert, G. Pineau, M. Laville, H. Vidal, E. Guichard, M.-C. Michalski and G. Feron, *International Journal of Obesity*, 2015, **39**, 1425-1428.
- 61. S. Camacho, K. Liu, A. Linden, M. Stieger and F. Velde, *Journal of Texture Studies*, 2015, **46**, 399-410.
- 62. S. Camacho, V. van Riel, C. de Graaf, F. van de Velde and M. Stieger, *Journal of Agricultural and Food Chemistry*, 2014, **62**, 5789-5795.
- 63. L. M. Bartoshuk, *The American Journal of Clinical Nutrition*, 1978, **31**, 1068-1077.
- 64. R. J. Contreras and F. A. Catalanotto, *Behavioral and neural biology*, 1980, **29**, 303-314.
- 65. J. Delwiche and M. O'Mahony, *Physiology & Behavior*, 1996, **59**, 605-611.
- 66. T. Morino and H. G. Langford, *Physiology & Behavior*, 1978, **21**, 45-48.

Table 1: presentation of the different blocks of variables used in the –MB-PLS Analyses. Y: variables to be explained, X: explanatory variables. a.u.: arbitrary units

Block	Abbreviation	Definition of the variable			
	Imax_S	Maximum intensity perceived (a.u.)			
	Tmax_S	Time to reach the maximum intensity (s)			
Y: sensory parameters	Vmax_S	Rate of perception (a.u./s)			
	Ideg_S	Maximum intensity at swallowing (a.u.)			
	Tend_S	Time to reach the end of perception (s)			
	A1_P	Area under the curve before 1 st swallowing (a.u.)			
	A2_P	Area under the curve after 1 st swallowing (a.u.)			
	A_P	Total area under the curve (A = A1_P + A2_P)			
X1: aroma	IMax1_P	Maximum intensity before 1 st swallowing (a.u.)			
parameters	tMax1_P	Time to reach maximum intensity before 1^{st} swallowing (s)			
	IMax2_P	Maximum intensity after 1 st swallowing (a.u.)			
	tMax2_P	Time to reach maximum intensity after 1 st swallowing (s)			
	Vmax _P	Rate of release (a.u./s)			
	Moist	Moistening of the bolus just before the swallowing (%)			
	sflow	Yield stress at flow phase of compression curve (Pa)			
	spart	Yield stress at particle phase of compression curve (Pa)			
X2:	hpart	Bolus height at the beginning of the particle phase of compression curve (mm)			
Bolus rheology	Kflow	Consistency at the flow phase, which reflects bolus consistency (Pa.s)			
	hflow	Bolus height at the beginning of the flow phase of compression curve (mm)			
	hend	Bolus height at the end of compression (mm)			
	Send	Area at the end of compression (mm ²)			
X3:	QRB	Amount of product remaining in the oral cavity after swallowing (%)			
Coating- oral vol	Oral_Vol	Volume of the oral cavity (cm ³)			
	Nber_Cycle	Number of chewing cycle			
X4:	Chew_time	Chewing duration (s)			
Masticatory	Ampl	Mean amplitude of contraction (mV)			
purumeters	W_tot	Energy expended in chewing (mV/s)			
	Flux_S	Salivary flow stimulated saliva (ml/min)			
	Prot_S	Amount of salivary proteins stimulated saliva (mg/ml)			
X5:	Lipolysis_S	Amount of Lipolysis in stimulated saliva (mU/mI)			
Properties of stimulated saliva	Amylase_S	Amount of Amylase in stimulated saliva (U/ml)			
	Proteolysis_S	Amount of Proteolysis in stimulated saliva (U/ml)			
	Na_S	Amount of sodium in stimulated saliva (mM)			

Table 2: oral physiological characteristics of the 14 subjects included in the study, mean, standard deviation, minimum and maximum values.

					Standard
		minimum	maximum	mean	deviation
Oral volume (cm ³)	Oral_Vol	26.85	62.16	39.03	9.73
Stimulated salivary flow (ml/min)	Flux_S	0.73	4.32	2.60	1.40
Protein (mg/ml)	Prot_S	0.57	1.31	1.04	0.31
Lipolysis (mU/ml)	Lipolysis_S	<0.01	1.32	0.90	0.74
Amylase (U/ml)	Amylase_S	1.27	30.6	20.86	11.00
Proteolysis (U/ml)	Proteolysis_S	0.04	0.51	0.13	0.12
sodium content (mM)	Na_S	2.09	37.82	15.82	11.03

Figure captions:

Figure 1. Time intensity curves obtained for one subject for blue cheese aroma (NO: nonan-2-one) for the four cheeses (hfS: high fat soft, hfF: high fat firm, lfS: low fat soft, lfF: low fat firm) showing the extracted parameters (Imax_S: maximum intensity, Tmax_S: time to reach maximum intensity, Ideg_S: intensity at swallowing and Tend_S: time to reach the end of perception.

Figure 2. Bibplot representation from the PCA performed on the 14 subjects and their physiological parameters.

Figure 3. Bar charts representing the relative importance (%) of the different blocks of variables (X1-X5) for the different components obtained by means of MB-PLS analysis performed on fruity aroma (EP) and blue cheese aroma (NO) sensory data set and for the high fat (hfF & hfS) cheese products. Blue chart: X1-Aroma release, Green chart: X2-rheology, Orange chart: X3-coating and oral volume, Red chart: X4-EM data, Violet chart: X5-stimulated saliva composition.

Figure 4. MB-PLS results on dim1/dim2 for high fat (hf) cheeses: relationships between the X-blocks of explanatory variables (Blue arrows: X1-aroma release, Green arrows: X2-rheology, Orange arrows: X3-coating and oral volume, Red arrows: X4-EMG data, Violet arrows: X5-stimulated saliva composition) and the Y block of variables to be explained (Black arrows: sensory data for fruity aroma (EP) and blue cheese aroma (NO). Top: firm high fat cheeses (hfF), bottom: soft high fat cheeses (hfS). The font size for each variable reflects the importance of the correlation coefficient with the two components.

Figure 5. Bar charts representing the relative importance (%) of the different blocks of variables (X1-X5) for the different components obtained by means of MB-PLS analysis performed on fruity aroma (EP) and blue cheese aroma (NO) sensory data set and for the low fat (IFF & IfS) cheese products. Blue chart: X1-Aroma release, Green chart: X2-rheology, Orange chart: X3-coating and oral volume, Red chart: X4-EM data, Violet chart: X5-stimulated saliva composition.

Figure 6. MB-PLS results on dim1/dim2 for low fat (If) cheeses: relationships between the X-blocks of explanatory variables (Blue arrows: X1-aroma release, Green arrows: X2-rheology, Orange arrows: X3-coating and oral volume, Red arrows: X4-EMG data, Violet arrows: X5-stimulated saliva composition) and the Y block of variables to be explained (Black arrows: sensory data for fruity aroma (EP) and blue cheese aroma (NO). Top: firm low fat cheeses (IfF), bottom: soft low fat cheeses (IfS). The font size for each variable reflects the importance of the correlation coefficient with the two components.



Figure 1. Time intensity curves obtained for one subject for blue cheese aroma (NO: nonan-2-one) for the four cheeses (hfS: high fat soft, hfF: high fat firm, lfS: low fat soft, lfF: low fat firm) showing the extracted parameters (Imax_S: maximum intensity, Tmax_S: time to reach maximum intensity, Ideg_S: intensity at swallowing and Tend_S: time to reach the end of perception.

254x190mm (96 x 96 DPI)



Figure 2. Bibplot representation from the PCA performed on the 14 subjects and their physiological parameters.

254x190mm (96 x 96 DPI)



Figure 3. Bar charts representing the relative importance (%) of the different blocks of variables (X1-X5) for the different components obtained by means of MB-PLS analysis performed on fruity aroma (EP) and blue cheese aroma (NO) sensory data set and for the high fat (hfF & hfS) cheese products. Blue chart: X1-Aroma release, Green chart: X2-rheology, Orange chart: X3-coating and oral volume, Red chart: X4-EM data, Violet chart: X5-stimulated saliva composition.

705x448mm (72 x 72 DPI)



Figure 4. MB-PLS results on dim1/dim2 for high fat (hf) cheeses: relationships between the X-blocks of explanatory variables (Blue arrows: X1-aroma release, Green arrows: X2-rheology, Orange arrows: X3-coating and oral volume, Red arrows: X4-EMG data, Violet arrows: X5-stimulated saliva composition) and the Y block of variables to be explained (Black arrows: sensory data for fruity aroma (EP) and blue cheese aroma (NO). Top: firm high fat cheeses (hfF), bottom: soft high fat cheeses (hfS). The font size for each variable reflects the importance of the correlation coefficient with the two components.

705x705mm (72 x 72 DPI)



Figure 5. Bar charts representing the relative importance (%) of the different blocks of variables (X1-X5) for the different components obtained by means of MB-PLS analysis performed on fruity aroma (EP) and blue cheese aroma (NO) sensory data set and for the low fat (IFF & IfS) cheese products. Blue chart: X1-Aroma release, Green chart: X2-rheology, Orange chart: X3-coating and oral volume, Red chart: X4-EM data, Violet chart: X5-stimulated saliva composition.

705x448mm (72 x 72 DPI)



Figure 6. MB-PLS results on dim1/dim2 for low fat (If) cheeses: relationships between the X-blocks of explanatory variables (Blue arrows: X1-aroma release, Green arrows: X2-rheology, Orange arrows: X3-coating and oral volume, Red arrows: X4-EMG data, Violet arrows: X5-stimulated saliva composition) and the Y block of variables to be explained (Black arrows: sensory data for fruity aroma (EP) and blue cheese aroma (NO). Top: firm low fat cheeses (IfF), bottom: soft low fat cheeses (IfS). The font size for each variable reflects the importance of the correlation coefficient with the two components.

705x705mm (72 x 72 DPI)